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## Scaling Technique for Web Based Management Systems in Bioinformatics

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**Abstract:** With the enormous growth in web based management solutions the number of transactions increases proportionally, as system grows the number of requests increase. With the increase in number of request there introduce scaling concept. There are number of scaling techniques available but all these techniques focus on scaling the server by distribute the load on different servers. Many techniques give solutions to reduce the number of request and load balancing. But all the existing techniques are limited as the number of transactions increases exponentially. As a remedy we introduce new technique, which helps give a scalable solution without server maintenance cost. In this technique all users actions stored in the local java script model which then will saved in the database after some point so that to reduce the traffic. Objective is to make some intelligent client who stores all transaction at the client side and send only one request to server for all transactions.

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**Keywords:** Techniques; Management System; Web Based; Solutions

### 1. Introduction

The main issue with web applications is inability to plan and predict the number of users that will be accessing this application. Sometimes this amount of users generate peak load in some special events or days. In such condition application remain to continue and work properly without any impact. And the response time must be average. So in the worst case application may chock or server crashes because of the amount of users that access the system. So the solutions would be such that it handles such worst condition and handle the dynamic behavior. Many techniques offer some dynamic behavior of the server, which distribute its load and give the average response time. In all techniques they might need some minimum number of servers but in the worst condition the number of servers might increase to infinite so it would increase the maintenance of server and cost of each instance. Many scaling techniques exists which helps to improve the server response and load on server, Like database Sharding which shards the data with respect to region using different shard key, Replication of servers, but all the available techniques have high cost of maintenance and hardware [3]. All available techniques focus on the server maintenance or to improve the middle layer. Some techniques are used to reduce the number of transactions but the problem of server maintenance still persists. Now we introduce the new technique that uses the JavaScript model to store the information at client side and then send all the transactions made by the user send to the server in a single request. This system helps to develop

the forms at run time, rich JavaScript capable to draw the form without compile or upload the page. All the existing technique makes the server scalable but in our purposed system we focus to fetch all the data from database in JavaScript model. This purposed techniques valid for single transaction system and partially offline mode.

### 2. Related Work

Client server model describes the communication between two instances of computers where one is termed as client who is on service requesting end and other is termed as server which is at service fulfilling end [1]. Client server architecture is all about sharing resources more specifically it's all about sharing server's resources by clients. Normally communication between two instances happens through a certain protocol. That protocol can be termed as Network. Client and server model can be applied within a single system or it can be over a computer network. Client triggers the communication by requesting server's resources while server fulfills entertains client's requests [5].

Client server architecture approach can also be applied in software engineering. Multi tier architecture is one very good example of this phenomenon. In which multiple layers (Presentation, Business logic, and database) can communicate with each other. 3-tier architecture is highly used multi-tier architecture.

Another example of client server architecture in real life can be online form submission in school,

College or university. In these type of transactions programs in computer is acting as client while the computer which is saving the forms is server.

Model-View-Controller is an architectural design in software engineering which can be used to separate the software programs in different logical layers. It separates the application layer and business logic layer from user interface. Adopting this approach can leads to less time input for maintenance. If any amendment is required then instead of going for the whole application change only specific tier can be amended.

Client server technique is good enough to be used for efficient communication but there is some limitation which needs to keep in view while implementing this architecture, as this architecture is based on mutual communication of client and server. All the clients are sending requests to server which in returns shares his resources. Too many requests from too many clients can put an impact on server's performance. For any action at client side server has to respond and bring in the latest data. Good client server model can be implemented if server is built with efficient resources. Server should be able enough to respond to all clients' requests on real time basis.

Scaling dynamic web sites has been gained much attention because of growing financial issues. Most of the existing research on scaling web applications focuses on either content replication [4], [5], [7] or dynamic content caching [8] that need site administrator or manual intervention of user. Furthermore, the dynamic content need page fragments via templates and database triggers [4]. Similarly, some current data replications techniques require specialized application uniformity schemes [4], [5], [8] and force the consumer to handle conflicting results [2].

### 3. Proposed Technique

In the early era of dynamic website development the request/respond architecture was very famous. The browser application used to send synchronous calls to the server and server used to send response against this request. Later on the Asynchronous post back or Ajax gained popularity due to its hidden request/response nature but still the number of request sent to the server had significance impact on the performance of the server. The purposed methodology can be implemented in single transaction system in which work of one user is not affected by other users. In these types of systems users normally work in partial offline mode management software solution are example of such system where we can implement this technique to enforce minimum request

on the server and to boost the performance of the server.

When the user logins into the system all its data is fetched into model layer and this model layers classes are then passed to Dynamic JavaScript Code generation layer that is generated Dynamic JavaScript code for the GUI this JavaScript code is basically build the global shared object of Script model layer and then GUI pages are call required to HTML function of that class that is generate dynamic HTML depending upon contents to be displayed on the page.

When the user leaves the page or he wants to save & reset the state of data the JS class model returns the XML with all the changes user have made in the state of data and then this xml is passed to XML parser which parses this XML and fill the model layer classes which can then be passed to Data access layer for further operations in database.

The proposed design method has the following components as shown in Fig. 1:

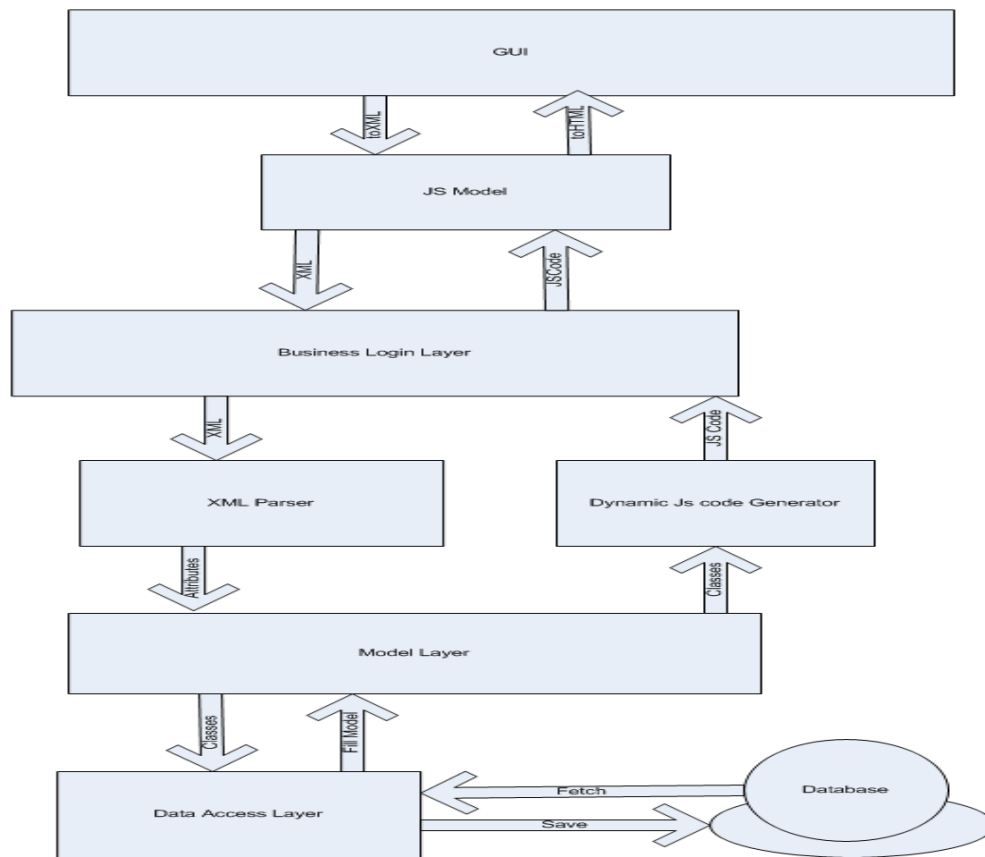
**GUI:** This component fetches data from JS Class model in the form of html and displays the content on the page. Other functions like validation, getting input from the user, is performed here and all the actions taken by the user is recorded in JS class model main object of JS class model is shared among all the pages and also redirection is also be performed on the client side to keep this shared object alive.

**Class Model Using JavaScript:** This component is replica of ERD that is used to tack all the changes users have made in the state of data it received from database. The whole model returns a global object that contains the user data and this object are shared on all the pages. Every change will be highlighted in this object and when user will leave the system or wants to Save and Reset state of data.

**Business Logic Layer:** The main purpose of this layer is to monitor the incoming and outgoing traffic between GUI and other components. This layer builds the JS class model after getting dynamic code from Dynamic JavaScript Code Generation Layer and when data has to be saved into database it fetches xml sting from JS class model and passes this string to XML parser for further processing.

**Dynamic JavaScript Code Generation Layer:** This layer gets the classes from model layer and creates JavaScript code to populate data into JavaScript classes. This JS code is thrown on the page dynamically to generate the contents of the page.

**XML Parser:** The XML parser gets the XML document as an input from GUI and populates all the data in Server side classes of model layer to be saved in database.



**Figure 1: Architecture of the Proposed Technique**

**Model layer:** Model layer models the ERD model on server side classes. These classes have same relationship as database entities and are used to populate user data into memory. If we are using Asp.Net framework then Un-typed dataset are best fit with the methodology.

**Data Access Layer:** This layer handles all the operations that are relegated to database like fetching data from database. Inserting data into database or deleting data from the database

**Database:** Database is the essential parts of dynamic web sites. All data and other supporting information are stored in the database as we store for a normal application. The main point that needs to be more focused while designing database is that all the entities and their relationship should be clearly defined. The same ER model is used to build model layer and Class model using client side scripting. Any change in any entity is needed to be incorporated into model layer and scripting class model.

The proposed technique has very appreciating values of certain parameters such as load balancing,

speed, server-interaction and client-intelligent behavior. Using this architecture the number of request sent to the server is less than ordinary request/response architecture so load on the server will be minimized. Everything is directly rendered on client and all the data will be available on client side as well so this will boost the speed of system as well. Request to the server will be sent either on the demand of user or when he will leave the system. Furthermore, all managerial tasks like adding, updating or removing any record will be handled on client side and save will be saved into database on logout or user's demand so user will be working in partially offline mode.

#### 4. Case Study

To verify the correctness and completeness of proposed technique, we have taken a library management system in which a librarian login into the system and perform various actions like issue new book, update book status and add new student into library etc, the some formal description of class model of case study is listed below:

```

function Grid (id, name, className, rowCount)
{
  this.ID = id;
  this.Name = name;
  this.ClassName = className;
  this.Rows = rowCount
  this.Columns=new Array ();
  this.RowResponse=new Array ();
  this.AddRowResponse = function (col)
  this.AddColumn = function (col)
    this.toXML = function (mod,SSsequence,label) {}
  this.toHTML = function (Mod, Header, FormID, SectionId,
  SubSectionID, label) {}
}
function Question (id, text, type, sequence, pageBreak, label)
{
  this.ID = id;
  this.Sequence = sequence;
  this.Text = text;
  this.Type = type;
  this.Label=label;
  this.Options = new Array ();
  this.Answers = new Array ();
  this.AddAnswer = function AddAnswer (Obj) {}
  this.GetAnswerIndex = function (parent) {}
  this.GetUniqueAnswerID = function GetUniqueAnswerID
  (QuestionID) {}
  this.AddOption = function (o) {}
  this.GetUniqueOptionID = function GetUniqueOptionID (QuestionID)
  {}
  this.toXML = function (mod) {}
  this.toHTML = function (mode, FormID, SectionId, SubSectionID) {}
}

function SubSection (id, name, className, sequence, type, label)
{
  this.ID = id;
  this.Name = name;
  this.Sequence = sequence;
  this.Label = label;
  this.Grid = new Grid ("", "", 0);
  this.Questions = new Array ();

  this.AddGrid = function (grid) {}
  this.AddQuestion = function AddQuestion (q) {}
  this.DeleteQuestion = function DeleteQuestion (id) {}
  this.GetQuestionByID = function GetQuestionByID (id) {}
  this.SortQuestions=function SortQuestions () {}
  this.toXML = function (mod) {}
  this.toHTML = function (mode, FormID, SectionID) {}
}

function Section (id,name, className, sequence, pageBreak, label)
{
  this.ID = id;
  this.Name = name;
  this.ClassName = className;
  this.Sequence = sequence;
  this.Label = label;
  this.SubSections = new Array ();
  this.AddSubSection = function AddSubSection (SS){}
  this.GetUniqueSubSectionID = function GetUniqueSubSectionID
  (SectionID) {}
  this.DeleteSubSection = function DeleteSubSection (id) {}
  this.GetSubSectionByID = function GetSubSectionByID (id) {}

```

```

  this.SortSubSections = function SortSubSections () {}
  this.toXML = function (mod) {}
  this.toHTML = function (mode,FormID) {}
}

```

```

function Form(id,title,sequence,istemplate, isComplete)
{
  this.ID = id;
  this.Title = title;
  this.Sequence = sequence;
  this.Sections = new Array ();
  this.AddSection = function (s) {}
  this.DeleteSection = function DeleteSection (id) {}
  this.GetSectionByID = function GetSectionByID (id) {}
  this.SortSections = function SortSections () {}
  this.toXML = function (mode) {}
  this.toHTML = function (mode) {}
}

```

```

function Study(id, name, xdate, nResponses, IsTemplate,
  ProtocoleNumber, ProjectId, StudyDescription, StudyShortName,
  Type, StartDate, EndDate, PlannedEndDate, NextFormID,
  NextSectionID, Mode)

```

```

{
  this.ID = id;
  this.Name = name;
  this.ExpiryDate = xdate;
  this.Responses = nResponses;
  this.IsTemplate = IsTemplate;
  this.StudyShortName = StudyShortName;
  this.Type = Type;
  this.StartDate = StartDate;
  this.EndDate = EndDate;
  this.Forms = new Array ();
  this.AddForm = function (f) {}
  this.DeleteForm = function DeleteForm (id) {}
  this.GetFormIndex = function GetFormIndex (id) {}
  this.GetFormByID = function (id) {}
  this.toXML = function (mode) {}
  this.toHTML = function (mode) {}
}

```

The GUI component can be build dynamically or statically we can use two approaches to build this system either we can create all the forms and can set the value of input/output field from JS Model Layer or we can get whole html from the JS model to show the contents on the page. Client model contains the classes of the entire table that have been created in the database. Of course the relation like inheritance/composition between different entities will also be maintained and all important method are implemented like for book class we implement different data manipulation functions. The Business Logic Layer governs the traffic between GUI and lower layers. it validates the xml that is given to XML parser and contains other business logic. The Dynamic JavaScript Code Generation Layer becomes in action when user will fetch data from database this will create dynamic JS code. This dynamic code will build the JS

Model.

### 5. Conclusion and Future Work

With the enormous growth in web based management solutions the number of transactions increases with the increase in the number of requests. To server so many requests scaling concept was introduced. There are number of scaling techniques available but all these techniques focus on scaling the server by distribute the load on different servers. The purposed study focus on to shift the load on client system and offers a model which can decreases the number of request sent to the server. This Software is best fit with those systems where more than one user does not work on the same data simultaneously. This technique can be further used to create readymade ERPs solutions. The model can be enhanced into two parts one for creation of the website and the other will be for the normal user of system.

### Acknowledgment

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## Anomalous Pattern Detection Using Context Aware Ubiquitous Data Mining

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**Abstract**— Due to the developments in technology number of applications emerged that produce huge amount of data in the form of streams. Dealing with this and extracting useful information from that data is a real challenge. In this paper, we have developed an architecture that can be used to manage data streaming applications and can extract useful information from that data in online fashion. To achieve mining results online, different phases in our model are parallelized. In this model we have also introduced the concept of context-awareness to improve performance of the proposed architectural model. In this model information from heterogeneous sources is gathered, fuse that information, and generate real-time results. These real-time results can be beneficial in different application area like web usage mining, online monitoring, fraud detection, network security, telecommunication calls monitoring, network monitoring and security, etc. To fulfill the objectives of this research, we incorporate lightweight online mining algorithms to extract useful but hidden information from the data gathered. Contextual information is exploited to detect anomalous behaviors. In this paper we have designed an architectural model to extract frequent patterns in the streaming data.

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**Keywords-** stream mining; context-aware; anomalous pattern mining; ubiquitous data mining

### I. INTRODUCTION

Developments in technology led us to the scenario where we have to deal with very voluminous data that is continuously arriving without limits. For example data being received from satellites, web-clicks, sensor network, stock exchange, shopping at big malls, telecommunication calls, etc. is very huge and continuous without limits. The data which is continuous, unbounded and without limits is called data stream. This data is fast changing, massive in nature and potentially infinite. To deal with this data, there is need to devise some architectural model and efficient techniques to extract useful information in timely fashion. Traditional data mining models and algorithms mainly focused on modeling [1], regression [1], clustering[5,6], classification [[2][3][4] and querying [5][6]. Unlike traditional data mining, stream mining need to extract information from continuously varying, huge and dynamic data therefore some complex challenges are need to tackle in streaming environment. Some inherent characteristics of stream mining are:

- data streams are in order and that cannot be controlled
- Data is coming continuously at some fixed or variable rate
- Concept drift might occur after some interval
- Storage of data being received is not possible or simply not required

- Only one scan of data is possible because of scarce computational resource
- Analysis of data should be instantly available when requested
- Error rate in outputs should be kept as small as possible

Due to these constraints, motivation for stream mining model and architectural design emerged. In other words, development of efficient algorithms and model for stream mining is direly needed. Many efforts have been made to develop efficient algorithms for stream mining [5] [7][8][9][10][11][12].

According to the stream mining model [13], there is a need to collect only sample data from the sensing devices to use their resources more effectively. Once the data is received, perform data mining operation on that with efficient algorithms and then store those results in the knowledge-base to improve learning ability of the system. Similarly, stream mining research can be categorized into *landmark window*, *sliding window and damped window* [14]. In the landmark window, transactions from some specific time marker to the present are considered to generate mining results. While in sliding window model, two types are time-sensitive and transaction-sensitive sliding windows. In time-sensitive sliding window, time slot expires while in transaction-sensitive window transactions are expired. The third type of sliding window model is



damped-window, deals with the concept-drift, meaning that recent-most transactions are more important than the older ones.

A large number of models and algorithms have been proposed for mining streaming data, many of those do not give importance to the recent transactions on the older ones. Similarly, most of those algorithms produce approximate results on streaming data [15][16][10][17]. To overcome this limitation of approximate results, a new CP-Tree based sliding window algorithm for streaming data has been proposed [18]. Although, sliding window based frequent pattern mining algorithm is efficient enough but it cannot be used in ubiquitous environment.

To overcome the problems in the domain for streaming data, we propose architecture for sensor stream mining. We have also incorporated the context-awareness to improve effectiveness and reliability of results. This framework will analyze the real-time contextual information of a particular environment although such information will of course impede the computational and communicative powers of ubiquitous devices. In the event of anomalous behavior, alerts and alarms caution security personnel to take necessary actions in a timely manner. These alerts help minimize the chances of disrupting the smooth flow as desired.

#### **Rest of the paper is organized as:**

Section 2 is dedicated to provide an overview of Ubiquitous Data Mining (UDM) and its potential for implementation in real-world scenarios. This emergent technology can play a vital role in predicting behaviors and intentions of an agent in an environment under observation. Applications and implementations of sensor streams and ubiquitous data mining are described in section 3 of this paper. Section 4 describes an example scenario for integration of ubiquitous data mining and contextual information for the prediction of human intentions. In Section 5, we have presented the design of the system, its sub-modules and schematic flow of data. Finally, Section 6 concludes and describes implications for future research.

## **II. PRELIMINARIES**

The process of extracting interesting, hidden and useful patterns is called data mining. With the help of data mining algorithms and techniques, we can identify clusters, classification of data and extraction of association rule can be performed. Statistical analysis and data mining tasks utilize considerable computational, memory and communicational resources. Therefore it is required and important to pay attention to the computational and communicative powers of systems. In conventional data mining systems, data is normally gathered at some central location in the form of a data warehouse to perform

data analysis by incorporating statistical techniques and machine learning algorithms [13]. The emergence of wireless and mobile devices has introduced a new dimension and enabled access to a large amount of data located at distributed and remote locations in the form of continuous streams. Ubiquitous Data Mining (UDM) is the process of analyzing data and information being received directly from the environment or retrieved from remote systems on mobile devices like cell phones, PDA or touch-pad [19]. Ubiquitous computing and data mining enables users to monitor, retrieve and analyze data from distributed and heterogeneous devices like sensors and mobiles [20][21][22][23].

As computational power of wireless and portable devices is continuously increasing so we are able to perform tasks that need high resources in terms of memory and computing power. Currently available portable devices can perform data mining operation on the bases of spatial and temporal constraints [24][22]. The basic techniques for analyzing data and extracting hidden patterns are usually derived from traditional data mining, statistical techniques and machine learning methodologies. However, the existing techniques cannot be used straightway due to resource limitations of these portable devices. There is a need to cater traditional data mining algorithms to fit in the ubiquitous environment.

Ubiquitous data mining normally need to perform pattern extraction task in online fashion. Similarly, the data in continuously arriving at high rate therefor very fast algorithms are required to be developed. These fast techniques need to compromise a bit on accuracy as compared with traditional data mining algorithms [25][26]. There is need of ubiquitous data mining (UDM) software to extract hidden but useful patterns from streaming data. Objective of this UDM module is to analyze data in real-time and then transfer that information to central location for further processing. Due to this functionality, usage of bandwidth can be improved with decreased traffic towards the central server. Personalization and aggregation tasks will also be performed locally.

Data-intensive applications are starting to appear on PDAs and cell phones such as cell-phone-based patient monitoring systems [27][28], vehicles and driving monitoring systems [29], and wireless security systems. In the near future, some of the applications to be exercised include monitoring and analyzing data in embedded devices for smart applications, and the use of Nano-scale devices for on-board monitoring. Thus, it is necessary to provide support for such applications in terms of advanced data analysis and prediction. Such applications pose various challenges and problems in order to analyze data and apply data mining techniques, which, in this domain, include:

- Efficient single-pass algorithms need to be

developed to analyze and extract useful information from streaming data in ubiquitous environments;

- How to visualize results on mini screens of smart phones and PDA's; This is the major area need to be worked so that extracted results and patterns can be presentable more effectively on these mini devices;
- Communication bandwidth in sensor network and cell phones is normally low as compared with the other computing devices like desktop or laptop computers. Channels used by normal computing devices for communication is fast enough though there is need to optimize utilization of bandwidth in that case too [22][30].

A lot of research is has been carried out to optimize battery usage in mobile devices and similarly researcher are also focusing to develop battery modules that can store more energy to provide longer backup time. In spite of all these efforts and research, limitation in terms of battery is still there. It also direly needed to use battery resources in optimal or sub-optimal way to increase network overall lifetime. Battery resource is still a barrier to extract useful information in ubiquitous data mining [31][32].

### III. LITERATURE REVIEW

Advances in technology and software enable us to gather huge amounts of data from various sources. To exploit the full potential of this data, we are in need to perform data analysis to extract useful patterns from data streams. A dramatic decrease in the cost of data storage technology has enabled us to store huge volumes of data streams. Extracting data from those data generating and storage devices for analysis and fulfilling users' queries is not efficient enough and has become an interesting area of research. To overcome this problem, researchers from artificial intelligence, machine learning and data mining community focused their attention to this specific problem. As a result technology of intelligent data analysis emerged to extract hidden and useful information by using automated or semi-automated means from data streams. Initially intelligent data analysis is incorporated in already existing statistical techniques to extract interesting patterns from historical and static data. Gradually with advances in computational devices and increase in database size, efficient and scalable machine learning and artificial intelligence algorithms were developed. This improved data analysis tasks both in terms of accuracy and reliability. In order to address the problem of very large databases, statistical analysis and machine learning techniques have been incorporated [33].

Developments in data gathering and wirelessly sending devices in last few years are exponentially high [34]. Voluminous data is being transmitted from satellites, sensors, web clicks, stock market, etc. and it is a sheer challenge to store, manipulate and analyze such huge quantities of data. Similarly, in time critical applications, analysis of data is important only in some specific time intervals. Storing all data might be of no use in future [35]. Data coming continuously in the form of streams needs to be analyzed as soon as it arrives at the processing unit. This online analysis is a challenge with the constraints of available storage, computational powers and communicational bandwidths. To overcome these problems some efforts have been made in last few years. To address the challenges of streaming data mining some systems, models and algorithms have been developed [35].

Incorporating contextual information in ubiquitous data mining is very useful. It is used to predict car accidents before it occur. Similarly the same information is used to warn drivers in smart cars. Sources of data for these alarms are both on-board sensing devices and sensors from the environment [36] [29]. This is a good breakthrough to develop such cars that use context-awareness to reduce number of accidents in smart cars. Analysis of test data reflects that most of the accidents are due to tiredness of drivers or the environmental conditions being logged as contextual information.

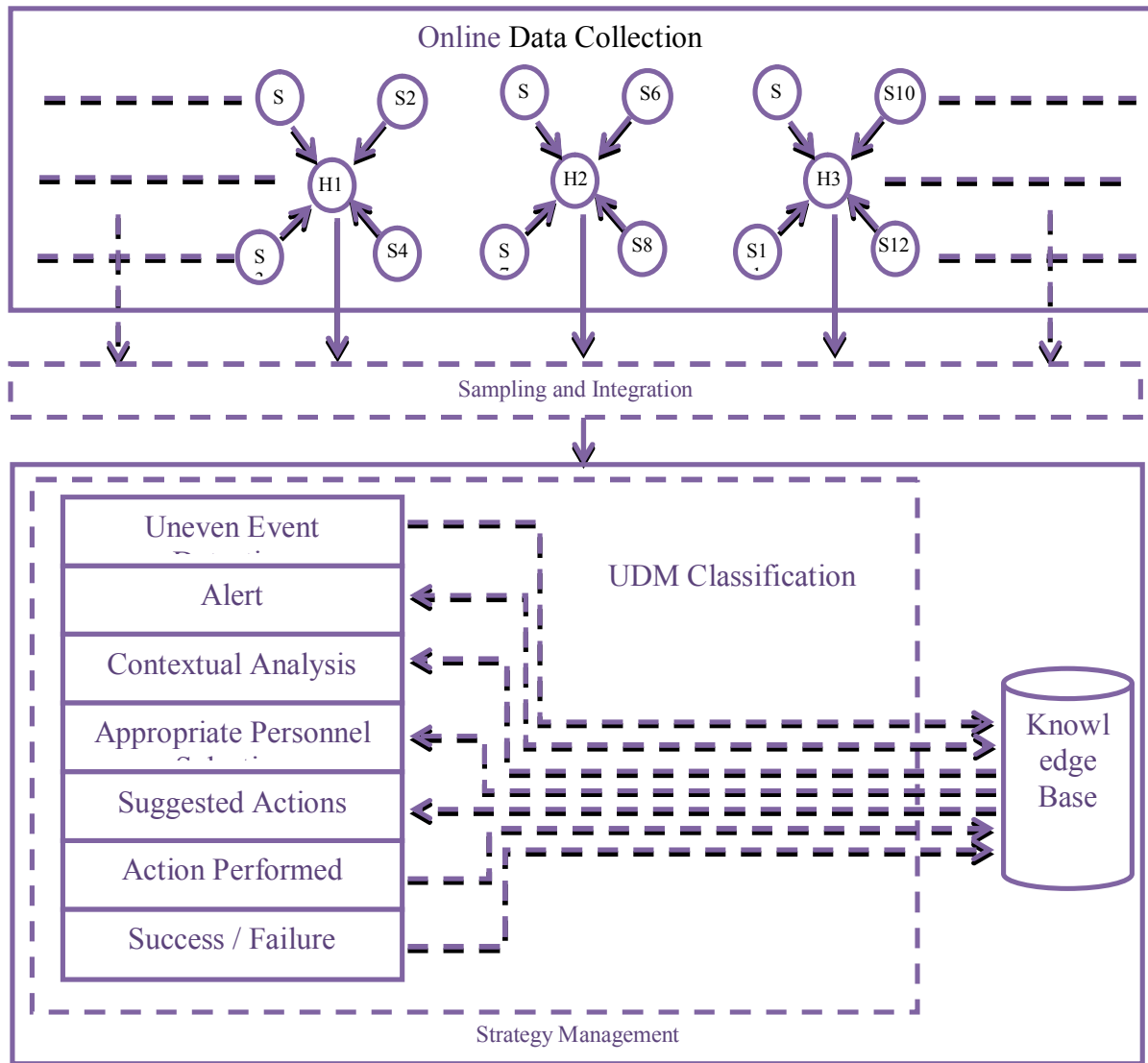
Monitoring patients who are suffering critical diseases like cardiac problem or asthma is one of the most important application area of UDM [27][28]. Using patient cell phone, home-based sensor network and then connecting with the hospital information system provide good opportunity for the better treatment of critical patients. Connecting patient information with the contextual circumstances, early treatment can be started if needed.

Link analysis techniques are used to detect human behavior in real-time. To achieve this feature extraction using unsupervised method is used and discrete human activities are extracted [37].

In this research we have developed an architectural framework to extraction pattern using portable devices. To improve performance, we have also incorporated context-awareness. Combining technological developments and data gathering techniques, our proposed architecture is expected to perform much better.

### IV. EXAMPLE SCENARIO

In this section, we explain our proposed architecture with example application of security and sustainability of society. There is need to define some basic things before explaining the example application.



*Situation:* The state of affair of an entity is called its situation i.e. what is happening inside or outside of an entity and how is the entity at the moment.

*Awareness:* Knowing exactly what is happening inside or outside of an entity under observation is called awareness. Awareness gives us some values about the entity and its surrounding.

*Context-awareness:* It means that getting information about the entity and its surroundings and then utilizing that information on the bases of context. It includes information about the location, person and the environment in which the person is currently located, among other things.

Security sensitive areas like bus stops, railway stations, airports, some events, etc. can be monitored using this UDM architecture. Environmental sensing devices can collect necessary information about the

persons in the area under observation. This live information is fused with the existing knowledgebase to extract useful patterns. Security personnel are provided with the UDM enabled smart-phones to analyze situation in online fashion. Unusual behavior of a person or vehicles or other moving things can be predicted in timely fashion to minimize chances of any devastation. Once the uneven behavior is detected, an alert will be generated on the smart-phone of the security personnel. These alerts will also consider context-awareness and will be generated or forwarded to the closer 'k' security personnel. These alerts will not only alarm security personnel but also suggest some preventive measures to be taken to avoid any miss happening. Along with the contextual information, history data can also be very useful for predicting intention of a person. Similarly, importance

of place and size of crowd also reflect useful information whether that place can be at risk or not. Uneven movements in one place cannot be similar to the other at some other place. So context-awareness will be playing vital role in these security applications.

#### IV. PROPOSED METHODOLOGY

The proposed architecture is novel as it integrates contextual information and performs ubiquitous data mining in online fashion. In this architecture, contextual information can be different depending upon the application area. In human security case, context may refer to historical data about criminal activities, personal profiles, current circumstances, place under observation, high profile official presence, etc. while in buying trend case context might be weather conditions, day of week, seasonal information, etc. These factors with ubiquitous data mining have not been used collectively to extract anomalous patterns from the streaming data as per our knowledge.

Fusing person's information like his/her bio data, area of residence, previous living records, etc. will be very useful to predict behavior of each individual with great accuracy. Current information in vicinity under observation is gathered using electronic devices like wireless sensors and cameras.

We also define usual patterns in each area so that unusual patterns can be distinguished easily. Here we apply Naïve Bayes technique to predict the occurrences of uneven events.

In real-time, we analyze our data on PDAs or handheld devices available from security personnel in order to assess the risk of criminal activities. Using past patterns of criminal activity, historic data, personal profiles, and current contextual information, the model detects odd events. For example, if a person had been involved in some criminal activity, has been identified as suspicious, or his or her current contextual information clusters him in a criminal category, the model alerts security personnel to take necessary countermeasures.

Initial training of the model can be performed using historical data about criminal activities. In case of unavailability of such data, synthetic data can be used to achieve this training objective. Once the training has been completed and system becomes live with the actual environment then the database will be automatically growing and subsequently knowledgebase will be building for future use. One of the important research goals is to find uneven events and crime patterns in this specific case therefore our proposed model will be mining that information. Knowledgebase built from these real world scenarios will be used for prediction of uneven events well before time and suggest necessary actions required to be taken.

We use probability theory to find out the chances of an unusual event so that the severity can be calculated. If severity level is some predefined threshold, then necessary alert will be generated on contextual bases and forwarded to the staff at duty for appropriate actions. Similarly, if calculated severity is below the threshold but there are some detected uneven patterns then those specific persons will be kept under keen observation to avoid any loss. The success and failure rates of the alerts generated, as well as the resulting predictions will be analyzed and the main repository database will be updated. This process of continuous learning improves system performance and future decisions become more accurate and reliable.

To save energy and communication cost, data sampling is performed. Only that data is recorded which is predicted as malicious and propagated towards main repository database for futuristic use. It will decrease network traffic and utilization of communication channel is expected to optimize. This mechanism increase overall lifetime as well as performance of network.

To convert this proposed model into practical shape, several issues need consideration and catering those factors is necessary to obtain expected results as well as accuracy. Few factors are:

- *Data:* multidimensional data from heterogeneous sensors arrive in the form on continuous streams at a high rate. To deal with such a substantial amount of data in real-time is a complex challenge. Moreover, classifying this continuous stream of data by incorporating a predictive model requires access to historic data about criminal events. Normally, data about a location where criminal action has occurred is available, as is information about the person who committed the crime. However, personal information about history of the criminal is often unavailable. Similarly, the movements and actions performed before committing a crime are not available as there is no existing system to record such information. To obtain such data, a simulator is ideal at initial stages; later, continuous learning processes enable models to become more realistic.
- *Analysis:* As lightweight algorithms have already been developed which perform well in resource constraint environment but there is a need to optimize those algorithms and cater those so that data obtained from integration of heterogeneous streams can be analyzed and process in effective manner.
- *Human Rights and Legal Issues:* Unfortunately, public organizations, constitutional rights, and basic rules of independence are core hindrances in the implementation of this model. To deploy

this model, constitutional shelter as well as acceptance by citizens is required, given that such a system will be perceived an invasion of their privacy due to modern ethics, which do not allow viewing an individual's personal details without sufficient and appealing proof.

## V. CONCLUSION AND FUTURE WORK

We have proposed an architecture based on information and communication technology to extract useful and uneven patterns from the streaming data. Our model uses state-of-the-art technological devices like smart phone, wireless sensors and imaging devices. This paper presented a novel approach to detect uneven patterns and suggestion for the actions to be taken to avoid from devastating results of those unusual patterns. It is expected that this proposed architecture will bring considerable improvement in finding uneven events in the streaming data environment. It will also enable researcher to perform ubiquitous data mining in streaming data to get results in real-time for better performance.

As a next step, we have developed a prototype of this system and it can detect anomalous patterns in the streaming data. It also generate set of suggested actions in case of unusual event has been detected. Once we are fully successful in prototype, development for real environment will be initiated on the bases of application areas.

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## Data Mining Methodology in Perspective of Manufacturing Databases

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**Abstract:** In recent years data mining has become a very popular technique for extracting information from the database in different areas due to its flexibility of working on any kind of databases and also due to the surprising results. This paper is an attempt to introduce application of data mining techniques in the manufacturing industry to which least importance has been given. A taste of implement-able areas in manufacturing enterprises is discussed with a proposed architecture, which can be applied to an individual enterprise as well as to an extended enterprise to get benefit of data mining technique and to share the discovered knowledge among enterprises. The paper proposes conceptual methods for better use of different data mining techniques in product manufacturing life cycle. These techniques include statistical techniques, neural networks, decision trees and genetic algorithms. An integrated and unified data mining platform is anticipated then to improve overall manufacturing process.

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**Keywords:** Data Mining, Manufacturing, Industrial application, Data Mining methodologies, Data Warehousing

### 1. Introduction

Computer integrated manufacturing systems as well as more simply controlled enterprises, generate huge amounts of data daily but even when companies appreciate the importance and value of this information, people seldom try to explore their databases thoroughly, because of other 'urgent' commitments towards their engineering and technical duties. The usual approaches for addressing and controlling problems that arise in manufacturing areas is through the application of engineering knowledge and experience. Yet these problems may also be tackled and solved by analysing the available operational information directly. This involves sifting through past data that exists in the enterprise's databases to see if any trends exist, or are emerging that may be causing specific defects or faults. Therefore, data mining techniques could be applied to improve exploitation of valuable information and knowledge sources, to better control the system and also to check the strategic gains and losses during the manufacturing.

In the section two we will describe, at a high level, areas in manufacturing enterprises where benefit can be gained from data mining technology. We shall also provide some details of design constraints and discuss how product life cycle data can be used to explore and discover knowledge by using data mining techniques. Section three covers data warehousing, as this plays an important part in

implementing data mining and saves time spent on the initial steps required for data pre-processing. The fourth section is about the real technology of data mining, a brief background and description of some of its popular tools are given, in the perspective of manufacturing enterprises. In the last section an integrated data mining model is proposed, keeping in mind the requirements of manufacturing enterprises and how a generic data mining system can fit within a manufacturing system.

### 2. Manufacturing System Design and Performance Improvement:

The importance of data oriented knowledge discovery techniques cannot be denied in any industry. Manufacturing enterprises in particular normally generate quantities of data at every step of the manufacturing processes from design through to the disposal of the product, and generally, most of this data is not fully exploited. Currently there is very little research being carried out in the manufacturing sector into the application of data-oriented knowledge discovery techniques.

Efforts have been made in the recent past to utilize the databases from manufacturing enterprises for design and quality control processes for example the factory data model [2][3] and data warehouses. The factory data model proposed in [2] promotes better exploitation of the information residing in the databases. It is however very difficult to design a data

supported manufacturing enterprise which can gain the benefits of their historical as well as their current databases.

In manufacturing systems involving small sized products in very large volumes, for example in the semiconductor industry, quality checking has always been a problem and detecting any process abnormality as early as possible becomes more crucial than ever. Similarly in the production of very large sized products with small volumes it is desirable to maintain the non-conformities level to a minimum. The aim of current manufacturing systems is to decrease the time from occurrence of a fault to its detection. The shorter the identification time the more controlled the manufacturing system is. It is therefore important to learn from both past problems and successes, and to use this existing knowledge to improve product designs.

#### ***Design and Re-usability of knowledge:***

Design can be defined as “effective allocation of resources”[4]. Alternatively, Pahl and Beitz [5] describe it as “a process of synthesis and integration”. The most comprehensive definition is given in [6] which states, “Systematic, intelligent generation and evaluation of specifications for artefacts whose form and function achieve stated objectives and satisfy specified constraints.” In their opinion, an engineering design does not directly result in a physical product compared with other design domains but rather, it provides a set of specifications to construct or fabricate the products.

Conceptual design, layout design, drafting and design analysis all create data, (which in turn may be used as the basic raw material for a data mining process). Product and process faults may occur at any stage of the product’s life cycle, hence, it is possible that certain faults can be traced back, using the data from the design and the design process. Earlier computer aided designs or old design drafts can be reused and be very helpful in the redesign of the product providing they can be analysed in a sensible way. A potential difficulty exists when attempting to re-use previous design knowledge that exists in the form of archival design documents, testing and analysis reports [7]. The difficulties lie in the forms in which the data has been archived, as some paper-based or hard forms are difficult and slow (and therefore expensive) to search and reuse. Therefore when designers or managers want to consult (and learn) from these designs or previous results the costs may be substantial in terms of time and overheads. Designers spend 20-30% of their time looking for information and same amount of time in handling information. Therefore any design system should incorporate all the designers’

own files, to make information easy to find and easy to use [7].

Data searching and retrieval become less of a problem when information is computer based. However substantially more efforts than are commonly used at present are needed to reuse such knowledge effectively. Data Mining could provide a solution to these problems by finding relationships between design problems and production problems or other aspects relating to the product life cycle. Manufacturing system design requires considerable amounts of information to be collected, and processed in order to improve the design and performance of the processes. Several information modelling techniques, and processing methodologies are already in use as described in detail within [2][3].

The popularity of computer aided design (CAD) and computer integrated manufacturing (CIM) have increased the amount of available digital data. This is easy to store and recover or search, helping the modern manufacturing enterprise to make better use of computing power in the analysis of its valuable data. Many researchers believe that computational approaches to design should enhance, not replace, human practice [8] [9]. With the help of knowledge discovery in databases, the causes and contributory factors to faults may be more clearly identified, enabling human designers to focus and concentrate their efforts on important problem areas and thus the time to design or redesign a product can be reduced.

It is generally important to consider how a product will be produced in parallel with the design of the product. Improvements to the manufacturing system can also result in improvements to the product. The manufacturing system, modelling and design, will therefore now be considered in the context of data mining.

#### ***Advanced Manufacturing Systems:***

Achieving absolute flexibility requires substantial quantities of information about the current processes and related activities, which directly or indirectly, link the whole manufacturing system. Information about the product life cycle also plays an important role in designing the flexible manufacturing process. The details of the product life cycle and employment of data mining on it are discussed at the end of this section.

Flexible manufacturing can be achieved by having tools, machining programmes and parts all quickly changeable and being able to respond rapidly by minimizing the part lead times. Machine utilization should be maximised together with an almost instantaneous response both to customers and to any problems that may occur. The philosophy



behind this arrangement simultaneously aims for minimisation of lead times for the parts to be processed along with the maximisation of the utilisation of the machines doing the processing [14]. But how can this be achieved successfully?

Since it is difficult to measure manufacturing flexibility, it is often hard to financially justify investments aimed at increasing the flexibility of a manufacturing system [15]. Manufacturing enterprises are struggling to make all the steps in their operation as flexible as possible, but often without proper knowledge and information sharing between the various stages of the manufacturing processes. The hurdles in making the flexible manufacturing systems can be analysed using data that has been recorded during individual processes within the whole system. Keeping in mind the problems data mining will give the solutions to the problems that are actually indirectly involve in the brittleness of the system. Once the information about the system's brittleness is discovered the system can be made more elastic by removing the obstructions in achieving the goal.

The first step towards applying data mining techniques for achieving the maximum flexibility in the manufacturing system is to make a system to archive the data recorded during different operational stages of the manufacturing organizations. Most advanced manufacturing enterprises record such data but it is often in the form of simple database files that are not well organized. A well designed database or model is required, otherwise substantial pre-processing of the data may be necessary before it can be mined and this will consume considerable resources. The first step towards achieving this goal is to develop a data warehouse, and this will be discussed further in section three.

If a data warehouse is utilized properly it can not only help in developing the new strategies but it can also be used for repairing the strategies of an enterprise in crisis. For ongoing fault detection, it will some times be necessary to have an on-line data mining system, which does not have to rely on the data warehouse. The on-line systems will work on fresh data direct from the manufacturing system to find out any developing trends towards bad quality or to help better scheduling of resources. In an advanced manufacturing system there are advantages to automating the data mining system to help directly in controlling the process.

#### ***Manufacturing Strategies and Data Models:***

An enterprise needs to redesign if its aims are not being achieved or if its aims or strategies change. Good strategy and a well structured enterprise result in profit whereas bad strategy or a

business that does not meet the competitors' challenges, damages the company in the marketplace. Therefore management decisions should be based on accurate and reliable information that is structured within a data warehouse and a factory data model [2]. A factory model focuses on operation and infrastructure in contrast to a data warehouse provides information about the behaviour of the existing enterprise. Both these source of information are vital for the design and redesign of an enterprise and for performance evaluation.

However, the existence of this useful information is only part of the solution. How it can be utilized effectively to produce the required results is equally important. Simple statistics normally work well to give a very good picture of the current overall manufacturing process but there may be much more hidden knowledge waiting to be discovered. Machine learning and artificial intelligence tools can be used to gain insight into the data and to discover hidden patterns and trends.

The above analysis regarding the ways of extracting information may not appear to provide adequate solutions as the results of the mining process cannot be predicted. Hence, we cannot be sure that the value of the knowledge that may be discovered in the data will be greater than the time and resources that need to be spent in the mining process. However implementations of the same technology in other areas of human sciences like banking, finance, marketing, insurance, telecommunication, health care etc. have given very good results [16] [17] and people are now benefiting from the knowledge they have gained in their respective fields – so why should manufacturing enterprise not also benefit?

#### ***Product Life Cycle and Data Mining:***

The product's life cycle is based on the design of the product and is important for a data collection point of view and for the analysis for the mining perspective. Information collected during a product's life cycle provides feedback on a product's performance that can be used to assess the quality of the design [18]. Nahmias [19] divided the product life cycle into the following four phases: start-up, rapid growth, maturation and decline. Hazelrigg [4] made a further in depth division and split the whole process into seven stages: engineering and design; test and evaluation; manufacturing; distribution and sales; operations, maintenance & repair and disposal. All these stages produce data, which should be more or less easily available for analysis to gain insight into the whole life cycle of the product. Knowledge gained through this analysis can be used for redesign or for the introduction of new products.

Once the production process is completed there are many reasons why it is difficult to collect information relating to the costs. This information is very important regarding the design or redesign of the products. Prasad [20] concluded from different studies that indirect costs could be as much as 4-5 times the amount of direct labour and material costs. In this situation if all the relevant data from the product life cycle is available then it is far easier to analyse the data and to target the areas for improvement in the design and the manufacturing process. This is the point where data mining comes into play. Figure1 shows a very high level architecture of collecting the data during the product life cycle and storing it in a data warehouse, which is actually coupled with a data-mining engine. The engine would use either one or a combination of the many available data mining techniques depending on the type of the data and the type of problem to be solved. A brief introduction and relevant details of a few of these techniques and their applicability can be found in the fourth section of this paper. A design improvement that takes into consideration all the

aspects of the product life cycle gives a much more controlled and efficient design compared with a design based on market demand and production capabilities. The advantages of mining data relating to the product life cycle are that it can improve the design or redesign of the product and also that it provides more information that is helpful for design of the production process, marketing strategy, environment effects, quality and reliability of the product. This information collected during a product's life cycle provides feedback on product's performance that can be used to assess the quality of the design [18].

Data for mining can be structured so that the overall data from all the major stages of the life cycle can be examined together. In addition, analysis should also be carried out on sections of the data relating to individual stages in the life cycle. This is necessary to detect any hidden relations and effects on subsequent life cycle stages so that corrective actions can be taken to improve the design of the product or the manufacturing process.

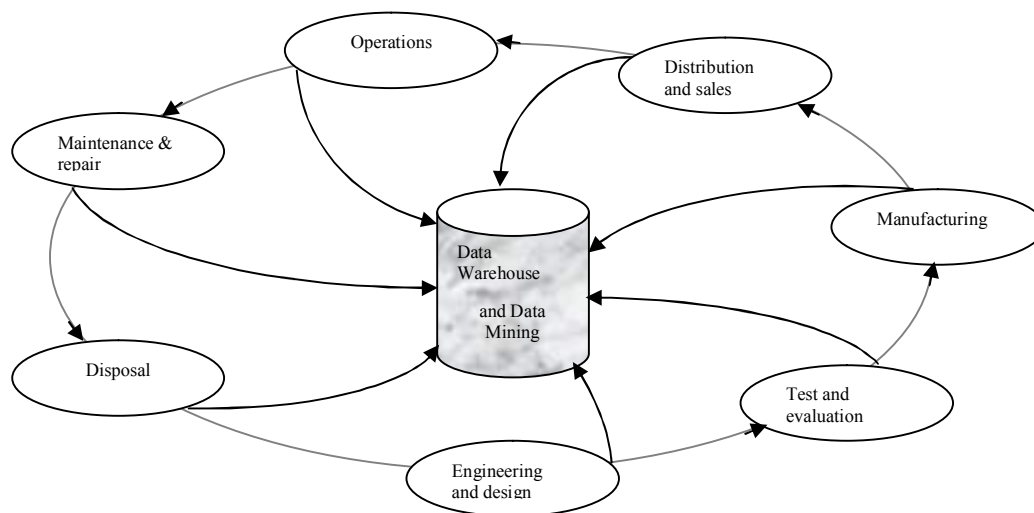


Figure1: Product Life Cycle's Data Collection Architecture.

### 3. Data Warehousing

Data warehousing has developed over the last 20 to 30 years, as they started emerging between 1984-88 [21]. The trends of today's enterprises towards data warehousing can be judged by a survey conducted by the META Group (Stanford, USA) that shows almost 95% of the corporations have plans to build data warehouses [22].

A database is a generalised and integrated collection of stored and operational data together

with their descriptions, and is managed in such a way that it can fulfil the differing needs of its different users [14]. A data warehouse can be defined as a single, complete, and consistent store of data obtained from a variety of sources and made available to end users in a way they can understand and use in a business context [21]. Mattison[23] explained data warehouse as a collection and organization of data to serve as a neutral data storage area that is non volatile, can readily be used for data

mining and/or other applications, and meets the specific business requirements.

The data warehouse must be structured to respond to queries related to different aspects of the process or business [29]. Users normally access operational databases using transactions that are also called On-Line Transactional Processes (OLTP), and often use executive information system (EIS). The disadvantage of OLTP is that the results of the queries of two users may be different if their queries are made at different times because the data is changing continuously and the second user may get updated results. However, with the data warehouse all the users will get the same results for similar queries, providing the queries are made during the intervals between updating the warehouse.

#### 4. Knowledge Discovery in Database

The term knowledge discovery in databases (KDD) was formalized in 1989 in reference to the general, 'high level' concept of seeking knowledge from data. This term (data mining) has been used by statisticians, data analysts and the management information systems (MIS) community. KDD has been mostly used by artificial intelligence and machine learning researchers.

Data mining is just one of several terms, used by the people in the data mining field, including knowledge extraction, data archaeology, information harvesting, and even data dredging, that actually describe the concept of knowledge discovery in databases.

Data Mining includes all methods and techniques, which allow practitioners to analyse very large data sets to extract and discover previously unknown structures and relations out of huge quantities of details. Information is filtered, prepared and classified so that it will be a valuable aid for decisions and strategies [30]. The most authentic definition of knowledge discovery in database or data mining is "non-trivial process of identifying valid, novel, potentially useful, and ultimately understandable patterns in data" [31]. With the explosive growth of data in databases the desire to extract useful information is also increasing. The manufacturing enterprises' databases contain treasures of information that tempt analysts to detect trends or patterns in them and react flexibly to them. However useful information may be hidden within the mountains of other data, and cannot be discovered using conventional database management systems. Data mining is becoming an increasingly important research area [32] [33] [34], since knowledge, e.g. extracted knowledge trends and patterns, can be used to help and improve business decision making.

#### *Data Mining:*

Data Mining normally works together with a data warehousing as this is necessary to organize historical information gathered from large-scale client/server bases applications as discussed in the previous section. Due to the explosive growth of data in the companies in the recent years and the non-availability of any proper technology to exploit this data in the past made data mining a very important research topic [31] [39] [40].

In order to understand how KDD can be implemented in manufacturing enterprises, it is important to understand the whole process, especially the discovery stage and its tools. For simplicity the whole knowledge discovery process is divided into six different stages: data cleaning, integration, selection, transformation, mining and evaluation/visualization [41] [42]. Fayyad [31] proposed five steps including, retrieval of data, selecting of data, sampling and cleaning, applying the appropriate transformations and fitting models to the proposed data. IBM [43] defined the four major operations for data mining as predictive modelling, database segmentation, link analysis and deviation detection. The above divisions show the actual discovery or mining process comes at the end and takes a very small fraction of the total time involved in the discovery process. Gonzalez and Kamrani [44] conclude that as much as 80% of KDD is about preparing data, and only the remaining 20% is about mining. Pre-processing is therefore very important and the details of how to implement the pre-processing steps can be found in lots of available data mining books in the market.

Some standard methodologies like SEMMA (Sample, Explore, Modify, Model, Assess) [45] and CRISP-DM (CRoss Industry Standard Process for Data Mining) [46] have also been developed for data mining process and to simplify its implementation in the industry.

Data mining in its traditional forms has been used to find patterns in the historical databases like banking, insurance, fraud detection, telecommunication data etc keep their old data for future strategy and planning. In the manufacturing process both the old and current trends of the process; policies & strategies and quality are important. The traditional way of data mining can help in finding the faulty processes and bad strategies in the manufacturing process and suggest remedies for them but finding the online or current trends is very important in any kind of manufacturing processes to control the whole process for better scheduling and quality. Data mining can also be used to solve this problem by embedding it in the process to find out

the run time errors of the process if they occur [47]. In this kind of mining process, all the pre-processing steps are eliminated by automating the whole process for collecting the data, analyzing it and making corrective actions.

### ***Data Mining Techniques for Manufacturing Enterprises:***

The analysis or mining of the manufacturing enterprise data can be done using all the popular data mining techniques. Some of the effective techniques for data mining like association rule, rule induction etc. are mostly used for retail market or basket analysis [48] [49] but are helpful in any kind of manufacturing databases too. The data mining techniques can be divided into three main categories, statistical techniques which uses simple to complex statistics to analyses the databases, the second category is artificial intelligence tools which become popular with the increase in the computing power over the last two decades and the third is machine learning tools which are actually a combination of statistics and artificial intelligence tools.

A very interesting survey has been done by [50] analyzing the efficiency and productivity of different data mining techniques with different kinds of problems. For example the survey shows that for data that has many attributes, like manufacturing data, and that is numeric in nature, the most suitable algorithms are decision trees, nearest neighbors and neural networks. But it really depends on what kind of problems are being examined in the manufacturing enterprises. The above techniques can be used to search for any kind of trends in the past data but in any specific problem the choice of the technique really also depends on other factors of the problem and algorithms.

A few of the most common data mining tools are listed here in the context of manufacturing databases. Only a brief introduction is given, as details can be traced back the references provided.

### ***Statistics:***

Statistics can be counted as a data mining tool since statistics is actually the origin of data mining. There are lots of statistical techniques including regression, discriminant analysis, classification, clustering and time series which are very popular in the data mining community and are extensively used for the large database analysis. With the passage of time these techniques are now mixed with the artificial intelligence tools to give even better, more reliable and faster results than the current simple statistical methods.

Statistics has always been a very popular tool in the manufacturing enterprises for process and

quality controls. In any kind of data mining process the preprocessing stage commonly uses different statistical techniques and the initial analysis of the data is also done using statistics and Structured Query Language (SQL). [42] [51] and [52] have stated that for the most part, about 80% of the interesting information can be abstracted from a database using SQL commands. However, as [30] stress, extraction of the remaining 20% of hidden information requires advanced techniques like expert systems, fuzzy expert systems, case-based learning, decision trees, neural networks, genetic algorithms etc. Hence statistics are a good starting point for the analysis of the data, to try to identify some trends for further detailed analysis.

### ***Decision Trees:***

Decision trees are normally used for classification purposes. These are tree shape structures resulted by the decision taken at each node. The database is divided into different fields that enable the analysts to look at the behavior of the database at different stages or to distinguish among different patterns present in the data. Different decision tree methods used as a data mining technique are Classification and regression Trees (CART) [53] and Chi Square Automatic Interaction Detection (CHAID) [54]. The first efficient decision tree model called ID3 [55] was based on the concept of entropy means the choice of the next feature used for branching should increase knowledge [56]. Decision trees are simple enough to understand and explain, and are easy to build, have relatively short training time and need very low memory [7].

### ***Neural Networks:***

Neural networks, is a very popular AI technique that mimics the working of neurons of human brain. Neural networks are not new as they trace back their history about 50 years ago when McCulloch and Pitts started working on them. [57], [58]. Neural networks are complex to interpret but very good in terms of accuracy. Carol [7] tabulated different data mining techniques such as neural networks, rule induction, decision trees, nearest neighbor etc and tabulated their important characteristics. It is a good idea to keep in mind the different characteristics of the data before choosing a specific data mining technique.

Artificial neural networks are simple computer programmes, which can automatically find non-linear relationships in data without any predefined model. According to [59], neural network-based database approach consists of three major-phases:

1 - Network construction and training: in this phase a layered neural network based on the number of

attributes, number of classes and chosen input coding method are trained and constructed.

- 2 – Network pruning: in this phase, redundant links and units are removed without increasing the classification error rate of the network.
- 3 – Rule extraction: rules are extracted in this phase.

### Genetic Algorithms:

This is one of the most recent methodologies used as a data-mining tool. Their basis is on the evolutionary computing which become very popular within the machine learning methodologies [60]. The basic concept of Genetic Algorithms comes from Darwin's theory of evolution. A genetic algorithm is reminiscent of sexual reproduction in which the genes of two parents combine to form those of their children and only the fittest will survive. The next generation improves and is better than the previous generation only if the strongest members of the population mate together to produce the next generation. The same principle can be applied to problem solving if the population consists of possible solutions to the problem. Each of these generated solutions have some characteristics that enable them to be categorized as a more or less fit as member of the next generation of offspring. The best members of a generation are given more chance for mating and producing the subsequent generation. In this way, each successive generation consists of better solutions, until an optimal solution is generated.

Genetic Algorithms can be very helpful in finding solutions that are very difficult to optimise. Another advantage of using genetic algorithms is that they can propose many possible solutions of a problem. The main advantage of using genetic algorithms (GAs) is, they can be synthesized without making use of the detailed, explicit knowledge of the underlying process. This means they will find a pattern if any exists even if the problem is new and no previous solutions are known. However, limited or noisy training data may result in inconsistent, meaningless output. This has been known to be a severe problem of genetic algorithms [61]. Another problem for genetic algorithms is they require lot of computing power to achieve a significant solution. In data mining problems specially to find out the relationships between the different entities genetic algorithms prove to be very effective. [62] shows a successful implementation of a search technique in big databases using genetic algorithms in solving a data mining problem.

The generic genetic algorithm consists of the following steps [56]

- 1- Each offspring (generation) is evaluated for fitness

- 2- The population is increased through mating and mutation of fit members to generate the new set of rules (generation)
- 3- Weak members from the generations are eliminated (reducing the size of the population)
- 4- A terminating condition is checked and if the optimal solution is not achieved then mating and mutation are done to produce the new generation.

There are many other popular data mining techniques including fuzzy logic, rule induction, association rule, k-nearest neighbor, intelligent agents etc, and this list continues to increase.

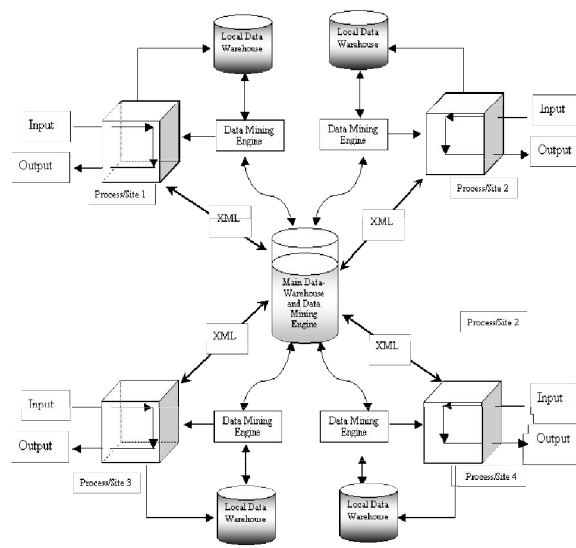


Figure 3. An Integrated Data Mining System Model

## 5. INTEGRATED DATA MINING

As explained in the first section of this paper a manufacturing enterprise can benefit from data mining in solving problems, but there is an important need for an integrated data mining system for diagnosing and solving online manufacturing problems. Here we introduce a relatively complex integrated data-mining architecture for a manufacturing enterprise. This architecture can be used to suggest solutions to particular problems, to learn from this, and subsequently to also help solve problems at other processes/sites with similar kind of parameters.

The KDD process is generally driven by individual user skills and experience and is not efficient to use in manufacturing applications where fast intervention may be required if things are going out of control. Since the production process in a factory is usually automated and manufacturing data is obtained continuously from the process, the data must be monitored without interruption so that any anomaly in the process may be detected and

eradicated immediately, therefore, the KDD process must run concurrently with the production process [47]. Enterprises aim for increasing levels of accuracy and improvement as manufacturing processes become more advanced and sophisticated and data is continuously recorded.

Manufacturing databases are dynamic with very regular updates made to the records. In the proposed integrated data mining model, data mining techniques can be applied at the micro level within sections of the manufacturing processes, whilst a Main Data Mining Engine may coordinate, share and exchange knowledge between the individual data mining engines. The main Data Mining Engine when connected to individual data mining engines establishes a data-mining network (see Fig. 3), which helps to mine the whole process. The whole manufacturing process may be divided into small steps and the data relating to each step and its adjacent steps are mined independently. The same principle can be applied to the extended manufacturing enterprises where the sites are not in one premises but are located at different places, cities, countries or even continents. The whole process is therefore supported and explored by the remainder of the network. Its activities and results are consistently communicated through the Main Data Mining Engine. Thus individual areas of data mining activity are kept to a manageable size whilst still supporting related areas of activity.

In an integrated manufacturing environment where the product is developed at separate locations or in discrete steps data mining can best be used to control any individual process or step through identification of hidden information in its associated data. The data-mining algorithm say rule induction can discover the relationships within the individual process, or dependent processes. It cannot however be utilized for the other discrete steps unless a common data warehouse collects the data. The concept of the proposed integrated data mining model works on the basis that rules; principles and concepts applicable to one manufacturing stage/site may also be utilized (tested and applied) for other similar stages, (by the exchange of activity and rules information via the Main Data Mining Engine). This integrated data mining model will work where a product goes into different stages and data for each and every step is collected and stored in a pre-designed data warehouse or in the pattern warehouse as knowledge is much more compact than data [63]. Data mining activities and the main data warehouse will work in parallel during the whole activity with the production process data and company data warehouses.

Each stage will have its own local data mining engine and data warehouse where the data will be

stored after cleaning. The data will also be transferred to the main data warehouse, which has a direct link with a pattern warehouse for the analysis or mining of the whole system's data.

If the same methodology is applied to an integrated system where production is being done at different sites then the central data warehouse will be built on a standard format. The data from the individual sites will be transferred to the main data warehouse using XML format where the data will be mined for the whole process and rules/knowledge extracted will be returned back in the same format.

The integrated data mining will be productive in the sense that if different rules are identified for two individual, but similar small manufacturing/production steps, the rules can be shared, and each data-mining engine can use its knowledge to refine the "best" one for its particular application. In this way, knowledge can be fed into the main data warehouse, so results can be reused in the future, as a pattern warehouse will be developed. Future applications can then make use of the stored patterns and rules instead of always having to return to the original manufacturing process databases.

The figure shows four different manufacturing processes or sites. The input and output of the manufacturing process is shown and the data from the manufacturing process is collected at the local data warehouse and is also transferred to the main central data warehouse in a neutral format say XML. The outcome of the data mining process (if any) is implemented to the manufacturing process and same information is also reported to the central system which analyze the kind of problem tackled by the local system and stores an index and the parameters for the problem occurred. If any of the other processes indicate similar kinds of problem then the central or Main Data Mining Engine first tries the same solution to that problem to see if it works. This methodology will help in future to minimize the time spent on understanding the problems and finding the solution. The data-mining engine can also suggest alternative solutions, but using an old solution and refining it to suit the present requirements should help to tackle the problem in more efficient, cost effective ways.

The integrated model should also be aided with the online visualization model so that the worker who is working on the machines/products can get a clear idea about the process and products. Visualisation should allow a user to discuss and explain the logic behind the model with colleagues, internal/external customers, and other users [64]. For example if there is a recurring problem with a particular step or process on a product then visual checks for previous trends/rules or current results at

other sites with the same or similar steps will help in getting a workable idea to fix the problem. Therefore visualisation of the whole mining process will help and enable the output of the data mining system to be understood qualitatively.

### Conclusion:

It has been showed that along with other areas, manufacturing enterprises can be benefited with the data mining techniques. There are lots of areas within manufacturing enterprises, few of them are explained in this paper, where data mining can find its ways of implementation and can give results comparable to any other corrective measures based on the engineering methodologies. The data mining architecture proposed in this paper can be refined from a small factory to an extended enterprise. Such kind of data mining approach will be an essential part during the designing of an advanced manufacturing process in future which can learn from its own mistakes and will do the corrective actions not only for its own processes but will help the other processes with its experiences.

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## Evaluating the Factors Responsible for Slow Rate of Technology Diffusion in Livestock Sector of South Asia and Developing a Framework to Accelerate this Process: A Case Study using data analysis for Pakistan's Livestock Sector

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**Abstract**— South Asia boasts a large number of livestock populations and is considered as a very important region in context of global milk and meat production. The development of Livestock sector in countries such as India and Pakistan can be considered as a vital source for alleviating poverty and for developing the economy of these countries. However, large number of animal population in this region suffers from low productivity when compared with developed countries. The main reason behind low productivity of this sector is the lack of technology acceptance among farmers. The dissemination of information regarding new innovations and technologies in livestock sector is carried out through agriculture extension services. Technology or innovation diffusion is a complicated process. Many times seemingly beneficial technologies and innovations fail to get large scale acceptance or are accepted at a very slow rate. The process of technology diffusion consists of three basic elements i.e. technology or innovation, communication channel and social system. To accelerate this process it is essential to understand the attributes of both technology and social system and then to built an effective communication channel. This study attempts to identify important factors and attributes that play a vital role in farmer's decision to adopt or reject a new technology. Through this study an attempt has also been made to determine the effectiveness and reliability of current extension services (communication channel) in livestock sector of Pakistan. Data analysis carried out in different livestock farms shows that both public and private sector have failed to increase awareness about new technologies and innovations among farmers. The analysis also reveals the important role played by technology diffusion attributes such as perceived usefulness, cost, personal innovativeness and social pressure in adoption or rejection of a technology. On the basis of these findings a model has been established to accelerate the process of technology diffusion in livestock sector of South Asian countries.

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**Keywords**- Technology Diffusion; Technology Acceptance Model; Livestock of South Asia, Livesock Sector of Pakistan

### I. INTRODUCTION

Agriculture forms the backbone of South Asian economy. The importance of agriculture sector for countries such as Pakistan and India cannot be over emphasized. According to Pakistan's economic survey 2009-10, agriculture sector in Pakistan accounts for about 21% of GDP and employs around 45% of the labor force. Agriculture sector can be divided into two major sub sectors i.e. crop sub-sector and live stock sub-sector. Livestock sector contributed about 53.2% of the agriculture value added in 2009-10. When compared with developed countries it becomes apparent that Pakistan's livestock sector suffers from low productivity. It is estimated that average productivity of dairy animal in Pakistan is about one fifth of Germany's average productivity and one third of New

Zeeland's productivity [1]. Similar observations can be made about the livestock sector of other countries in this region such as India and Bangladesh. One of the main factors that contribute to this low productivity is the slow rate at which farmers adopt any new technology or innovation.

Technological improvement and innovations are a result of research and development (R&D) efforts. However, the outcome of these efforts can only be appreciated once the new technologies make their way to regular farmers. This process is known as 'technology diffusion'. No new technology can have a significant impact on a country's economy until it becomes widespread [2]. Sometimes seemingly beneficial technologies take an amazingly long time to get adopted. Many researchers have tried to explain this

phenomenon and the literature available on technology diffusion sprawls over many disciplinary boundaries [3].

For the growth of livestock sector in Pakistan it is imperative that the process of technology diffusion among farmers is accelerated. Diffusion of innovation or technology can be a complicated process. The rate of diffusion varies depending upon the nature of technology and characteristics of targeted social system. For livestock sector, it is the responsibility of agriculture extension workers to ensure that farmers are aware of new technologies that can help improve productivity in this sector.

A farmer's decision to adopt or reject a technology is affected by several factors. The main objective of this research is to establish a better understanding of these factors. For this purpose two livestock technologies were chosen and data analysis was carried out in livestock farms of Pakistan. In this research an attempt is made to understand the key factors behind the farmer's decision to either accept or reject these technologies and to measure the performance of extension services with regards to introducing these technologies to the farmers. Afterwards, a comprehensive model for accelerating the technology diffusion process in livestock sector of South Asian countries is developed which can be helpful for agriculture extension services to operate more effectively

## II. LITERATURE REVIEW

### A. Technology/Innovation Diffusion

Diffusion of innovation can be defined as "A process by which an innovation is communicated through certain channels over time among the members of a social system." Diffusion can be considered as a special type of communication in which messages are concerned with new ideas [4].

Evert Roberts in his book "diffusion of innovation" defined the four elements central to this process:

- **Innovation:** An innovation is an idea, practice or object that is perceived as new by an individual or other unit of adoption.
- **Communication Channel:** Any new idea or practice reaches its potential adopters through certain communication channels.
- **Time:** An innovation's *rate of adoption*, the relative speed with which an innovation is adopted by members of a system.
- **Social System:** Social system is defined as set of interrelated units that are engaged in joint problem solving to accomplish a common goal. Diffusion occurs within the social system

### B. Technology Acceptance Model

Many researchers have come up different models for explaining the behavior of consumers towards a new technology or innovation. Technology Acceptance

Model (TAM) was proposed by Fred D. Davis in 1986. TAM initially aimed to explain computer adoption among individuals. After its introduction many researchers tried to replicate TAM with other technologies and research settings to verify its consistency. The researchers concluded that TAM maintains its consistency and validity in explaining user's acceptance of different technologies and innovations [5].

Some of the variables associated with TAM are as follows:

**Perceived Usefulness (PU):** Perceived Usefulness is defined as the degree to which an individual believes that using a particular technology will enhance his or her performance [6].

**Perceived Ease of Use (PEOU):** Perceived Ease of Use is defined as the degree to which an individual believes that the use of a particular technology will be free of physical and mental effort [7].

**Personal Innovativeness (PI):** Personal innovativeness can be considered as an individual trait reflecting a willingness to try out any new technology [8].

**Subjective Norm/Social Influence (SN/SI):** It is a measure of a person's perception that most people who are important to him think he should or should not perform a particular task [9].

**Accessibility (Acc):** Physical Accessibility is the measure of the extent to which someone has the physical access to the technology [10]. Information accessibility refers to the accessibility to the information regarding the usage of a technology.

## III. OVERVIEW OF LIVESTOCK SECTOR OF PAKISTAN

Pakistan has almost 8 million farming households with a total herd size of approximately 50 million animals. The livestock sector of Pakistan has shown consistent growth in production over the years but this is mainly due to the increase in number of animals as opposed to higher productivity per animal. "Table 1" shows the livestock population of Pakistan has increased consistently over the years.

TABLE 1. LIVESTOCK POPULATION IN PAKISTAN (THOUSAND NUMBERS)

	1960	1986	1996	2006
<b>Cattle</b>	16624	17541	20424	29559
<b>Buffaloes</b>	8161	15705	20272	27335
<b>Sheep</b>	12378	22655	23544	26488
<b>Goats</b>	10046	28647	41166	53787
<b>Total</b>	47209	84548	105406	137169

Source: Agriculture statistics of Pakistan 2008-2009  
*Average Milk Productivity Across the World*

A. When compared with developed countries the productivity of livestock sector of Pakistan is extremely low. "Table 2" shows a comparison between developed countries and Pakistan in terms of average milk production per animal as recorded in year 2005. One of the main reasons behind this difference in productivity is faster technology diffusion at farm level in developed countries as compared to Pakistan [1].

TABLE 2. AVERAGE MILK PRODUCTIVITY PER ANIMAL ACROSS THE WORLD

Countries	Milk Production per Animal per year (kg)
USA	8395
Germany	7117
UK	6886
New Zealand	3947
Australia	4926
India*	1450
Pakistan*	1900

Source: FAO (Food and Agriculture Organization)

\*Milk Production for Buffalo only

#### B. Agriculture Extension Services in Pakistan

The most important goal of extension services is to provide a reliable channel through which technology can be transferred from R&D institutes to farmers [11]. Agriculture extension in Pakistan was a sole public funded service for almost 40 years, during the period since its independence in 1947 till 1988. During this period successive governments experimented with several different models and styles of extension with the view to increase its efficiency but limited success was achieved [12]. In 1988 private sector was allowed to participate in extension activities. Currently in livestock sector of Pakistan extension services are being provided by both public and private sector through veterinary officials, doctors and marketing personnel etc.

#### C. Social Influence on Farmers

Along with traditional extension service activities, social influences (opinions of peers and colleagues) also have a great impact on the process of technology diffusion. In Pakistan, studies reveal that farmers' attitude towards adoption of new technologies are interdependent. In other words a farmer is easily influenced by the actions and opinions of his colleagues and peers. Farmers appear to be more strongly influenced by their social peers rather than physical neighbors. Social peers are defined as farmers within the same village who share similar economic and social standing [13].

### IV. DATA COLLECTION AND ANALYSIS

As mentioned before there are three important factors that affect the process of technology diffusion. These include technology/innovation, social system and communication channel. In the context of technology diffusion in livestock sector, these factors can be

replaced by more appropriate terms i.e. livestock technologies/innovations, livestock farmers and extension services.

To evaluate the factors that affect the rate of technology diffusion in livestock sector of Pakistan, two questionnaires were developed, for two different livestock technologies that were chosen in consultation with NARC experts. These technologies are:

- Balanced dairy concentrate feed
- Urea as protein source

Through these questionnaires livestock farmers' feedback was collected about the two livestock technologies and the role of extension services in introducing these technologies.

#### A. Questionnaire Design

The questionnaire used to collect data on two livestock technologies, social system and extension services consists of following sections:

- The general information about the farm
- Familiarity with the technology
- Actual usage of the technology
- Measure of extension service variables such as public sector extension services, private sector extension services and other channels such as TV, Radio and internet etc.
- Measure of technology variables such as Perceived usefulness, Perceived Ease of Use, Cost, Accessibility.
- Measure of Social System Variables such as Social Influence and Personal innovativeness.

The data was collected by carrying out the survey across different livestock farms located in Islamabad, Rawalpindi, Chakwal, Gujjar Khan and D.G Khan. The survey consists of direct visits and telephonic interviews. The sample size was 150 farms that were divided into small (1-6 animals) and large (>6 animals) categories. For analyzing participant's responses five-point Likert scale ranging from 'strongly agree' or 'excellent' to 'strongly disagree' or 'poor'.

#### A. Data Analysis: Balanced Dairy Concentrate Feed

The first technology chosen for the survey was 'balanced dairy concentrate feed'. The entire sample population showed a basic level of familiarity with the technology. "Table 3" shows the actual level of adoption for this technology. The data shows that most of the farmers have continuously rejected this technology. Large farms show a slightly better adoption rate than small farms.

TABLE 3. ACTUAL ADOPTION OF BALANCED DAIRY CONCENTRATE FEED

Level of Adoption	Small Farms	Large Farms
Continued Adoption	29%	37%
Continued Rejection	61%	58%
Discontinued Adoption	10%	5%

To better understand the decision of farmers to adopt or reject ‘balanced dairy concentrate feed’, the participants of the survey were asked to rate different variables associated with the technology. These variables include Perceived Usefulness, Perceived Ease of Use, Accessibility and Cost (in comparison to traditional feed). The result for small scale farmers is shown in “Fig. 1” and for large scale farmers in “Fig. 2”.

The data indicates extremely low level of Perceived Usefulness of technology among farmers. Slightly better scores were recorded for Perceived Ease of Use and Accessibility of the technology. Most of the small scale farmers found the cost of ‘balanced dairy concentrate feed’ on the higher side when compared with the cost of traditional feed. Whereas, for most of the large scale farmers this cost was lower or normal in comparison with the traditional feed they utilize. This difference exists because traditional feed used in livestock farms of Pakistan vary from farm to farm based on the resources of farmer.

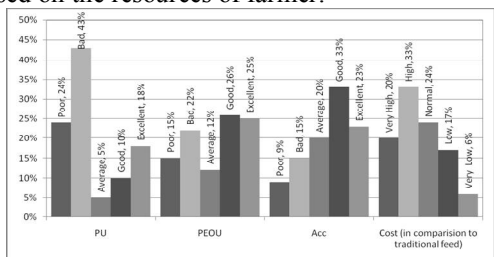


Figure 1. Scores for Technology Variables as Recorded by Small Scale Farmers

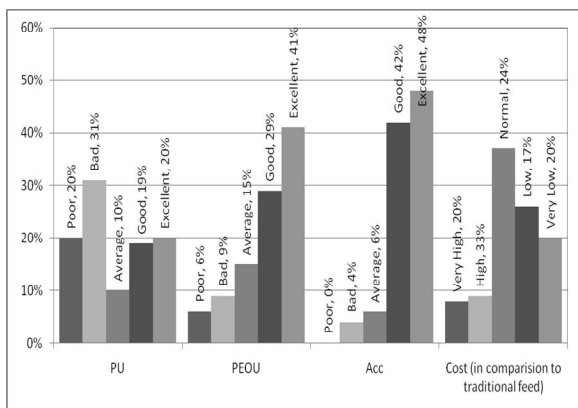


Figure 2. Scores for Technology Variables as Recorded by Large Scale Farmers

In addition to these variables related to technology, another important variable associated with social system is Personal Innovativeness. The Personal Innovativeness of the participants was measured as their willingness to try out new technologies and innovations. The result is shown in “Fig. 3”.

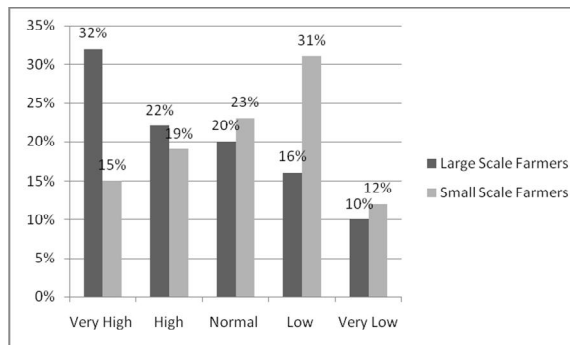


Figure 3. Personal Innovativeness of Small and Large Scale Farmers

Correlation matrix was generated to highlight the relationship among actual usage of technology and other variables used in the questionnaire. The correlation matrix for small and large farms is given in “Table 4” and “Table 5” respectively.

TABLE 4. CORRELATION MATRIX: USE OF BALANCED DAIRY CONCENTRATE FEED IN SMALL FARMS

	Usage	PU	PEOU	Acc	PI	SI/SN	Cost
Usage	1						
PU	<b>0.91</b>	1					
PEOU	0.57	0.63	1				
Acc	0.3	0.33	0.1	1			
PI	0.64	0.52	0.061	0.26	1		
SI/SN	<b>0.82</b>	<b>0.86</b>	0.49	0.25	0.56	1	
Cost	<b>0.81</b>	0.79	0.51	0.3	0.55	0.74	1

The correlation matrix shows that the variables Perceived Usefulness, Social Influence/ Subjective Norms and cost highly affect the actual usage of this technology for small farms. In other words these three factors play a decisive role in small scale farmer’s decision to adopt or reject this technology.

TABLE 5. CORRELATION MATRIX: USE BALANCED DAIRY CONCENTRATE FEED IN LARGE FARMS

	Usage	PU	PEOU	Acc	PI	SN	Cost
Usage	1						
PU	<b>0.92</b>	1					
PEOU	0.59	0.52	1				
Acc	0.22	0.16	0.09	1			
PI	0.47	0.36	0.05	0.11	1		
SI/SN	0.69	0.68	0.22	0.24	0.12	1	
Cost	<b>0.76</b>	0.71	0.57	0.19	0.26	0.52	1

“Table 5” shows that factors of cost and social influence/subjective norms play a relatively small role in large scale farmer’s decision to adopt or reject the technology.

#### B. Data Analysis: Urea as Protein Source

Only 32 out of 150 farmers showed familiarity with the second technology ‘urea as protein source’. All of these farmers belonged in the category of ‘large farms’. “Table 6” shows the actual level of adoption for this technology among these 32 farmers.

TABLE 6. ACTUAL ADOPTION OF UREA AS PROTEIN SOURCE

Level of Adoption	Large Farms
Continued Adoption	76%
Continued Rejection	24%
Discontinued Adoption	0%

The participants of the survey familiar with this technology were asked to rate different variables associated with ‘urea as protein source’. The correlation among actual usage of technology ‘urea as protein source’ and other variables used in the questionnaire is given in “Table 7”.

TABLE 7. CORRELATION MATRIX: FOR UREA AS PROTEIN SOURCE

表格 1

	Usage	PU	PEOU	Acc	SI/SN	Cost
Usage	1					
PU	0.94	1				
PEOU	0.78	0.61	1			
Acc	0.71	0.12	0.69	1		
PI	0.76	0.79	0.64	0.15		
SI/SN	0.45	0.51	0.06	0.2	1	
Cost	0.24	0.11	0.09	0.13	0.39	1

‘Urea as protein source’ can be considered as a relatively new technology when compared with ‘balanced dairy concentrate feed’. The correlation matrix shows that unlike the first technology, social influence and cost do not play an important role in farmer’s decision to adopt or reject this technology. Instead perceived ease of use and personal innovativeness along with perceived usefulness possess a high relationship with actual usage of the technology.

#### C. Data Analysis : Extension Services

This section is used to analyze the role of extension services in technology diffusion in livestock sector of Pakistan. The participants in the survey were asked about their primary source of information for the two livestock technologies mentioned above. The results are summarized in “Table 8”.

TABLE 8. PRIMARY SOURCE OF INFORMATION FOR THE TWO LIVESTOCK TECHNOLOGIES

	Balanced Dairy Concentrate Feed (Small Farms)	Balanced Dairy Concentrate Feed (Large Farms)	Urea as Protein Source (Large Farms)
Private Sector	21%	41%	5%
Public Sector	11%	20%	29%
Peers and Colleagues	60%	29%	35%
Others (TV, Radio and Internet etc)	8%	10%	31%

The participants were asked if they received information regarding new technologies from either public or private sector. The result is shown in “Table 9”.

TABLE 9. PRIMARY SOURCE OF INFORMATION REGARDING NEW TECHNOLOGIES FOR LIVESTOCK FARMERS

	Private Sector	Public Sector
Small Scale Farmers	30%	11%
Large Scale Farmers	45%	18%

In addition the participants of the survey were asked about their trusted sources of information regarding new livestock technologies. The results are shown in “Table 10”.

TABLE 10. TRUSTED SOURCE OF INFORMATION FOR LIVESTOCK FARMERS

Sources of Information	Level of Trust among Farmers
Private Firms	15%
Public Sector Veterinary Officials	70%
Peers and Colleagues	65%
Observation	90%
Others (TV, Radio and Internet etc)	18%

#### D. Result and Discussion

Some of the important points that appeared after data analysis are listed below.

##### 1) Important Factors and Variables of Technology

- The correlation matrix shows that for ‘balanced dairy concentrate feed’ the farmer’s decision to adopt or reject the technology is influenced mostly by factors of perceived usefulness, social influence/subjective norms and cost.
- For ‘urea as protein source’ (relatively newer technology) the factors of perceived usefulness, perceived ease of use and personal innovativeness are more important.

##### 2) Important Factors of social System

- The data shows that farmers tend to be influenced by the opinions of their peers and colleagues.
- For large scale farmers the factor of social influence/ subjective norms plays a smaller role as compared to small scale farmers.

- Farmers also showed lack of personal innovativeness meaning they were unwilling to adopt new innovations and technologies on their own.

### 3) Role of Private Sector in Technology Diffusion

- Aside from peers and colleagues, private sector is the most important component of extension services as far as familiarizing the farmers with new technologies is concerned.
- Low familiarity with technology 'urea as protein source' is a result of lack of involvement of private sector. As most of the urea manufacturers do not see livestock sector as an important market for their product.

- But at the same time farmers showed a lack of trust on the private sector as compared to their trust on public sector veterinary officials and doctors.

### 4) Role of Public Sector in technology Diffusion

- Farmers showed a willingness to adopt new technologies if they were advised by public sector veterinary officials and doctors.
- But at the same time data analysis shows that public sector extension services have failed to reach most of the farmers.

## V. MODEL FOR ACCELERATING TECHNOLOGY DIFFUSION PROCESS

In this section a model has been proposed that can be used to accelerate the process of technology diffusion in livestock sector of South Asian countries. This model is based on data analysis carried out in livestock farms of Pakistan but in the opinion of these authors it is also applicable in other developing countries with similar conditions to Pakistan such as India and Bangladesh.

The process of technology diffusion consists of three fundamental elements i.e. social system, innovation or technology and communication channel. For accelerating the process of diffusion in any sector all of these three elements need to be examined and managed simultaneously. The case of livestock sector is not any different. "Fig. 1" shows the basic framework for accelerating the process of technology diffusion in livestock sector.

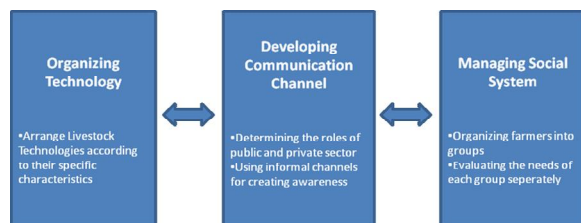


Figure 1. Basic Model for technology Diffusion in Livestock Sector

### A. Organizing Livestock Technologies

Technology or innovation can be a result of either local R&D efforts or it can be imported from foreign sources. In either case it is essential to categorize the technologies according to their key features. Some livestock technologies might be too complicated or expensive for an ordinary farmer. For effective transfer of technology to the farmers, livestock technologies can be arranged according to their benefits, cost and complexity as shown in "Fig 2".

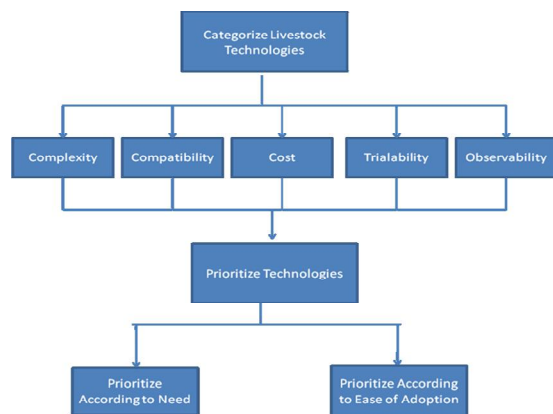


Figure 2. Organizing Livestock Technologies

### B. Managing Social System

Social system is formed by the targeted users of any technology. Each social system has its own characteristics which should be understood for effective technology diffusion. The social system that is the target for livestock technologies consists mainly of farmers with limited resources that possess low level of personal innovativeness and are highly influenced by subjective norms. "Fig. 3" shows the steps that can be taken to better prepare the social system for technology adoption.

- **Identify Clusters:** The first step in managing the social system is to identify clusters where large numbers of farming households are located. These clusters of farmers should be the high priority target for technology diffusion activities. Through this approach the official extension workers or private marketing staff can reach more farmers by utilizing relatively less resources.

- **Form Groups of Farmers:** Through the survey conducted in this research paper, it can be concluded that most of the small holding farmers are strongly influenced by opinions of their peers and colleagues. This means that most of the farmers refrain from adopting any new innovation on their own initiative and wait until their colleagues also show some sort of interest in that innovation. To overcome this

obstacle, groups can be formed consisting of farmers in same vicinity that share similar farming practices.

- **Identify HR Needs:** After forming groups of farmers, the number of extension workers needed for effective communication can be identified.
- **Assess Technology Needs for each Group:** Technology needs of livestock farmers in Pakistan vary vastly throughout the country. These variations exist because of difference in land structure and climate, available resources and level of awareness. But it is unfeasible for extension services to focus on each farmer separately according to his unique circumstances. Instead the extension workers can consider a group of farmers with similar needs as a distinct unit for technology diffusion activities.

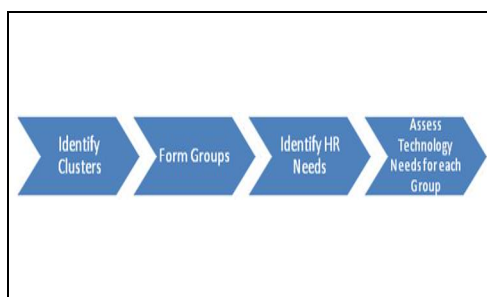


Figure 3. Steps for Managing Social System

### C. Developing Communication Channel

To ensure effective transfer of technology to farmers, a proper communication channel needs to be developed. This channel should ensure continuous communication between livestock development agencies and farmers. "Fig. 4" shows an effective communication channel developed by the authors that can be utilized for transferring livestock technologies to farmers. Public sector extension services consisting of livestock doctors and assistants should form the backbone of this channel as farmers show more trust on public sector as compared to private sector.

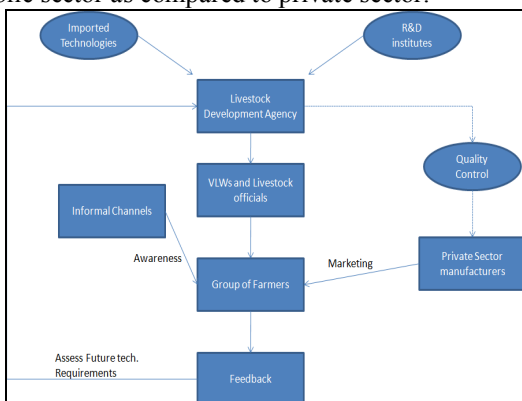


Figure 4. Elements of Communication Channel for Technology Diffusion in Livestock Sector

## VI. RECOMMENDATIONS AND CONCLUSION

### A. Recommendations

The enormous livestock population in the region of South Asia suffers from low productivity. Technology diffusion at a faster rate is the best solution available for ramifying this situation. The responsibility for increasing the productivity of livestock sector mainly lies with the many Govt. institutions and departments that are operating at federal or provincial level in these countries. The authors recommend the following steps that can accelerate the process of technology diffusion in livestock sector.

#### 1) Redefine the Roles of Public and Private Sectors

- Farmers show lack of trust on private firms.
- It is difficult for private sector to convince farmers into adopting any new technology or innovation.
- Public sector extension workers (veterinary officials) usually possess farmer's trust.
- Veterinary doctors that have an ongoing contact with farmers should encourage them to adopt a new technology.

#### 2) Quality Control

- Almost 10% of sample population discontinued the use of 'balanced dairy concentrate feed' because of poor results.
- The main reason behind it is the lack of quality control on livestock products.
- The provincial livestock and agriculture departments should ensure strong quality control on all livestock technologies.

#### 3) Carry out Trials for Farmer's Observation

- Carryout trials of new technologies by selecting small number of animals from each group.

#### 4) Understanding the Different Aspects of Technology

- For new innovations and technologies the variables such as 'perceived ease of use' and 'accessibility of information' become very important.
- It is imperative that all the personnel participating in extension activities completely understand the use of technology.

#### 5) Utilize Mass Media

- Use of radio and TV programs to create awareness among farmers.
- Can be extremely useful in the case of new technologies such as 'urea as protein source'.

**B. Conclusion**

The true potential of livestock sector of South Asia is yet to be realized. Most of the farmers in countries like Pakistan and India are still utilizing traditional farming approach and are continuously rejecting new technologies and innovations.

Currently both private and public sectors are involved in extension activities in Pakistan. This research highlights the shortcomings and inefficiency of extension services in Pakistan. The extension activities of private firms are the main source of information about new technologies for the farmers. On the other hand, public sector veterinary officials and doctors have more influence on farmer's decision. The rate of technology diffusion among farmers can be increased if information regarding new technologies reaches the farmers through proper channel.

There are many important factors that influence a farmer's decision to adopt or reject any new technology such as perceived usefulness of a technology, perceived ease of use, cost, personal innovativeness of the farmers and subjective norms. It is imperative for both public and private sector to understand the importance of these factors while introducing any new livestock technology to farmers.

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2/19/2012



## An Analysis of Relationship between Total Quality Management and Kaizen

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**Abstract:** Total Quality Management (TQM) and Kaizen- a continuous change toward betterment are two fundamental concepts directly dealing with continuous improvement of quality of processes and performance of an organization to achieve positive transformation in mindset and action of employees and management. For clear understanding and to get maximum benefit from both of these concepts, as such it becomes mandatory to precisely differentiate between TQM and Kaizen. TQM features primarily focus on customer's satisfaction through improvement of quality. It is both a top down and bottom up approach whereas kaizen is processes focused and a bottom up approach of small incremental changes. Implementation of TQM is more costly as compared to Kaizen. Through kaizen, improvements are made using organization's available resources. For the effective implementation of kaizen, the culture of the organization must be supportive and the result of continuous improvement should be communicated to the whole organization for motivation of all employees and for the success of continuous improvement program in the organization. This paper focuses on analyzing the minute differences between TQM and Kaizen. It also discusses the different tools and techniques under the umbrella of kaizen and TQM Philosophy. This paper will elucidate the differences in both these concepts as far as their inherent characteristics and practical implementations are concerned. In spite of differences in methodology, focus and scale of operation in both the concept, it can be simply concluded that Kaizen is one of the Technique of the TQM for continuous improvement of quality, process and performance of the organization.

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**Key Words:** Total Quality Management, Kaizen Technique, Continuous Improvement (CI), Tools & Techniques

### 1. Introduction

Continuous Improvement (CI) is one of the important elements of competitive advantage in production system and for the existence of organization (Dean and Robinson, 1991). Continuous improvement is an on going process of improvement which involves all the stakeholders at all level in the organization (Malik and YeZhuang, 2006). TQM and Kaizen are two fundamental concepts directly dealing with continuous improvement of quality, standard way of accomplishment of a job and performance in an organization to achieve positive transformation in thoughts and action of employees. Owing to similarity in practical application, confusion exists while comprehending the core issues related to both the concepts. Therefore, before embarking upon the discussion of continual quality improvement, it is necessary to be precisely clear about both the concepts and to discern the differences between them.

Kaizen is a Japanese word that indicates small continual improvement as routine functioning of the organization (Chen *et al.*, 2000). Kaizen is a combination of two Japanese words, Kai mean change and Zen mean toward betterment (Palmer, 2001). Kaizen is also known as *Gemba* Kaizen mean 'Continuous Improvement' (CI) at actual work place.

The ultimate goal of Kaizen is to make a good learning organization through small incremental changes toward betterment known as kaizen events. These events are performed through cross functional teams. To involve the people in kaizen means, go beyond their contracted role to continually identify and develop better way of doing a routine job and enhance the organizational performance (Brunet & New 2003).

Kaizen was initially introduced in Toyota Motor Company by Imai in 1986, to improve quality, productivity, and competitiveness of its product due to increasing competition in the world. With the implementation of Kaizen, the manufacturing sector of Japan has earned a lot and become a world class. Since then Kaizen has become a part and parcel of Japanese's manufacturing system (Ashmore, 2001). On the other hand, Total Quality Management (TQM) refers to the management's approach which focuses on the quality and involve all the employees at all levels of an organization. ISO: 8402 defines TQM as "*Management approach of an organization, centred on quality, based on the participation of all its members and aiming at long-term success through customer satisfaction, and benefits to all members of the organization and to society*".

The focus of this paper will be on analysing of the main differences between the two concepts. The main objectives of this research are;

- To analyse the relationship between TQM and Kaizen and hence establish the difference between two concepts
- To identify the differences in tools and techniques of both the concepts.

The research merits attention as that, the concept of TQM and kaizen would be easier to comprehend. The reader would be able to identify the differences between the two, besides being guided with the tools and techniques identified by different researcher in this field.

## 2. Literature Review

### 2.1. The Kaizen

Masaki Imai (1997), in his book *Gemba Kaizen* defines Kaizen as; “KAIZEN means continuous improvement. The word implies improvement that involves everyone—both managers and workers—and entails relatively little expense. The kaizen philosophy assumes that our way of life—is it our working life, our social life, or our home life—should be the focus of constant improvement efforts”. This concept is so natural and obvious to many Japanese that they often do not even realize that they possess it (Imai, 1997).

Thus kaizen is a continuous improvement and struggle for betterment in our daily working and personal life. Imai gives principles of Kaizen in comparison with other continuous improvement practices and techniques in an organization. Anthony C. Laraia (1999), in his book *Kaizen Blitz* defines Kaizen as; “The Japanese technique of continuous improvement involving everyone, managers and workers. In manufacturing, kaizen relates to finding and eliminating waste in machinery, labour or production methods”. Everyone involved in manufacturing process is responsible for the identification of wastes in the process, and workplaces. The elimination of these waste improve the quality and performance of the organization. Suzaki (1987) considers Kaizen (continuous improvement) as a philosophy which is widely practiced in manufacturing processes and quality circles. Kaizen is based on concept that there is always room for improvement of the process. Process can be made better through small incremental changes towards betterment also called Kaizen events. This small improvement project consists of many development phases. Kaizen was originally used for improving manufacturing processes. Now this concept is widely used and practised in every sphere of life in Japan and other industrially developed countries of the world. Teian (1992) considers Kaizen more than just a means of improvement; the reason is that it represents the daily effort of the workers at the workplace to improve their

processes and the way this effort is being made. The concept of Kaizen can be implemented at every place in the organization which requires improvement. Either it may be an individual department workplaces manufacturing process or whole organization. Bassant and Caffyn (1994), define Kaizen as ‘It is a process of focused and sustained innovation through out the organization that is in the form of small incremental projects known as kaizen events’. It means systematic way of small incremental changes toward betterment in each place and each department. These small changes are brought through workers working on their own jobs or by a cross functional team of workers given a special task of improvement. Similar concept was given by Cheser (1998), who believed that Kaizen is based on small incremental changes in routine functioning of the organization, which further reduces waste and improve productivity and quality of the product. As a continuous improvement Kaizen gain popularity when it was considered as an over arching concept for TQM. (Lillrank & Kano, 1989). In any organization, improvement through Kaizen depends upon its employee’s cooperation (Malloch, 1997). Team importance is a fundamental design of kaizen approach. Hyland et al (2004) highlighted prospective benefits of Kaizen, as organizational performance improvement in the form of reduction in waste, breakdowns, lead time, setup time, and as human resource development, in the form of enhancement in skill level attitude, knowledge, empowerment, and quality of life of the worker.

### 2.2. Fundamental Elements of Kaizen

The foundation elements of Kaizen as given by Imai (1986) are appended as follows

- Teamwork.
- Self discipline
- Improved morale
- Quality circles
- Suggestions for improvement
- Elimination of waste (muda) and inefficiency
- The Kaizen 5S framework for good housekeeping.
- Standardisation of the processes

### 2.3. Types of Kaizen

According to Imai (1997), Kaizen may be of following type

- Individual versus team Kaizen
- Day to day versus special event Kaizen
- Process level versus sub process level Kaizen

#### 2.3.1. Individual versus Team Kaizen

Mostly, in Kaizen a team approach is used however another method called “Teian Kaizen’ or personal Kaizen” is also adopted. Kaizen in which the individual employees reveal improvement areas in their

daily work activities and give ideas/suggestions about its improvement is known as Teian Kaizen. This method focuses only on the suggestion for change. Making change for improvement require approval at appropriate level. However, at Toyota motor company the employee suggesting the change is the one who always makes the change either individually or as team member.

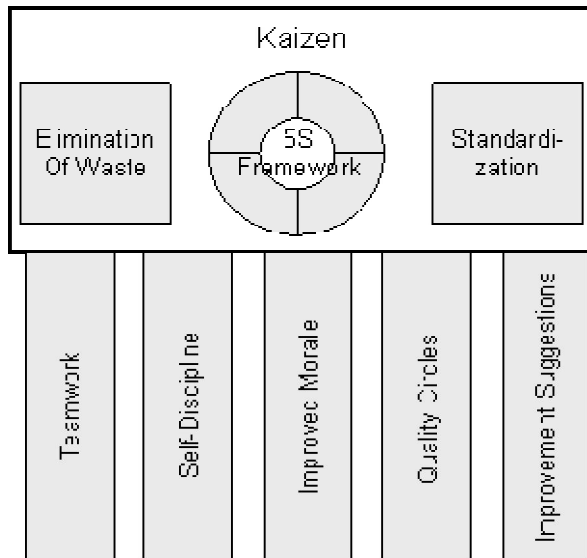


Figure: 1 Elements of Kaizen

Source: - 12 manage.com

### 2.3.2. Day-to-Day Versus Special Event Kaizen

Quality Circles are illustration of a day-to-day Kaizen. In this method, a natural work team identify opportunities for improvement by observing work processes. Team meets at the end of the week for selection of a problem as a kaizen event. They try to identify the sources, (root causes) of the problem and give their suggestions to eliminate these sources, Accepted suggestions or implemented to solve the selected problem. Improvements in work process are made during regular working hours without using over time. Special event Kaizen plans for future and then executes. Improvement process takes two to five days and takes place at the work site. Normally workers identify waste in the processes and eliminate this waste as a kaizen event.

### 2.3.3. Process versus Sub process level Kaizen

Mostly, Kaizen make improvements at the sub process level that is at component level work process. These sub process may includes the activities acquiring material from suppliers, processing them into useful product and providing these products to the end user. Gemba Kaizen, referred to as Point Kaizen, is an example of sub process level Kaizen. On the other hand, there is Flow Kaizen or Kaikaku Kaizen, in which

improvement activities takes places as radical change towards betterment at the value stream or business level. Basing on the literature review, Kaizen technique can be summaries as:

- Kaizen is a small incremental change toward betterment (Cheser, 1998).
- It empowers & involves everyone in the organization to participate in problem solving activities (Imai, 1997).
- Cross functional teams are used to achieve these incremental changes (Imai, 1997).
- Kaizen improve methods or standard way of working of an organization through identification and elimination of waste.
- Save money by focusing on small improvements through organization's own workers and spend the saved money on further improvements within the organization (Imai, 1986)
- Ask suggestions for improvement from all the employee of the organization.
- Never stop trying to improve as improvement has no limits.
- Kaizen may be implemented at process or sub process level as a day to day improvement or as a special kaizen event to improve the quality, process and performance of an organization through elimination of waste. It also reduces cost of production and develops human resources of the organization.

### 2.4. Kaizen Techniques under Kaizen Umbrella

The Kaizen umbrella consists of the collection of Japanese tools. The Kaizen umbrella includes the following tools and techniques (Imai, 1986).

- Customer orientation
- Total Quality Control
- Robotics
- QC Circles
- Suggestion systems
- Automation
- Discipline at the workplace
- Total Productive Maintenance
- Kanban
- Just-in-time
- Zero defects
- New product development
- Small group activities
- Productivity improvement
- Statistical Quality Control
- Cooperative labour /management relations

### 2.5. Description of Kaizen Tools & Techniques.

Kaizen practitioners use various tools during kaizen implementation relevant to the area of

application. A brief introduction of these tools is given in table below.



Figure 2: Kaizen Umbrella  
Source: Imai, (1986).



Figure 3: Kaizen Tools and Techniques  
Source: - (Singh & Singh, 2009)



Figure 5: The 7 Wastes  
Source: - Toyota production system

S/No	Name of Tool with brief description
2.5.1	<b>Single Minute Exchange of Die (SMED):</b> Technique which refer to significant reductions in set-up times. In this technique main emphasis is given on reduction in set-up time, like “changeover of die, clamping and unclamping of work piece/die on the machine.
2.5.2	<b>Total Productive Maintenance (TPM):</b> TPM enhance equipment efficiency through establishment of a preventive maintenance system of equipment throughout its working life. It involves and empowers every employee, from shop floor worker to top management to initiate preventive and corrective maintenance activities.
2.5.3	<b>Kanban:</b> Kanban is a specially designed box/container having a kanban card in it, which moves from workstation to store on requirement bases. This Kanban card is a green signal for store to forward material to workstation for processing. Toyota motor used Kanban system to reduce the work in process inventory.
2.5.4	<b>5 S Practice:</b> It is a housekeeping technique. 5 S represents five words in Japanese language (Scotchmer & Andrew, 2008).these words & their meanings are:- <b>Seiri (Sort out):</b> It mean that at workplace all the irrelevant items /things should be sorted out/ removed. <b>Seiton (Set in order):</b> Items should be arranged properly so that they can be identified and approached easily. <b>Seiso (Shine):</b> Shine means cleaning the workplace till it is spic and span. <b>Seiketsu(Standardise)</b> This mean developing and maintaining standard work practices. <b>Shitsuke (Sustain):</b> Sustaining the progress made. To ensure success in 5S, discipline must be maintained. Progress mad in above four points must be maintained.
	<p>Kaizen 5S1 framework Workplace Organization</p>
	<b>Figure 4 5 S Framework</b> Source:- 12manage.com
2.5.5	<b>Poka Yoke/Jidoka:</b> It is mechanisms used to make mistake-proof an entire process Poka-Yokes ensure that proper conditions exist before actually executing a process step. This prevents defects from occurring in the first place. Where this is not possible, Poka-Yokes detect and eliminate defects in the process. Stop the machine when ever problem occurred. This ensures the reliability of the process.
2.5.6	<b>Standardised Work:</b> A work in which the successive activities have been properly structured so that it can be done efficiently, is called standardised work. The aim of standardised work is to bring the process under control by reducing variation. This in tern eradicates wastages and increases the productivity.
2.5.7	<b>Value Stream Mapping:</b> A value stream mapping is a flow diagram of all the activities required to bring a product from raw materials to delivery to the customer. The objective is to identify and get rid of the waste in the process.
2.5.8	<b>Takt time:</b> Takt time is time taken from the receipt of order from customer till the product is hand over to him. It should be minimize through reduction of waste in the processes.
2.5.9	<b>Standard operating procedure:</b> Means standardise all operating procedures for comparison and further improvement purpose.
2.5.10	<b>Kaizen blitz/Kaizen Event:</b> Kaizen event or kaizen Blitz is a focused small incremental improvement projects completed by cross functional team in a limited time frame (Doolen et al, 2008).
2.5.11	<b>7 W (waste):</b> Seven Ws are 7 commonly accepted wastes out of the manufacturing operations. They include waste from over-production, waste of waiting time, transportation waste, inventory waste, over processing waste, waste of motion and waste from production defects.

### 3. Total Quality Management (TQM)

Johnson (1998), define TQM, as *“An ongoing process whereby top management takes whatever steps necessary to enable everyone in the organization in the course of performing all duties to establish and achieve standards which meet or exceed the needs and expectation of their customers, both external and internal.”* Deming prize, describe TQM as: *“A set of systematic activities carried out by the entire organization to effectively and efficiently achieve company objectives so as to provide products and services with a level of quality that satisfies customers, at the appropriate time and price.”* American Federal office of Management Budget Circular define TQM as; *“TQM is a total organizational approach for meeting customer needs and expectations that involves all managers and employees in using quantitative methods to improve continuously the organisation’s processes, products and services”.*

According to Dale (1999), TQM is the mutual co-operation of everyone (management, workers, suppliers, customers) in an organisation and associated business processes to produce products and services, which meet and, hopefully exceed the needs and expectations of customers. TQM is a philosophy and guiding principles of management of an organisation. Quality experts (Deming, 1986; Juran, 1993; Scholtes & Crosby, 1992), are of the opinion that organizations must seek quality improvement in a long term perspective. Wilkinson (1998) argued that TQM emphasised on empowerment of employees which will enhance an organization's efforts to improve the quality of their products /services.

According to Oakland (1998), to meet the challenges of technology and environment continuous training is the most substantial component in quality improvement. According to Juran (1993), both the external customers (clients, government regulatory bodies, and the public) and the internal customer (employees within different departments) are linked with the quality of product/service. External as well as internal customers have their own needs as for as quality is concerned. TQM give importance to satisfying needs of both kinds of customers. In an organization, goal of TQM is to increase both internal as well as external customer satisfaction through continuous improvement (Anderson, Erikson & Torstensson, 2006), Torrington and Hall, 1998; Dale, 1999; Ahire & O'Shaughnessy, 1998), described that Top management has leading role and provide guidance and direction to the other employees within the organization for the successful implementation of TQM. The company in which top management play leading role, produce high quality products as compared to those where top management is not committed to quality objectives of the organization. Supportive organizational culture helps in

implementation of TQM principle. Oakland (2007), described TQM as an effort to improve the whole organisation’s competitiveness, effectiveness and structure. This is achieved through involvement of all the persons in all quality improvement activities of the organization. Continuous process improvement is a natural evaluation of TQM (MacDonald, 1995; Chung, 1999). Haapasalo (2008) describe TQM as a Management Philosophy that is collection of quality management methods and techniques. Liu, Wu & Chen, (2010), Describe that TQM pay attention to procedural and structural elements.

Deming (1986) declared that “in God we trust - all others we must use data.” This statement stresses on the importance of data management techniques, tools, and systems. (Goetsch and Davis, 1994), explain the management tools as the means of *“collecting and displaying data in a way that help the human brain to grasp and perceive the thoughts and ideas that, when applied to physical processes, cause the processes to produce better results”.* The important aspects of TQM are identified as TQM principles, concepts, tools and techniques. The TQM tools and techniques are known as Hard aspects while the principles and concepts are refer to the Soft side of TQM. Both are briefly discussed as under:-

#### 3.1 The “Soft” Side of TQM

The “Soft” side of TQM as identified from literature review are given as under:-

- TQM involve all employees at all level of an organization.

- It is continuous improvement Philosophy. Continuous process improvement is a natural evaluation of TQM.

- Continuous training of employee is necessary for the successful implementation of TQM in an organization.

- Top-management commitment and support is an essential element of successful implementation of all the principle of TQM.

- TQM is a customer focused management approach

- TQM is a system approach through a processes management. Processes must be improved to improve the results of an organization.

- Cultural change is necessary for the successful implementation of TQM in organization.

- TQM based on actual data that is a factual approach to decision making. TQM develop a mutually beneficial supplier relationship.

#### 3.2 TQM Tools (Hard aspects of TQM)

Hard aspect of TQM consists of its different tools, techniques and systems (Hanson, 2003). Different tools and technique of TQM commonly found in quality management literature (Hyland P W et al, 2004), are given in table below:-

S/No	Name of Tool with brief description
3.2.1	<b>7 Basic QC Tools:</b> These are seven basic tools used for data collection, data presentation and data analyses, for the improvement of quality of the products and processes. They include Check sheets, Pareto Diagram, Histogram, Control Charts, and Cause & effect diagram. Scatter diagram, Graphs (Ishikawa and Kume, 1985).
3.2.2	<b>Fishbone or Ishakawa Diagram:</b> It is a brain storming method to guess different causes of problems related to each, man, machine, material and method, without using statistical methods.
3.2.3	<b>The Matrix Diagram:</b> This tool is used to grade the relationship among different variables. It encourages them to think in terms of relationships, their strengths, and patterns (Bester field-Michna, & Bester field -Sacre, 1999).
3.2.4	<b>Tree diagram:</b> According to Dale (1999), it is a tool Which arranges targets, problems, or customer's needs in a specific order.
3.2.5	<b>Critical Path Analysis (CPA):</b> CPA seeks to establish a sequential order of activities including time and their priority for the completion of a project, through the use of a network of arrows or nodes.
3.2.6	<b>Statistical Process Control:</b> This tool is used to reduce; both assignable as well as un assignable variation in the process e.g. control charts. It helps the managers to control the production process
3.2.7	<b>Pareto Analysis:</b> Pareto Analysis helps the management teams to identify major 20% causes which are giving 80% variation in the production or service processes. Management team should concentrate on these 20% causes first to improve the quality and performance of the system.
3.2.8	<b>ISO 9000 Series:</b> ISO series is an International standard written by a worldwide organization known as the ISO/Technical Committee 176 (Lamprecht, 1992). This set of standards requirement ensures that a company has a specific quality improvement policy, which makes it more competitive in the market.
3.2.9	<b>Bench marking:</b> It involves selecting a demonstrated standard of product or process, costs or practices that represent the very best performance for processes or activities very similar to the company's own.
3.2.10	<b>Just In Time (JIT):</b> It is one of the cost, time and inventory reduction techniques. It is designed to produce products or deliver services just as and when they are needed
3.2.11	<b>Quality Lost Function(QLF):</b> It identifies all cost associated with poor quality and show how these costs increases as the products / services moves away from being exactly what the customer wants
3.2.12	<b>Quality Function Deployment (QFD):</b> QFD is the process of determining customer's desires/ requirements and translating those desires into the target product design. A graphic, yet systematic technique for defining the relationship between customer desires and the developed product or service is known as House of Quality.

#### 4. Methodology

The methodology adopted for this paper is qualitative and descriptive in nature. As the topic demands the comparative analysis of the TQM and

Kaizen, the theoretical perspective was pertinent to be employed. Viewpoints of different researcher and proponent about TQM Philosophy and Kaizen have been highlighted. Different tools and techniques of both TQM and Kaizen are briefly discussed to analyse the main difference between the two. The sources used for information collection were research papers; Books, online journals and web based links. The analysis is based on the result of through study of literature on these topics.

#### 5. The Relationship between TQM & Kaizen

TQM and Kaizen are interdependent. In the literature, Kaizen-Continuous improvement has been broached as an important element of TQM. Kaizen is one of the reference points in the Deming's 14 points regarding TQM. Deming's point "Improve constantly and forever" infer the need for some sort of continuous improvement methodology such as Kaizen. So this makes Kaizen a subset of TQM

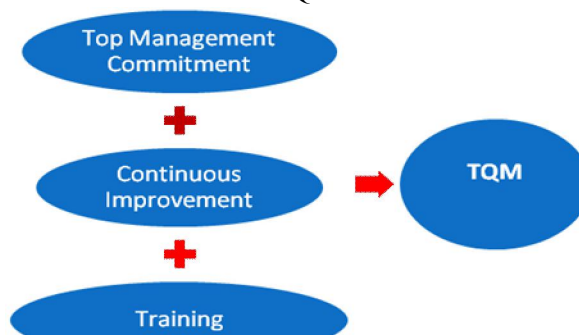


Figure 6: Kaizen as a subset of TQM

Kaizen means continuous process improvement. Some researcher considered continuous processes improvement is a natural evaluation of TQM and CI perspective. Both can be distinguishing as kaizen focused on small and gradual improvements where as TQM involve radical improvement of important and crucial process to get large effects (Davenport & short, 1990). According to literature both concepts are complementary and share same philosophy (Imai, 1986). The best organization always applies both the concept together to get maximum benefits of continuous improvement. Implementation of only one concept will not be so fruitful. The difference between continuous improvement (CI) and Continuous process improvements (CPI) is given in figure below.

#### 6. The Differences between TQM & Kaizen

TQM is a philosophy of what makes up a quality organization, and Kaizen is a methodology that one can apply to encourage improvements to existing processes.

The main differences between the concepts of Kaizen and TQM are highlighted below

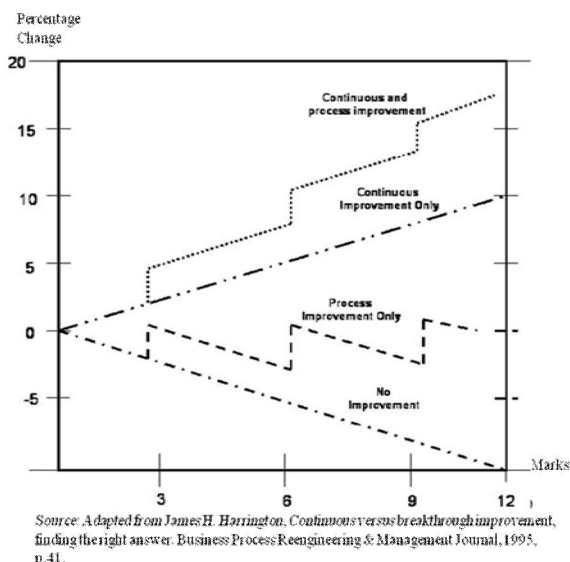


Figure: 7, Differences between continuous improvement & Continuous process improvements

**6.1 Definitions**

TQM is a management approach that aims at long-term success by focusing on customer satisfaction, based on the participation of all members of an organization through improvement of quality, processes, services, and the culture in which they work. Whereas, term Kaizen is "to take apart and put back together in a better way". Kaizen is "small incremental but continual improvement" in order to improve process, quality and of course performance of the organization.

**6.2 Focal point**

Kaizen is a process oriented concept. It focuses on the improvement of the process to get improved results in every sphere of life. On the other hand, TQM is a product oriented and customer focus concept. It focuses on the quality of the product to satisfy the customer.

**6.3 The Scope**

Kaizen can apply to encourage improvements to the existing processes. The scope of Kaizen is limited to selected project however; the scope of TQM is spread throughout the organization. It works on every process at every department of an organization all the time for achieving quality product and services.

**6.4 Implementation Method**

Kaizen is implemented in the form of small incremental projects in a selected area in order to make changes in the work standard towards betterment. These

small increment projects are known as kaizen events. Kaizen event can be selected for each department of the organization separately also for each kaizen event independent cross functional team is selected which works on the improvement of one project at a time in focus area for a limited time frame. The workers or the team should work on one process at a time only. Consequently, in TQM improvement is to be made on all the processes in all the business department of the organization involving all the persons at a time.



Figure 8: The different business department of an organization where TQM can be implemented at a time.

**6.5 The Approach**

Kaizen concept follows bottom-up approach. The suggestions for improvement are put forward by the workers. Whereas TQM concept follows both top-down and bottom up approach, the need for improvement is suggested and introduced by top management as well as by the workers of the organization.

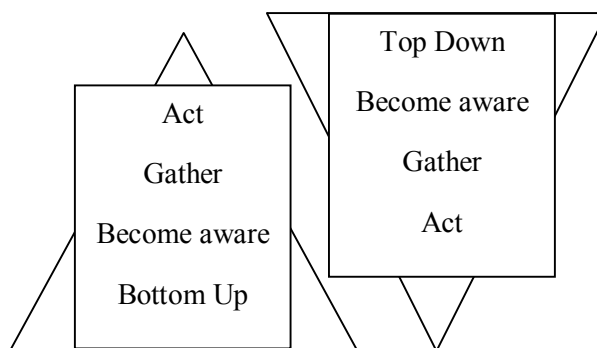


Figure 9: The Approaches of TQM and Kaizen

**6.6 Importance of Resources**

TQM is little more expansive to implement as compared to Kaizen. Kaizen focuses on the improvement within the available resources of the organization (Imai, 1986). It doesn't encourage large investment from the organizational resources for improvement. Whereas, in TQM Investment has to be made to improve the quality of product or process like investment on new or updated machinery e.g. Automation, Innovation etc.

### 6.7 Involvement of People

Kaizen involve all stake holders at all level of the organization when asking suggestions for improvements of the organization, but it does not necessary that everyone in an organization should participate in the improvement project also. The people linked with a particular process on which improvement project is taking place are involved. e.g. cross functional Kaizen team. Whereas in TQM, Whole organization including all employees at all levels are responsible and involved in improvement of quality of the product at all time.

### 6.8 Implementation Mechanism

Kaizen focuses on step by step improvement. While TQM focuses on simultaneous operations in all the processes In TQM sustain and improvement goes parallel to each other. Another difference between the two concepts is that, in Total Quality Management there is no discontinuity in the process of continuous improvement, the whole organization should always work on the improvement process. On the other hand, Kaizen focus on small scale improvements in steps. After every step, there lies a discontinuity for sustaining the improvement, afterwards the process continues toward the second step and so on. This is called continual improvement

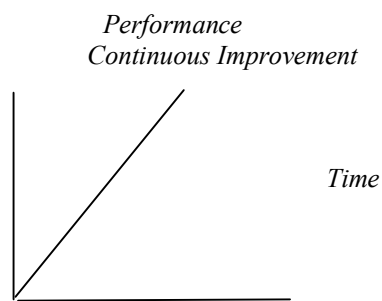


Figure.10 Continuous Improvement in TQM

### 6.9 Improvement Strategy

Kaizen strategy is made for small permanent improvements in processes. However, TQM focuses on long term improvements. TQM means organized Kaizen activities involving everyone in a company, managers and workers in a totally universal and integrated effort toward improving performance at every level.

### 6.10. Improvement of Quality

Kaizen and TQM both deal with the quality. TQM focuses on improving quality by value addition, making the product perfect, improving productivity, reducing the variation in measurements, and processes while Kaizen focuses on improving quality through small incremental changes in processes, and workplace result in reduction in different types of wastes.

### 6.11 Improvement through Innovation

TQM involves continuous improvement of process through kaizen and innovation Whereas Kaizen philosophy stresses on continual improvements in existing standards rather than innovation. This process leads to better utilization of R & D resources of a company and better productivity.

### 6.12. Decision Making

Quality decisions are made based on measurements in case of TQM. After the decision is implemented, the output is compared with the standards, but in Kaizen, decisions are made, when an employee makes errors in his job, it is not seen as an occasion to blame, but is seen as a chance to find out what went wrong with the process. The continuous improvement by removing the errors and minimizing the chances of reoccurrence is the baseline to make the decisions in Kaizen

### 7. Summary TQM & Kaizen

- To get maximum advantages, knowledge of both TQM and Kaizen concepts is necessary for the workers, middle management and top management of the organization.
- Continuous improvement of quality should be the concern of every one in an organization. Training should be imparted to every employee regarding how to continuously improve the quality, process and performance of the organization using both the concept.
- There should be a brainstorming on the level of problem that occurred in the process, so that the pertinent improvement mechanism should be evolved and implemented.
- The effect of Kaizen activities should be circulated to the whole organization, so that it should work as a motivator for the top management and employees to carry out further improvements.

### 8. Conclusion

There is ample of literature available on the TQM philosophy and Kaizen concept. But very less has been talked about the relationship between the two concepts. So there was need to elaborate the relationship and main differences between these two concepts for the purpose of implementation. Although, in the literature the philosophers and experts do have differences of opinion regarding the difference between TQM and Kaizen, Yet an effort was put in to extract few main differences on the basis of the literature and concepts given by the experts. A selected researchers consider both as the tool of continuous improvement and can be compared while others consider continuous improvement and kaizen as a



part of Total Quality Management. It is evident from the literature that Kaizen or continuous improvement is one of the points in the TQM model by Deming. In spite of differences in the methodology of continuous improvement, focus of improvement, scale of operations, etc in both the concepts, it can be concluded that the Kaizen is one of the technique of TQM for continual improvement of process, quality and performance along with the development of human resource of the organization.

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## Internet Development Opportunities for Small to Medium Sized Enterprises in Ukraine

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**Abstract:** It has been argued that the development of the small business sector in Ukraine is central to economic reforms and the creation of wealth and employment. Given the inherent instability and general weakness of the Ukrainian economy to date, there have been many structural and political obstacles to entrepreneurial activity and the development of small enterprises in a variety of business sectors. However, the relatively recent emergence of the Internet as a vehicle for business transformation has already provided opportunities for small to medium-sized enterprises (SMEs) in Ukraine to enter global markets. The extent to which this has been the case for developing economies is a continuing research issue, given that various studies have already identified tangible benefits for the growth of SMEs in developed economies. This paper looks at the development of SMEs in Ukraine and outlines the factors that have affected their growth. The paper then focuses on the role of the Internet in promoting the development of SMEs in Ukraine, and considers the facilitation of opportunities provided by the available infrastructure. Recent progress towards developing a better infrastructure to support entrepreneurial activity and SME growth are discussed, including the need for further research.

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### 1. Introduction

It is widely accepted that two main types of small business were in existence in the republics of the former Soviet Union: small enterprises and co-operatives (Isakova, 1997). Co-operatives were first established from 1986 onwards during the early stages of *Perestroika*, and they had significant privileges enabling them to operate successfully within the framework of the Soviet Union prior to its dissolution in 1991. Small enterprises in Ukraine effectively started in 1990 following a resolution affecting the development of small enterprises by the Soviet of Ministers of the Ukrainian SSR. This resolution stated that during their first two years of operation small enterprises were not required to pay any taxes. Ukraine immediately experienced a twenty-fold increase in the number of registered small enterprises. The owners of large state-owned enterprises were initially very active in launching small enterprises because this was particularly profitable at the time. Then in 1992 a law governing private property was introduced, and the number of small businesses began to grow mostly on the basis of private capital, gradually replacing co-operatives. By 1994 both were still in existence, but many co-operatives attempted to equate themselves legally with the status of small enterprises. Following the introduction in 1994 of the law on enterprises, profit tax levels were set at 30% for small enterprises. Ukraine subsequently saw a rapid decrease in the number of new small enterprises, detrimented by the

fact that by 1997 these enterprises had to pay 13 different taxes amounting to between 70 and 90% of their profit. This forced many firms to consider either bankruptcy or operating in the shadow economy. In total over 90,000 small enterprises were registered, but only 45,000 of them had reported as operating (figures cited in Isakova, 1997). According to official data for 1995 provided by the Ministry of Statistics in Ukraine, the level of small business growth in Ukraine not only lagged behind the developed economies, but also the transition economies. The slowdown of the small business sector from a high growth rate of 52% in 1991 to 0.8% in 1994 is important, given that by 1995 this sector still accounted for approximately 60% of Ukraine's GDP. Statistical data suggested that the majority of these small enterprises operated primarily in 'retail and catering' or 'transport', followed by 'industrial enterprises' and 'construction firms'. Note that this data does not include investment companies and funds as these are considered business infrastructure institutions by the Ministry of Statistics in Ukraine. However, based on the number of employees in these firms, they can be considered as small to medium-sized enterprises (SMEs) that have a major role to play in the economic development of Ukraine. This is consistent with the definition adopted by the European Commission that describes SMEs as companies that typically have less than 250 employees. Globally, SMEs contribute greatly to national economies e.g. in the USA where they

generate more than half of all employment (Baldwin et al., 2001). It is clear that the activity and development of SMEs in Ukraine will have an influence on the development of the Ukrainian economy in general.

## 2. Growth of the Small Business Sector in Ukraine

The majority of employees in the small business sector in Ukraine have predominantly worked in 'retail and catering' or in 'services to the population', and these have traditionally been considered the easiest for small business start-ups. In recent years, the 'research and development' sector (i.e. technology infrastructure firms) has been a relatively entry resistant sector for small businesses in Ukraine for various reasons. Although Ukraine has high research potential, the reduction of R&D allocation in the state budget combined with factors affecting the gradual transformation of scientific research has meant that the demand for intellectual products within Ukraine is less than the supply. This means that technology-based enterprises have often faced greater constraints to their development than firms involved in other activities. This does not mean that such firms do not operate in Ukraine, as they are arguably a major presence in the shadow economy. Some Ukrainian experts have estimated the 'legal to illegal' small business ratio in different sectors as follows: retail trade - 30:70, services - 40:60, manufacturing - 50:50 (Isakova, 1997), although it is known that authorities have since tried to close this deficit given the loss of tax revenues and the global perception of Ukraine as a place to undertake business (Yushchenko, 2006). There are various opportunities and threats for firms in these different sectors, and these have influenced the development of SMEs. The purpose of this paper is to consider the role of the Internet in developing entrepreneurial opportunities within such SMEs in Ukraine. The main opportunities for these firms have traditionally been as follows (Isakova, 1997):

- Greater business activity due to the legitimisation of private small business
- Until the late 1990s, a shortage in the marketplace of consumer goods and foodstuffs
- Great demand for business and professional services
- Abundance of a qualified and well educated labour force
- Low internal competition and niches in manufacturing and services
- Comparative independence from the former Soviet Union market
- High availability of natural and technological resources

It can be assumed that most of these opportunities are applicable to all sectors, but the availability of a highly qualified labour force and the availability of technological resources are particularly relevant for the development of technology-based enterprises. The main threats for small Ukrainian businesses have been described as follows (Klochko & Isakova, 1996):

- An absence of small enterprise legislation, with inconsistent and ineffective regulations
- Restrictive currency controls and burdensome taxation policies
- Low access to equity and loan capital due to lack of sources of capital, and an inadequately regulated banking system
- Poverty within some areas of the population
- High cost of commercial space/equipment
- Shortages of some necessary suppliers and raw materials
- Underdeveloped business and legal infrastructure
- Crime, corruption, bribery, racketeering
- Problematic relationships with large state enterprises
- Lack of government support programmes for SMEs

The technology sector has been particularly susceptible to these threats, thereby limiting the growth of technology-based enterprises. Isakova (1997) argued that one of the best ways to facilitate the transition to market and restore economic health in Ukraine would be to use technology to create value-added jobs and foster worldwide exports, achievable by generating innovation and diffusing new technologies. Until recently this aspect has not been possible because of the absence of customers, long-term loans, adequate banking and business infrastructure, and a lack of venture capitalists seeking involvement with Ukraine. This situation has been worsened by the lack of support organisations and foundations seeking to assist business innovation, which when coupled with uncertainty about intellectual property and the cost of patents may have acted as a deterrent for many entrepreneurs. More recently, some management consultants have given a more positive perspective on the investment and business potential of Ukraine, e.g. Arthur Andersen (2000) and Pricewaterhouse Coopers (2001). A lack of entrepreneurial activity has not been the case in developed economies where major technological changes and business opportunities have occurred, particularly during the emergence of Internet-mediated growth especially in the creative industries

within the 'digital economy', as described in the next section.

### 3. Internet Enterprises and the New Economy

One of the biggest challenges facing large and small enterprises in the developed world is the need to find ways to increase the value from their customers rather than from their products, and to gain revenue growth that is continued instead of purely short-term (Vandermerwe, 2000). The aim is to make customers want the services of a particular company as their sole or dominant choice because they provide superior value at lower cost, from which the company benefits by obtaining a deeper and broader share of customer purchases for longer periods of time. This 'economic transformation' has been driven by electronic business, and more specifically electronic commerce (e-commerce) that has provided a powerful way for many organisations to achieve tangible benefits based on web-enabled transactions and use of the Internet (Hartley & D'Cruz, 2001). It is clear that e-business has opportunities in terms of improving the efficiency and effectiveness of an organisation, but also has a more strategic and competitive aspect: the need to find and attract customers, fulfil their expectations with good customer service, and continue that relationship with them. This has meant that customer relationship management (CRM) is an area that has received much attention, and many small firms initially struggled to develop effective CRM systems (see Whittle, 1999; Phillips, 2000). This paper is particularly concerned with the growth of SMEs and the role of the Internet in facilitating new opportunities. The argument here for e-business is that it could help SMEs located in Ukraine to grow and compete with similar organisations throughout the world, a view supported by the experiences of SMEs in developed countries (e.g. McWilliams, 1995). There have been various guidelines and indicators proposed for the successful adoption of e-business (e.g. Warren, 2000; Baldwin et al., 2001) but implementing these and evaluating subsequent performance has been problematic (Laughlan, 2000). Some of the changes to political and technological infrastructure have made this a more realistic possibility for Ukraine, although there is still much to be done in terms of establishing a legitimate business infrastructure that customers and partners can rely upon. This includes the need for reliable and secure payment systems, timely and well monitored distribution systems, effectively regulated import and export arrangements, and better consumer protection. Large multinational organisations generally have the resources and established strategic business planning processes to account for these, but the smaller

enterprises have a different set of issues that need to be considered, as discussed in the next section. When coupled with the global downturn and recent financial/energy crises, this has not made the outlook for smaller organisations in Ukraine any brighter, and they continue to face challenges that make the need to use technology to reduce their costs and increase access to global customers even more important.

### 4. The Impact of the Internet on Small Business Sector

There have been various studies into the impact of the Internet on business transformation in large companies (e.g. Dutta & Segev, 2001), but less research has specifically focused on the implications for SMEs. Baldwin et al. (2001) suggested that SMEs are associated with the relative quality of their customer service, generally an aspect that many larger companies cannot match. This comparative advantage can be of tremendous benefit to SMEs as Internet technology now allows SMEs in geographically disparate locations to attract and retain customers they would otherwise not have been able to reach at all. It can be argued that despite varying estimates, the majority of SMEs in developed countries have Internet access. Barriers to greater and more effective use of Internet technology within these SMEs typically includes lack of time and an absence of specialised technical expertise. However, opportunities for SMEs provided by the Internet include export generation, niche marketing, better information technology utilisation, and development of strategic partnerships/alliances. The Internet influences the way that SMEs can communicate with contacts and customers, the way they manage information, and also how they project their image. This change often means that business processes and operations impact on different levels including external contacts, the industry, internal organisation, administration and manufacturing tasks, and will influence areas such as productivity, information retrieval, communication, knowledge management and the environment (Baldwin et al., 2001; Hamill & Gregory, 1997). The governments of many developed countries such as Britain, USA and Ireland have heavily invested in plans to develop the underlying technical infrastructure and encourage greater usage of e-commerce by SMEs over the coming years. Similarly, the government of Ukraine officially promotes the process of 'informatisation' i.e. the general penetration of IT into state-owned enterprises, educational bodies and Ukrainian society. In addition, there has been legislation introduced to improve the environment for small businesses in Ukraine. Sikorsky (2002) reports on measures for deregulation of entrepreneurial activity (decree no.

817 in July 1998) that orders many governmental/state bodies to ensure support and development of entrepreneurial activity. Similarly, measures to provide for support and development of enterprise (decree no. 906/2000 in July 2000) stipulated personal liability for officials that adopt decisions that worsen the business environment. This was clearly progressive, and as the underlying business and legal infrastructure develops, so will the adoption of e-commerce and the creation of Internet-mediated opportunities. However, even if an infrastructure that promotes entrepreneurial activity is available, not all SMEs will adopt the Internet for the same purposes. The success of Internet-enabled commerce is an area of substantial research in developed economies, for example Daniel & Myers (2000) conducted a study of UK SMEs to consider how e-commerce adoption has already realised appreciable benefits in organisations. The report from their study considered the responses of 688 companies, and considered the reasons for and impact of e-commerce on the activities of these companies. The main findings were:

- The main reason for companies to adopt e-commerce is to differentiate themselves from competitors and improve relationships with customers, e.g. services to customers (63% of respondents), brand building (42%), to find new customers (41%), and to hold dialogue with customers (38%)
- The rationale for e-commerce adoption is similar across different sized companies, although companies with more than 100 employees are more likely to want to use e-commerce to hold a dialogue with customers, or to communicate and share knowledge within the company
- Most SMEs are yet to realise significant benefit from their e-commerce adoption, and e-commerce is predominantly bringing benefits to internal communication between employees and customers (almost 90% of respondents) and finding external information (almost 80%)
- Very few SMEs have found significant benefits from using e-commerce to find suppliers (less than 7% of respondents) or recruit staff, suggesting that larger competitors still have an advantage here
- Fewer companies are taking orders online (28%) and even fewer are receiving payment online for goods sold (13%) or purchasing online (12%), this suggests many are taking a staged approach to e-commerce adoption from low risk customer-focused activities to more complex tasks
- Factors stated for the successful adoption of e-commerce include senior management support,

staged implementation, and a clear understanding of how e-commerce can benefit the business

- The industry that leads e-commerce usage is the IT and communications sector
- Regional variations are not clear, although greater benefits and e-commerce adoption have been reported in and around the UK capital
- Younger companies are more likely to be using e-commerce than older established companies, but this lead is only slight as older firms now appreciate the value of electronically-mediated forms of business

These findings are interesting if they are considered with respect to the economic conditions and factors affecting SME growth in Ukraine. SMEs in Ukraine are likely to have very different experiences as they grow, given that they have not had the same stable and reliable infrastructure that underpins the business activities of UK SMEs. Indeed the Digital Britain (2009) interim report looks at how the UK infrastructure can be further enhanced to improve the social and economic benefits of ICT networks. For Ukraine, the reasons for e-commerce and e-business adoption may perhaps be similar, but it is necessary to consider the business environment of Ukraine where capital is often difficult to obtain, equipment and business premises costly, and legal and consumer protection in need of further development. Many UK SMEs have adopted e-commerce and reported benefits from business-to-consumer (B2C) activities rather than business-to-business (B2B) activities. These benefits are consumer-oriented such as improvements in communication with customers and raising brand awareness, or internal such as finding external information or customers, sharing knowledge within the company, etc. According to Daniel & Myers (2000) study, very few UK SMEs reported benefits associated with reducing costs of administration, staff management and supplier relationships, mostly due to the comparative scope of operations within these companies. That is probably no longer the case with remote working, videoconferencing, Skype, wireless networks, the need to reduce energy costs of local data centres and data management that are all benefited by technological advances and use of Internet infrastructure. SMEs in Ukraine are also likely to consider consumer-based activities as a priority as these are most likely to generate much needed revenue streams, but the number of customers having access to the Internet within Ukraine and developing countries is significantly smaller than in the UK and other developed countries. Telecommunications in Ukraine were under

government control and penetration by foreign operators was slow and problematic (Mishustin, 2002). Given the limitations of technical infrastructure, Ukrainian SMEs initially adopted e-commerce for reasons more likely to be rooted in potential access to foreign markets, raising product and brand awareness, and finding particular competitive niches within which they can operate. Although technology-based SMEs within Ukraine have been slow to develop, there are now numerous companies possessing technical expertise and suitably qualified people able to contribute to the growth of SMEs, e.g. by developing 'front-end' systems and content often located on overseas web servers. These sites need to be designed to reflect the multi-lingual and multi-currency requirements of a global market, and also specific cultural and social factors that have been described as 'psychic distance' (Dicht et al., 1990). Whether or not Ukrainian SMEs will choose to take a cautious and staged approach to e-commerce and e-business adoption with government support similar to that of UK SMEs is not clear, given the uncertain yet dynamic changes occurring in Internet technology and electronic business generally. As the technological infrastructure in Ukraine develops to facilitate global access to e-business and the digital economy, these companies must have appropriate 'back-end' billing, distribution and inventory systems if they are to avoid many of the problems faced by companies in developed countries. Additionally, the focus of much of the current electronic business activity in developed countries is on electronic marketplaces and trading exchanges that link related companies to their suppliers, which potentially includes SMEs. Research is needed to investigate whether Ukrainian SMEs are aware of these issues, and to explore the rate of e-commerce and e-business adoption within Ukrainian SMEs further.

##### **5. Developing Infrastructure to Support Entrepreneurial Activity**

This paper has looked at the importance of SMEs in Ukraine and outlined the factors that have affected their growth. The paper has also considered the role of the Internet in the development of SMEs, and considered the potential opportunities for Ukraine. The need for research into the extent to which Ukrainian SMEs have adopted e-commerce, e-business and Internet technologies has been emphasised, and implications put into context. There are various difficulties in conducting this research, particularly the availability of information and the readiness of SMEs to openly discuss their activities given the influence of the shadow economy in Ukraine. Many firms now have dedicated 24-hour

access to the Internet with acceptable transmission speeds, supplied to them at a low. Ukrainian SMEs have traditionally focused on faxes rather than email and file attachments, necessitated by a business culture that relies heavily on formal documentation and the security of signed documents. Email and Internet-based communications are now widespread, although arguably not to the extent that a relationship with customers can be fully managed via the Internet. Electronic customer payments and Internet-mediated banking systems are accessible (e.g. Nunan, 2001), but this is set against a global backdrop of recession, cybercrime and organised criminal activity. There is still a lack of trust in the banking infrastructure of the former Soviet Union owing much to historical and cultural precedents (Jones et al., 2000). The Internet is accessible to Ukrainian customers through the growing number of companies offering Internet and related services provision, although transmission speeds tend to be much slower and less reliable. Some SMEs have Ukrainian, Russian and occasionally English language web sites, and these are sometimes listed in a directory of business services or through business portals, and there are a large number of available domain names that can even be localised to individual Ukrainian cities. This demonstrates that many Ukrainian SMEs already have an awareness of the business opportunities created by the Internet, and this needs to be developed further. It has been argued that global Internet business transformation is not simply a matter of changing existing marketing channels involving a language translation process, but of effective content management and technical support underpinned by strong manufacturing and distribution capability (Heikkila, 2002). This is where many SMEs in the developed world have had difficulties, and has resulted in the emergence of companies that specialise in the globalisation of Internet activities through networks of affiliated companies. Ukrainian SMEs and entrepreneurs have the opportunity to become involved in this process by utilising such networks for enterprise development, and focusing on their capabilities and the development of relationships with customers and suppliers. The use of social networks and Web 2.0 is also an interesting area as sites such as 'odnoklassniki' have emerged to rival 'facebook.com' and 'linkedin.com' for useful social and business networking, and their business potential is yet to be fully evaluated, particularly with the advent of Twitter and converged mobile devices. An investigation into the factors that influence the development of web-based ventures was conducted by Cantarella (2001) which explored the background, education and supporting environment for the

founders of US-based enterprises. A similar study looking at developing economies such as Ukraine and emerging technologies is needed in order to put the findings into context, and is an opportunity for further research.

## 6. Summary

Much has been speculated about the role the Internet will play in developing countries in terms of how services to citizens are provided, and how entrepreneurial opportunities similar to developed nations can be created. This is an important issue for individual government policy, particularly as bodies such as the World Bank and the World Trade Organisation are in favour of greater global e-commerce regulation within less restrictive international e-business and trade frameworks (Hallett, 2002; Hartridge, 2002). According to Risaburo Nezu, Director of Science, Technology and Industry at the Organisation for Economic Co-operation and Development (OECD), there is “a need for governments to create an open, predictable and transparent telecommunications market, backed up by open, free foreign investment regimes” (Nezu, 2002). There are some nations within the developing world that have been particularly successful in supporting this, i.e. the lack of initial intervention in the activities of Indian SMEs by its government resulted in the opportunity for India to be considered a potential ‘IT Superpower’ (Barker, 2000). Numerous IT-related projects in the developed world are now successfully outsourced to the mature software industry in India, even though India continues to struggle to retain its best people. India has the largest pool of English-speaking IT professionals outside the US, and the Indian government has adopted a liberalising and deregulated policy that encourages overseas high-tech companies to locate in India. Some of India’s comparative success can also be attributed to Indian companies with government support establishing effective strategic alliances with local and overseas partners, and attracting direct foreign investment enhancing their entrepreneurial activities (D’Cruz, 2008). Despite political, cultural and business infrastructure problems, SMEs in Ukraine and the former Soviet republics have similar opportunities given the wealth of intellectual and technological resources they have at their disposal, assuming any prior negative images of problematic project delivery and systematic business corruption can be overcome and protectionism deterred (McCue, 2007; Project Eye, 2009). It is likely to be an issue of the value of the products and services that these companies can offer, and investment into the infrastructure to support entrepreneurial activity. Coupled with this

will be the speed at which Internet-based practices are adopted within competitive international frameworks that ultimately dictate success or failure.

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**Pathology Induced by *Sphaerostris picae* (Acanthocephala, Centrorhynchidae) in the Hooded Crow *Corvus corone cornix* (Aves: Corvidae) from North Delta of Egypt**

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**Abstract:** The present study describes the pathological manifestations of the acanthocephalan, *Sphaerostris picae* (Rudolphi, 1819) Golvan, 1960 in the small intestine of the hooded crow "*Corvus corone cornix*" collected from the northern parts of Nile Delta in Egypt. Histological and histochemical alternations of infected ileum were illustrated using hematoxylin and eosin stain, alcian blue method for mucin and Malaty's modified simultaneous coupling azodye method for acid and alkaline phosphatases. Examinations of 25 birds showed that the infected ileum only harbored the acanthocephalan worm, no other helminthes were observed. The proboscis of the acanthocephalan pierced the mucosal epithelium, its lamina propria and reached the external muscularis causing compression and erosion of the villar epithelium, shortening and abrasion of the intestinal villi and destruction of the glands (crypts) apposing the everted worm proboscis. Noticeable cellular infiltration, hemorrhage and marked destruction, thickening and vacuolation of stromal connective tissue surrounded the acanthocephalan preasoma, as well as in the submucosa were detected. A marked increase in the number of goblet cells in both crypts and villi was observed. The intestinal epithelium exhibited a detectable increase in acid phosphatase activity in both villi and crypts while alkaline phosphatase showed moderate decrease in the villi and detectable decline in the crypts epithelium.

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**Key words:** *Sphaerostris picae*, Acanthocephalae, *Corvus corone cornix*, Histopathology, Phosphatases, Egypt.

## 1. INTRODUCTION

The pathological changes in the parasitized tissues brought about by acanthocephalans form an important factor in determining the ability of these parasites to adapt at their environment. The body of acanthocephalans consists of a trunk or metasoma, freely lying in the intestinal lumen and the praesoma (proboscis and neck) that has the ability to penetrate into the intestinal wall of the host. **Wanstall et al. (1986)** revealed that the common consequence of acanthocephalan infection in vertebrate host is damage to the mucosal epithelium of the gut by the main body (metasoma). In addition, the attachment organ (praesoma) can damage the gut at the attachment site.

**Palmer and Meerveld (2001)** referred to the inflammation of the host induced by intestinal parasites, resulting in altered gastrointestinal function, namely enhanced secretion and propulsive motility of the gut. **Sanil et al., 2010** reported that the acanthocephalan histopathology in the intestine as revealed by loss/degeneration of the intestinal villi, formation of granular tissues and capsule are associated with host immune responses. Depending on their attachment mechanism, they are able to seriously disrupt the integrity of the mucosal gut layer, inducing lesion of wide degree from shallow erosions to deep ulcerations with hemorrhage and perforation of the gut wall. Invasion/migration of the acanthocephalans into uncommon locations has also been reported (**Nickol, 2006**).

The hooded crow is a widely distributed bird and a common resident inhabiting cultivated land and open and wooded terrain in the Nile Delta and Valley in Egypt (**Tharwat, 1997**). Although its food habits include wide range of intermediate and paratenic hosts (insects, mollusks, worms, small vertebrates and carrion), its entire intestine has been reported as microhabitat of only one acanthocephalan species; *Sphaerostris picae* with no infection of any other helminth species (Radwan, personal communication). To the best of our knowledge, no information is available due to the pathology induced by *S. picae* infection in the hooded crow.

The present study was undertaken aiming to determine the histopathological changes in the intestine of the hooded crow *Corvus corone cornix* by the invasive action of the well armed proboscis of the big-sized acanthocephalan; *S. picae*. The mucopolysaccharides and phosphatases have been localized in the intestinal tissues.

## 2. MATERIALS AND METHODS

### Sampling

Twenty five hooded crows were collected from the agricultural areas around Kafr El Sheikh Governorate. Birds were transported alive to the laboratory, anesthetized and dissected. The intestines were incised longitudinally and examined in 0.7% physiological saline for the parasite infections. Some acanthocephalan specimens attached to the wall of the

intestine were dissected out with the aid of fine needles and forceps, and then fixed in cold 70% ethanol.

#### **Whole mount preparation**

For preparing whole mount of collected acanthocephalans, specimens were stained in Mayer carmine and cleared in Terpinol according to **Amin, 1998**. Acanthocephalan specimens were identified according to **Dimitrova et al., 1997 and 2000**.

#### **Histopathological examination**

Pieces of 1 cm in diameter of infected ileum were fixed in Bouin's solution, dehydrated in an ethanolic series, cleared in xylene and embedded in paraffin wax. 5 µm paraffin sections were stained with haematoxylin and eosin (HE) and mounted in Canada balsam. Slides were examined with Olympus CX31 equipped triocular light microscope and representative photomicrographs were taken with E-330 DC 7.4V digital camera. For comparison uninfected host tissue was also sectioned and examined.

#### **Histochemical examination**

Alcian blue method for demonstrations of acid mucopolysaccharides was done (**Steedman, 1950 and Lison, 1954**).

Suitable parts of both infected and uninfected ileum were used for the preparation of cryostat sections of 8µm thickness. The frozen sections were treated according to Malaty's modified simultaneous coupling azodye method (1971) for detection of the activity of acid and alkaline phosphatases. Red granules of the Azo dye deposits indicate sites of acid phosphatase and bluish granules indicate sites of alkaline phosphatase activity.

### **3. RESULTS**

All the examined birds appeared healthy without any external clinical manifestation concerning the weight, activity and feather appearance.

*S. picae* is a large-sized acanthocephalan (Figure 1a) with well armed proboscis (Figure 1b). It was restricted to the ileum; no infection was detected in the duodenum, jejunum or caecae. Heavily infected parts of the ileum appeared swollen with the anterior ends of some worms deeply embedded. Along all the number of examined infected intestine (25), no worms were found outside the intestine. At the site of parasite attachment, the surface of the ileum appeared thickened (Figure 1 c).

#### **Histopathological observations**

Sections of the ileum of uninfected hooded crow showed the normal features of bird ileum consisting of an outer serosa, muscularis externa with outer longitudinal muscle fiber and thick inner circular muscle fiber. The intestinal glands (crypts) are closely packed in the demarcated lamina propria. The villi are tall and regular arranged with columnar epithelial lining included mucus secreting goblet cells and the lumen is narrow (Figure 2a). Strips of smooth muscle

fibers forming the muscularis mucosa extend in-between the intestinal glands (crypts) and villi. A core of lamina propria with diffuse lymphatic tissue and small blood vessels extend inside the intestinal villi (Figure 2b, c).

When the penetration of *S. picae* proboscis through the wall of the ileum is shallow, it reaches the mucosal and submucosal layers only, and this is accompanied by blunting, shortening and destruction of the intestinal villi (Figure 3a), and compression and erosion of their columnar epithelium apposed the everted worm proboscis, with noticeable increase in the number of goblet cells which open in the inter-villous space (Figure 3e). A marked destruction of intestinal gland (crypts) and an increase in the number of goblet cells were detected (Figure 3c, d).

Marked cellular infiltration was seen in the stromal connective tissue surrounding the everted proboscis in both submucosa and muscularis (Figure 3a). The serosa and muscularis presented destruction, thickening and vacuolation of the stromal connective tissues (Figure 3b, f). Dilation and congestion of blood vessels in stromal connective tissue between the muscle bundles have been detected (Figure 3f).

The deep penetration of the worm preasoma to reach muscularis layer was accompanied by full thickness of mucosa and submucosa with cellular infiltration and hemorrhage surrounding the penetration site which enclosed poorly differentiated lymphocytes (Figure 4a).

The tissues around the proboscis were hemorrhagic and granulated (Figure 4b). The epithelial cells of the intestinal glands near the site of proboscis attachment, exhibited crypts hyperplasia and hyperchromatic nuclei and mitotic activity (Figure 4 b&c). There was noticeable fibrosis and dense fibrous stroma around the worm proboscis (Figure 4d). In the submucosal layer, dilation and congestion of blood vessels and stromal lymphocytic infiltration was clearly observed (Figure 4f). The presence of aggregation of lymphocytes and large number of eosinophil granulocytes and fibroblasts in the inflammatory sites, suggest inflammatory responses (Figure 4e).

#### **Histochemical study**

##### **I- Acid mucopolysaccharides**

A moderate alcian blue stain revealed the presence of acid mucopolysaccharides in the mucus secreting goblet cells in the villi and crypts of uninfected sections (Figure 5a). Due to the erosion of the mucosal epithelium facing worm attachment sites, negative reaction of mucopolysaccharides was detected in these areas. However, villi and crypts near but not facing the attachment sites showed strong alcian blue reaction as a result of the increase in number of goblet cells (Figure 5b).

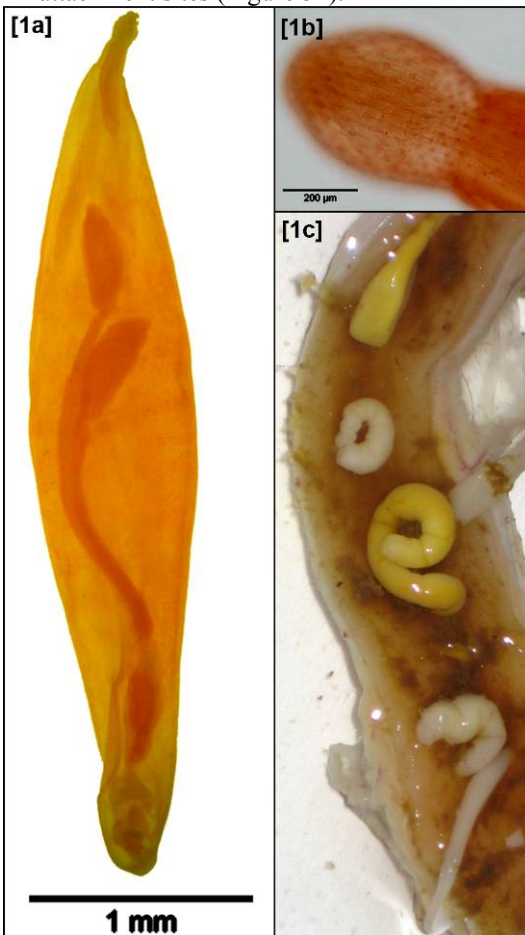
## II- Enzymes

### Acid phosphatase

Acid phosphatase activity in the control ileum was intensified in the epithelial cells lining the villi and crypts (Figures 5c, e). In infected ileum, the intestinal epithelium exhibited a detectable increase in acid phosphatase activity in both villi and crypts (Figures.5d, f). Strong reaction was noticed at the worm attachment sites (Figure 5d).

### Alkaline phosphatase

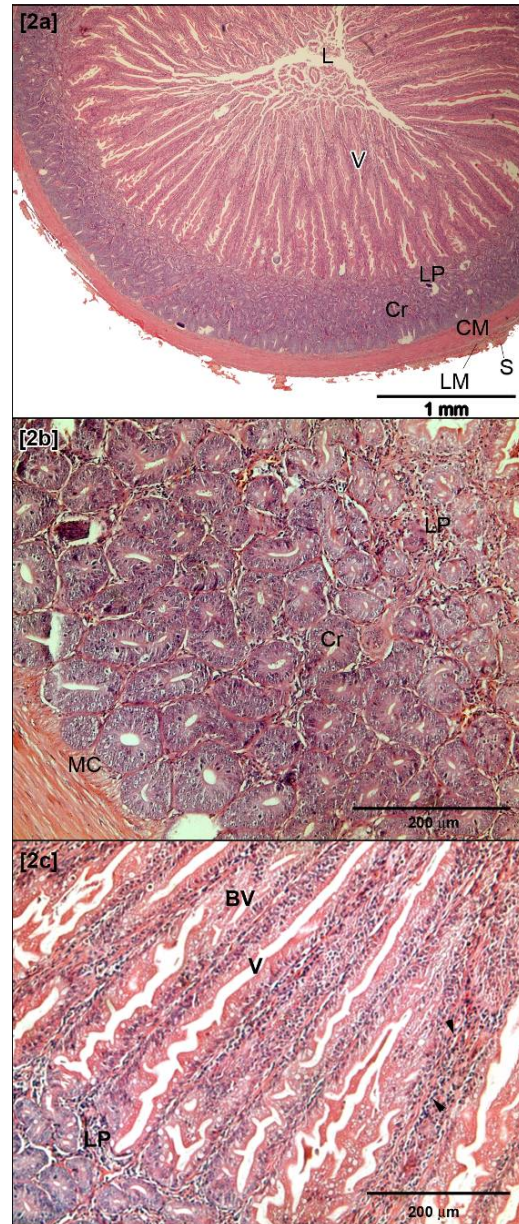
Alkaline phosphatase was detected in the intensely stained border of the intestinal epithelium of the villi and crypts (Figures 5g, i). In infected sections there was moderate decrease in the enzyme activity in the villi and detectable decline in the crypts cells (Figures 5 h, j ). No reaction was detected around the worm attachment sites (Figure 5h).



**Figure 1:** Whole mount of adult male *S. picae* (Mayer's carmine stain).

**Figure 1b:** Enlarged heavily armed proboscis of *S. picae* (Mayer's carmine stain).

**Figure 1c:** Infected intestine of the hooded crow opened to show thickening at the site of *S. picae* attachment.

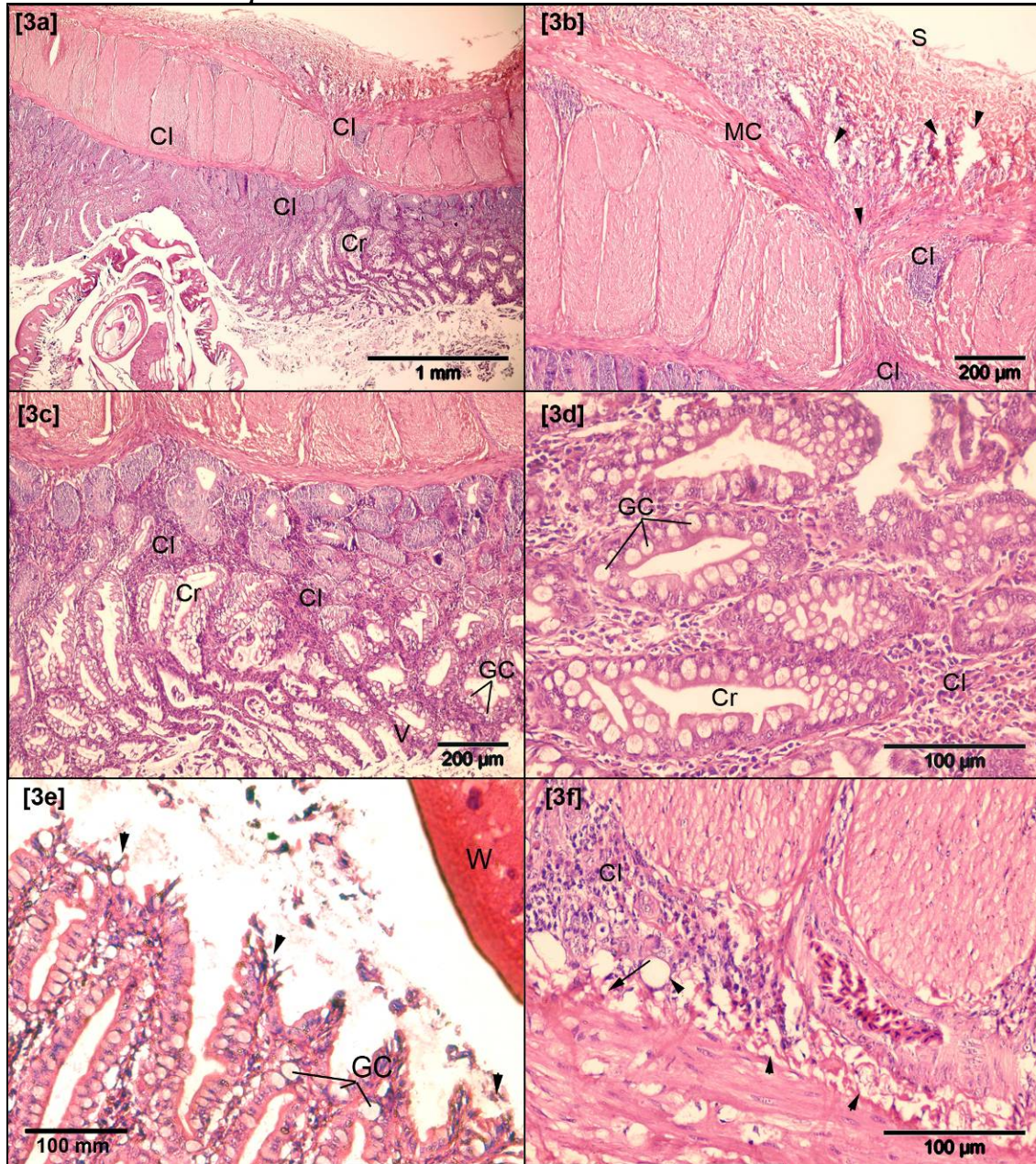


**Figure 2.** Light micrographs of histological sections through the ileum wall of hooded crow (hematoxylin and eosin preparation)

**Figure 2 a:** Transverse section of uninfected ileum to show normal features; outer serosa (S), muscularis externa with outer longitudinal muscle fiber (LM) and thick inner circular muscle fiber (CM), cross sections of intestinal gland (crypts) (Cr) closely packed in demarcated lamina propria (LP), tall regularly arranged villi (V) and narrow lumen (L).

**Figures 2 b, c:** Magnified parts of the previous section to show strips of smooth muscle fibers; muscularis mucosa (MC) extended in-between the intestinal glands, tall regular intestinal villi (V) exhibiting a columnar lining epithelium with strait border and goblet cell (GC), and core of lamina propria with diffused lymphatic tissue (arrow head) and small blood vessels (BV).

**Figures (3-6): Light micrographs of histological sections through the ileum wall of hooded crow naturally infected with *S. picae***



**Figure 3a:** Longitudinal section exhibit invasion of the worm to the ileum wall causing noticeable destruction of its layers by *S. picae* preasoma. Note the blunting, shortening, and destruction of the villi, compression and erosion of epithelial cells (arrowheads), marked cellular infiltration (CI) and heamorrage (H) in the stromal connective tissue.

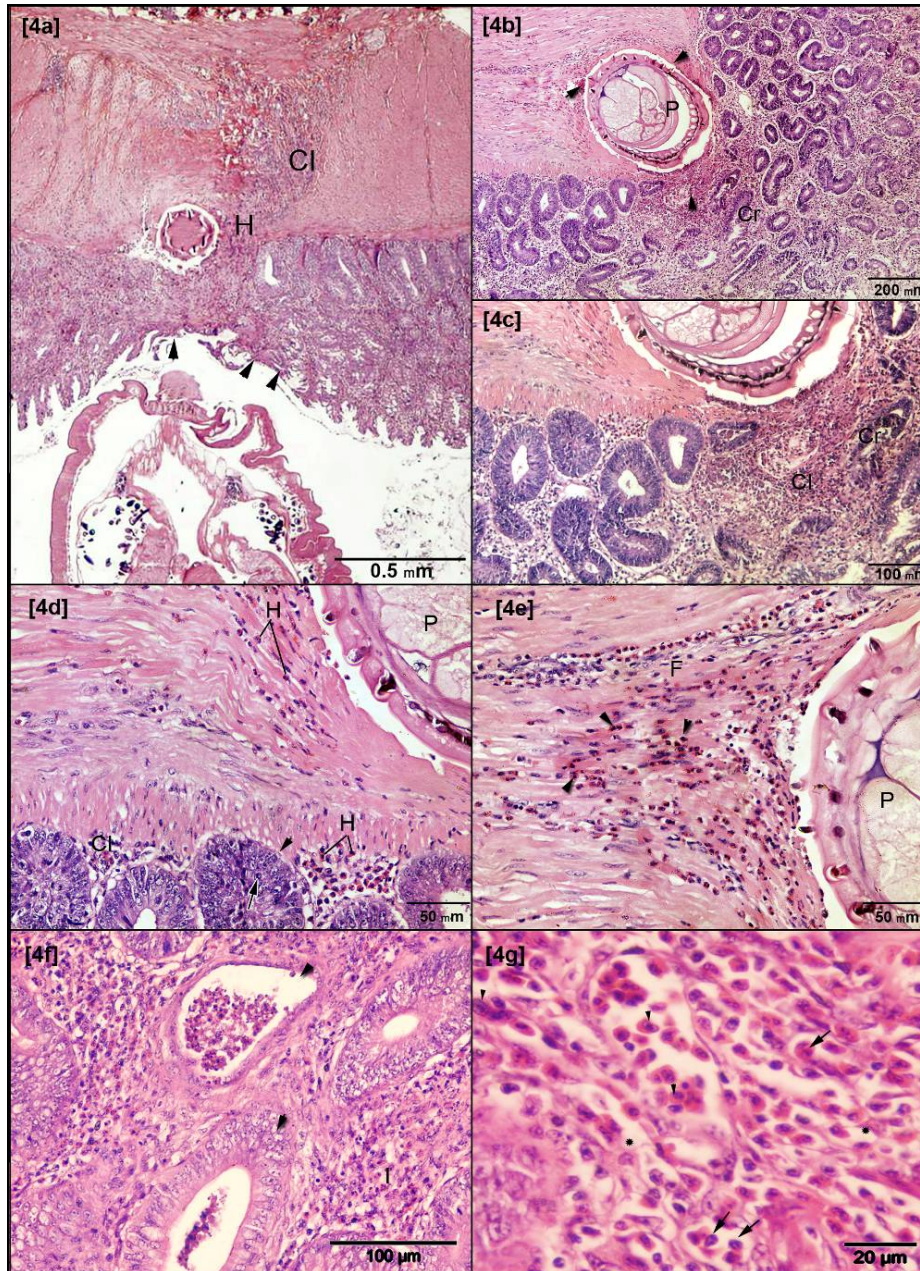
**Figure 3b:** Magnified parts of figure 4a showing the muscularis mucosa (MM) and serosa (S) layers with destruction, thickening and vacuolation of stromal connective tissue (arrowheads), and marked cellular infelttration (CI).

**Figure 3c:** Magnified parts figure 4a showing the mucosal layers with disorganization and destruction of both villi (V) and crypts (Cr), notable cellular infelttration (CI).

**Figure 3d:** High magnification of intestinal glands (crypts) (Cr) showing the increase in number of goblet cells (GC) and remarkable cellular infiltration (CI) between the crypts.

**Figure 3e:** High magnification of intestinal villi showing destruction of villi with erosion of villous epithelium (arrowheads) facing the integument of the worm (W) and increase of the goblet cells (GC) that open into the intervillous spaces.

**Figure 3f:** Magnified parts figure 4a showing dilation and congestion of blood vessels (arrow) and vacuolization and destruction in stromal connective tissue between muscle bundles (arrowheads).



**Figure 4a:** Longitudinal section shows that deep penetration of the worm to reach the muscular layers is accompanied by destruction and blunting of the villi and crypts (arrowheads) and full thickening of the mucosa and submucosa with cellular infiltration (CI) and hemorrhage (H) surrounding the penetration site which encloses poorly differentiated lymphocytes.

**Figure 4b:** Magnified parts of figure 4a shows the destructed intestinal glands (crypts) (Cr), villus (V) atrophy and crypts hyperplasia (arrows). Note that the thickness of mucosa and submucosa surrounding the worm is replaced by poorly differentiated lymphocytes (arrowheads)

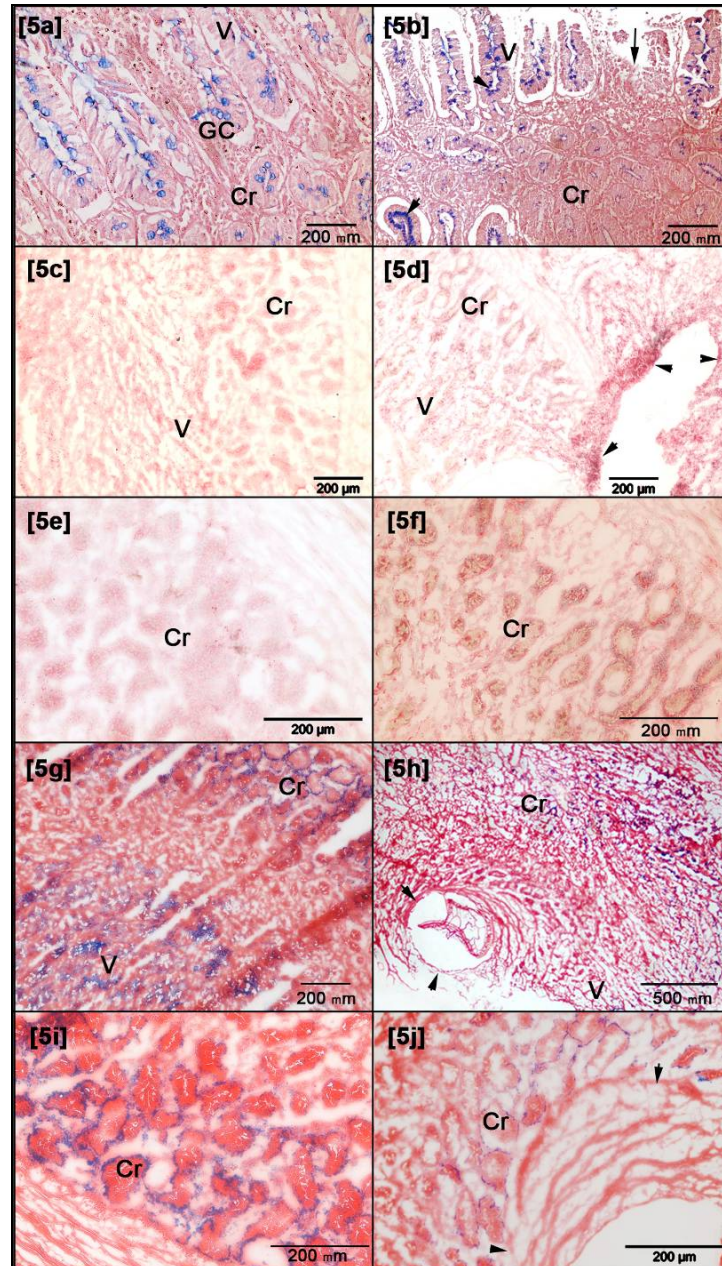
**Figure 4b, c:** Magnified parts of figure 4a shows high stromal cellular infiltration (arrowheads) around the proboscis (P) and destruction of crypts (Cr) towards the invasive proboscis.

**Figure 4d:** High magnified part of figure 4a exhibits hemorrhagic reaction (H) in muscle bundles and at the base of crypts surrounding the proboscis (P). Note that the epithelial cells of intestinal glands exhibit crypts hyperplasia (arrows) and hyperchromatic nuclei and mitotic activity (arrowheads).

**Figure 4e:** High magnified part of figure 4a exhibits noticeable fibrosis (F) around the worm proboscis (P), active fibroblasts (arrowheads) and dense fibrous stroma (arrows).

**Figure 4f:** Another magnified part of intestinal mucosa to show crypts hyperplasia (arrows), dilated and congested blood vessels (arrows) and stromal lymphocytic infiltration (I).

**Figure 4g:** Magnification of cellular infiltrated area surrounding the proboscis, aggregation of lymphocytes (arrowheads), eosinophil granulocytes (arrows) and fibroblasts (star) at the site of inflammation.



**Figures 5a, b: Histochemical localization of acid mucopolysaccharides**

**Figure 5a:** Intestinal mucosa of uninfected crow exhibits normal distribution and activity of mucus secreting goblet cells (GC) in both crypts (Cr) and villi (V).

**Figure 5b:** Intestinal mucosa of infected crow show decrease in number of mucus secreting goblet cells (GC) in the site of worm attachment in both destructive atrophied crypts and villi (arrows), while those nearby the worm site show high active goblet cells (arrowheads)

**Figures 5c-f: Histochemical localization of acid phosphatase:**

**Figures 5c and 5 e:** Acid phosphatase reaction in uninfected intestine presenting moderate activity intensified in the epithelial cells lining the villi (V) and crypts (Cr).

**Figures 5d and 5 f:** Acid phosphatase reaction in infected intestine presenting detectable increase in activity mainly in crypts cells (Cr), and epithelial lining of the villi (V). Strong reaction is noticed in the proboscis attachment sites (arrowheads).

**Figures 5g-j: Histochemical localization of alkaline phosphatase**

**Figures 5g and 5i:** Alkaline phosphatase reaction in uninfected intestine presenting strong activity in the strained border of the epithelium of the villi (V) and crypts cells (Cr).

**Figures 5h and 5j:** Alkaline phosphatase reaction in infected intestine presenting moderate decrease in enzyme activity in the villi (V) and detectable decline in the crypt cells (Cr). Negative reaction is noticed in the proboscis attachment sites (arrowheads).

#### 4. DISCUSSION:

**Persson (1974) and Galaktionov and Bustnes (1996)** proposed that the pathogenic effects of helminths on birds might be manifested as a reduction in their populations. **Sala and Martorelli (2007)** referred to many underlying processes, including, food resource shortage, environmental contaminants, inclement weather, infectious diseases, sibling competition, and internal parasitism which may interact together and lead to render a host more susceptible to the effects of parasitism.

Acanthocephalans have been reported in recurrent mortality events in many birds such as the common eider (**Camphuysen et al., 2002**), mute swan (**Sanford, 1978**), Olrog's Gulls (**Sala and Martorelli., 2007**) and ducklings (**Hollme'n et al., 1999**). **Taraschewski, 2000** and **Sanil et al., 2010** observed that the histopathological changes due to acanthocephalan infections depend on various factors such as species of parasite and host, nature of the infected tissues and host-parasite interactions. The nature and thickness of the various host tissue layers, length of the neck and proboscis, presence or absence of a proboscis bulb and the nature of spination in the acanthocephals also affect the pathological outcome.

Although the present observations revealed that *S. pica* is injurious to the hooded crow's ileum tissue and create histological alternations, bird with unusually moderate parasite load (2-5 worm/cm<sup>2</sup>) appeared healthy without any clinical manifestations. In agreement with **Taraschewski (2000)**, the probosces of penetrated worms appeared more or less evaginated. The author reported that the proboscis of the adult would not completely evert during the penetration of host tissues.

The penetration of the intestinal wall seems to be both mechanic and chemical. The advancement of the large-sized *S. picae* with well armed proboscis through the wall of intestine may be due to partial evagination and invagination of the praesoma in combination with contractions of the metasoma. The degree of penetration depends on the probosces orientation relative to host intestinal wall. If the proboscis is directed transversally to the mucosal layer, shallow penetration will happen, and the pathological effect will be limited in the mucosal and submucosal layers. This action may induce blunting, destruction and fusion of the intestinal villi and glands (crypts), compression and erosion of their columnar epithelium apposed the penetrated worm, as well as an increase in the number of goblet cells in both villi and crypt epithelium. In this case the inflammatory infiltration will poorly develop especially in muscular and stromal connective tissue layers. On the other hand if the proboscis is directed obliquely, the penetration will be deeper reaching the muscular layer where the host inflammatory reaction will be more pronounced

and associated with hemorrhage and extensive cellular infiltration including aggregation of lymphocytes, presence of numerous eosinophilic granulocytes and the appearance of fibroblasts. This inflammatory response may be either induced directly by *S. picae*, or indirectly by bacteria and other gut pathogens introduced the damaged intestinal wall. This reaction did not appear to damage the worm, where the inflammatory cells were not seen within the integument of *S. picae*. This observation is corroborated by the findings of **Krasnoshchekov and Lisitsyna (2009)** on the cystacanth of *S. picae* in the tissue of its paratenic host; *Lacerta agilis* (**Linnaeus, 1758**).

According to **Schelhaas (1980)** and **Cortan et al.(1999)**, these tissue changes may represent building up of cell mediated immunity to the causative agent, and in the initial stage, the neutrophils and macrophages aggregate at the site of infections. The authors also suggested that macrophage engulf the necrotic tissue and dead cells, and such development of cell mediated immunity, lead to the generation of specifically sensitized lymphocytes. **Nickol, 2006** explained that the parasite which induces fibrosis in the intestinal wall along with the associated biochemical reactions will induce loss of gut motility. Furthermore, **Sanil et al. (2010)** referred to reduction of the absorptive area available for the digestive and absorptive functions of the animal due to damage of the intestinal folds.

The present investigation revealed that dilation and congestion of blood vessels and stromal lymphocytic infiltration often emerge at considerable distance from the worm praesoma. This agrees with the finding of **Thurston et al. (1998)**, who explained such change as a sign associated the inflammatory reactions and infiltration in this layer. In addition, the pronounced thickening of the ileum surface around the site of attachment could be due to the detectable hyperplasia in the crypts. This finding agrees with the study of **Sanil et al. (2010)** on the intestinal wall of red snapper infected with *Tenuiproboscis* sp.

The present findings are corroborated by the report of **Amin et al. (2010)** on the same species infecting the intestine of *Pica pica* Linnaeus, 1758 (Magpie). The author referred to the possibility of the acanthocephalan to migrate through the musculature into the abdominal cavity causing more destruction to the penetrated layers.

**Krasnoshchekov and Lisitsyna (2009)** suggested that such mechanical injuries would be purulent but does not produce inflammation associated with eosinophils and neutrophils formation. On the other hand, there are evidences in literatures on the modifying effect of the parasite, in particular, on production of substances inducing eosinophilic taxis



and neutrophilic migration (**Linghtowlers, Rickard, 1988 and Taraschewski, 2000**).

In the present study, the erosion of the host intestinal epithelium towards the worm proboscis may be also a result of proteinases secretion through the pores of channels in the acanthocephalan tegument. Some acanthocephalans (*Pomphorhynchus laevis*, **Müller, 1776**) have been reported to secrete trypsin-like proteinases that were necessary for the complete and quick perforation of the fishes' intestinal wall (**Polzer and Taraschewski, 1994**).

Helminth infections are typically associated with considerable goblet cells hyperplasia (**Artis et al., 2004**) which is related to intestinal protection and worm expulsion (**Amin, 1998**). As expected, a detectable increase of goblet cells in the intestinal epithelium was recorded near the attachment sites. In support of the role of goblet cell derived mucus in worm expulsion, *in vitro* experiments have demonstrated that increased intestinal mucus viscosity at sites surrounding *Nippostrongylus brasiliensis* inhibits worm movement (**Ishikawa et al., 1994**). Moreover, isolation of the goblet cell secreted protein RELM $\beta$ /FIZZ2 and its incubation *in vitro* with parasitic nematodes, resulted in impaired chemotactic function in the worm (**Anthony et al., 2007**).

To the best of our knowledge, the present study is the first to detect the enzymatic histochemical changes in the bird's parasitized intestine with adult acanthocephalan. The results showed an increase in the level of acid phosphatase in the ileum of infected birds which may reflect a muscular damage and cellular infiltration at the penetration sites. Acid phosphatase is a lysosomal enzyme that play a vital role in the physiology of the intestine. The increase of the activity of this enzyme may be related to autolysis of any foreign substances and microbial agents. Similar observations have been reported in the intestine of birds and rodents with a trematode infection (**Bassiouni et al., 1985 and Abo-Shafey, 1992**).

Regarding to the role of alkaline phosphatase in transport of glucose 6- phosphate from the intestinal lumen, the moderate decrease in the level of this enzyme in the infected ileum, especially in the villi, may be explained by the destruction of the intestinal epithelium and altering of its absorption power. Similar observation has been detected by **Boulus et al. (1981), Hamdy and Saleh (1983) and Abo-Shafey et al. (1992)** on the small intestine of mice had a trematode infection. Otherwise **Matta (1980)** reported that the alkaline phosphatase activity of *Ascaridia galli* infected intestine was found to increase around the parasite and at the sites of the damaged tissues.

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3/2/3012

## Awareness and use of agricultural market information among small scale farmers in Ngaka Modiri Molema District of North West Province

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**Abstract :** The study determined awareness and use of agricultural market information among small scale farmers in the Ngaka Modiri Molema District in the North West Province. Simple random sampling techniques was used to select 120 farmers from the population of study and data were collected through the use of structured questionnaire and subjected to analysis using frequencies, percentage and multiple regression analysis. The results of the study show that majority of farmers were between the age category of 51-60 years, married (58.8%); Christians (80.7%), males (56.3%), married (58.8%) and had studied up to high school level (47.1%). The results also shows that agricultural market information provides farmers with knowledge of the prices of the produce, provides knowledge of who to buy the produce, the quantity to be produce and knowledge of different outlets such as fruit and vegetables, respectively with 67.2% and 66.4%. Farmers have indicated that they are aware of agricultural market information and they use radio with 68.9% and newspaper with 68.1% respectively. Significant determinant of use of agricultural market information were knowledge ( $t= 6.464$ ) and awareness ( $t= 6.963$ ).

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**Keywords:** agricultural market information, small scale farmers South Africa, information sources

### Introduction

Agriculture is considered critical to social, economic growth and poverty reduction and has been described as the backbone of the economies of most African countries and the main economic base for small-scale farmers in Africa. Empirical evidence from various country case studies conducted in Africa indicated that pro-growth and pro-poor performance of agriculture will continue to depend on the broad participation of smallholder farmers, and that food staple growth generates more to poverty reduction than other agricultural subsector (Diao et al. 2007:). About 2/3 of developing countries depend on agriculture for their livelihood and 75% of these farmers are small-scale farmers (Bunders and Broerse 1991). About 3/4 of Africa's population is found in rural areas and 60-90% of the total labour force is employed in the agricultural sector. According to the Economic Commission for Africa and the African Union, agriculture employs about 70 per cent of the work force and generates on average 30 per cent of Africa's GDP (ECA and African Union 2007). In sub-Saharan Africa, small-scale farmers constitute about 73% of the rural poor. Agriculture is linked to food security and will remain a primary source of growth and means of poverty reduction and backbone of rural economy in Africa (IFAD 2001). The agricultural sector could also be the main contributor to poverty reduction (ECA and African Union 2007). Diao et al. (2007) posited that linkages between agriculture to the rest of the

economy can generate employment and intensive patterns of development. However, the ability of agriculture to reduce poverty and generate growth varies across and within countries. Africa has battled with food insecurity and agricultural production for a long time. It is noteworthy that although poverty levels have fallen globally, Sub-Saharan Africa is the only developing region where there has been a decline in per capita food-grain output and where the number of people living below poverty line has doubled over four decades. About 46% of the population lives on less than a dollar a day (UNDP 2005; ECA 2006). Less than 6% of Africa's arable and permanent crop land is irrigated (ECA 2005) and most of the land on agriculture is not arable.

World Bank (2006), states that Africa needs to make agriculture more productive and sustainable, connect poor people to markets, enhance human development, get services to poor populations (especially women) and use natural resource assets well if the MDGs are to be achieved. There are a number of initiatives at the continental, regional, sub-regional, national and local levels working towards increasing agricultural productivity in Africa. All these indicators make initiatives on ICTs and small-scale agriculture in Africa urgent if the set milestones are to be achieved. All agricultural business activities involved in the movement of commodities from production to consumption is marketing (Batcheller, 2005). This means that the farmers' market information needs are those that enable them

make rational and relevant decisions. Market information is vital to market participation behaviour of small-scale farmers. Availability of market information boosts confidence of households who are willing to market their produce. In other words, market information allows farmers to take informed decisions. Thus, farmers who are more informed are more likely to participate in marketing. Also of equal importance is the source of market information because it determines accuracy of the information (Cook, 2003).

Information is an essential ingredient in agricultural development programmes but small-scale farmers seldom feel the impact of agricultural innovations either because they have no access to such vital information or because it is poorly disseminated (Zhang, 2000). In agriculture, ICTs are being used for accessing agricultural information, financial information, market information, surveys and agri-business (May et al, 2007). Maru (2004) pointed out that the use of ICTs is ubiquitous in national agricultural research systems in Sub-Saharan Africa, while Grimshaw (2005) observed that there was consensus that ICTs play an important role in development by linking users to up-to-date information, skills and markets. In Uganda, ICTs are used to disseminate local agricultural information and knowledge to small-scale farmers (Akiiki, 2006) while in Senegal, women are using tele-centres (linked to the internet via mobile phones) to access market prices (May et al, 2007). The literature reveals that in many instances information provision in agricultural science is exclusively focused on policy makers, researchers, and those who manage policy decisions with scant attention paid to the information needs of the practising small-scale farmers who are supposed to be beneficiaries of the policy decisions. A further neglect in the information provision is the concentration of information supply on production practices to the exclusion of the marketing information.

It is therefore clear that if the approaches to agricultural development programmes are to work, governmental and non-governmental organizations need to take new approaches to information dissemination and management that grow out from a clear understanding of what information needs of small-scale farmers. If provided with the right inputs, feasible technology and relevant information, small-scale farmers are capable of transforming traditional agriculture, which they normally adhere to if not provided with new information on the latest agricultural technology (Mabota et al, 2003). The main objective of the study is to identify and analyze the awareness and use of agricultural market information among small scale farmers in Ngaka

Modiri Molema District of North West Province. The specific objectives of the study were to determine the socio-economic characteristics, investigate the sources of information and determine awareness and use of sources of information. The study also explored the significant relationship between socio-economic characteristics and use of market information among small scale farmers

### Materials and Methods

The study was conducted in Ngaka Modiri Molema district in the Northwest Province. The main economic activity in the Ngaka Modiri Molema of the Northwest Province is Agriculture, mainly producing crops and cattle. Temperatures range from 17° to 31°C (62° to 88°F) in the summer and from 3° to 21°C (37° to 70°F) in the winter. Annual rainfall totals about 360 mm (about 14 in), with almost all of it falling during the summer months, between October and April. A descriptive research design was used to analyze awareness and use of agricultural market information by small scale farmers. The population of study is all small scale farmers in Ngaka Modiri Molema district. From the list of farmers obtained from North West Department of Agriculture, Conservation, Environment and Rural Development, simple random sampling technique was used to select 120 farmers representing five percent of the population. Data were collected through the use of structured questionnaire that consists of demographics, knowledge of agricultural market information and different channels of communication. Data collected were analyzed with statistical package for social science using frequencies, percentage and multiple regression analysis.

### Results

Table 1 presents personal characteristics of small scale farmers covered in this study. Table 2 indicates the knowledge and use of farmers on agricultural market information, Table 3 Determinants of use of agricultural marketing information and Figure 1 compared the Awareness and use of information sources on agricultural market.

### Discussion

In Table 1, the majority of farmers were between the age category of 51-60 years, married (58.8%); Christians (80.7%) and had studied up to high school level (47.1%). Mabe *et al.* (2010) who revealed that majority of the farmers in North-West Province had low educational level. Majority of the respondents were males (56.3%).

**Table 1: Personal characteristics of small-scale farmers**

Variables	Frequency	Percentage
<b>Age</b>		
<30	22	18.5
30-40	23	19.2
41-50	22	18.4
51-60	28	23.5
Above 60	15	12.6
<b>Gender</b>		
Male	67	56.3
Female	52	43.7
<b>Marital status</b>		
Single	40	33.6
Married	70	58.8
Divorced	8	6.7
<b>Religion</b>		
Christianity	96	80.7
Islam	4	3.4
<b>Education</b>		
Primary	27	22.7
High school	56	47.1
College	18	15.1
University	14	11.8
<b>Sources of land</b>		
Personal	90	75.5
Rented	20	16.8
<b>Organizational Membership</b>		
No	84	70.6
Yes	15	12.6
If yes name them	1	0.8
No	15	12.6
<b>extension contact</b>		
No	17	14.3
Yes	93	78.2
<b>labour sources</b>		
Self	63	52.9
Family	38	31.9
Hired	17	14.3
<b>Farm income</b>		
<10 000	59	39.1
10 000-20 000	24	20
Above 20 000	6	4.8
<b>Farming experience</b>		
<10 years	53	44.4
10-20 years	42	35.3
Above 20	24	20
<b>Household size</b>		
1-3	43	36.2
4-6	50	42
Above 6	9	7.5

The results suggest that farming is still dominated by males and 44.4% have less than 10 years of farming experience. Moloi (2008) who reported that despite the gains that have been made with respect to gender equality, the redistribution of resources and power has not shifted the structural forces. The gendered nature of the social, culture, economic and policy systems may have limited women farmers from participating in the study (Logwa et al., 2010). Table 1 further revealed that 58.8% of the farmers are married and having between 4-6 persons per households. Modise (2008) stated that large households may have an advantage to farm labor, and this might have a positive impact on the farm income. About 75% of the farmers are farming on personal land while 71% have organizational membership with farmers' groups and association within the study area. In terms of contact with extension services, about 78% of the farmer indicated contact with extension officers, while only 25% earn income more than R10,000. Schwalbach *et al.* (2001) revealed that majority of farmers earn low income per year from their farming activities.

Table 2 indicates the knowledge and use of farmers on agricultural market information, most farmers have knowledge of market information and it provides them with the quality that is demanded by the consumer. The results also shows that agricultural market information provides them with knowledge of the prices of the produce, provides knowledge of who to buy the produce, the quantity to be produce and knowledge of different outlets such as fruit and vegetables, respectively with 67.2% and 66.4%. Most farmers have proved that small scale farmers still have or know little access about the international markets as the results shows only 14.3% know on how to enter international markets. The results also show that small scale farmers are still not familiarized with the JSE SAFEX which helps the farmers with future prices and provide them with knowledge of what maize cultivar is mostly demanded by the international markets. This may be due to the environment in which these farmers dwell, on average most of these farmers are not exposed to internet and other sources of information also taking into consideration literacy level is another bottleneck. In terms of the use of agricultural market information, the prominent use of agricultural information are in terms of quality demanded (61.3%); time of production (57.1%); awareness of risks associated with production (60.5%) and knowledge of how to control farm gate buyers (57.1%).

**Table 2. Knowledge and use of agricultural market information**

Statements	Knowledge		Usage	
	Yes	No	Use	Non Use
Market information provide prices of produce	80(67.2)	39(32.8)	58(48.7)	61(51.3)
Market information provide buyers of produce	80(67.2)	39(32.8)	59(49.6)	60(50.4)
Market information provide location where the produce is demanded	82(68.9)	37(31.1)	62(52.1)	57(47.9)
Market information provide what quality is demanded	82(68.9)	37(31.1)	73(61.3)	46(38.7)
Market information provides knowledge of when to produce.	71(59.7)	48(40.3)	68(57.1)	51(42.8)
Market information provide knowledge of different outlets such as fruit and veg.	74(62.2)	45(37.9)	66(55.5)	53(44.6)
Market information provide what to produce	73(61.3)	46(38.7)	66(55.5)	53(44.5)
Market information provide and quantity to be produced	79(66.4)	40(33.6)	55(46.2)	64(53.8)
Market information provide knowledge of auctions	45(37.8)	73(61.4)	50(42.0)	69(58)
Market information provides knowledge of how to enter international markets.	17(14.3)	102(85.7)	42(35.3)	77(64.7)
Market information provide knowledge of JSE SAFEX	19(16.0)	100(84)	42(35.3)	77(64.7)
Market information provide knowledge of the seasonality of the produce	48(40.3)	71(59.6)	63(52.9)	56(47)
Market information provide awareness of risks	60(50.4)	59(49.6)	72(60.5)	47(39.5)
Market information provide knowledge of producing multiple different produce within different seasons.	56(47.1)	63(53)	63(52.9)	56(47.1)
Market information provide knowledge of middlemen activities	48(40.3)	71(59.7)	62(52.1)	57(47.9)
Market information provide knowledge of how to control farm gate buyers	53(44.5)	66(55.5)	68(57.1)	51(42.8)

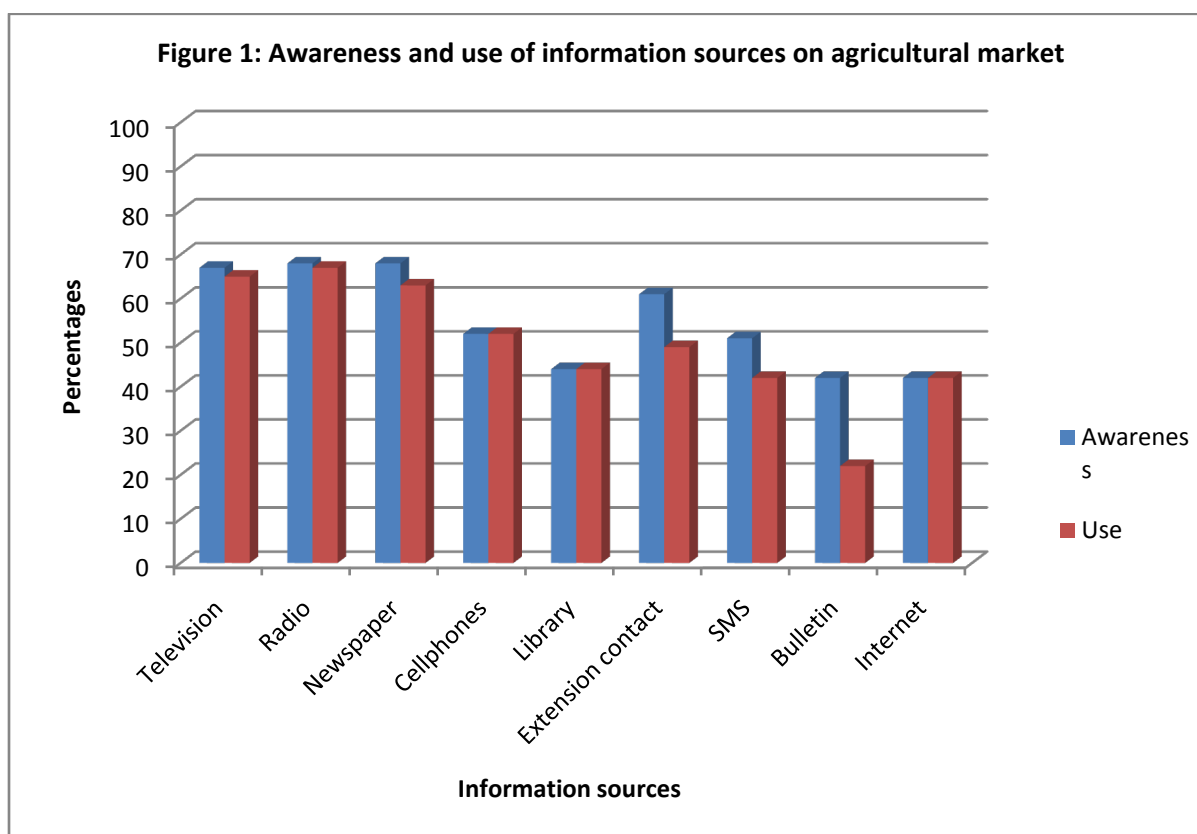
**Figure 1: Awareness and use of information sources on agricultural market**

Table 3. Determinants of use of agricultural marketing information.

	B	Std Error	Beta	T	Sig
Constant	-3.025	3.822		-.791	.431
Age	.064	.043	.130	1.488	.140
Gender	-.773	.921	-.052	-.839	.403
Marital status	.435	.730	.037	.596	.553
Religion	-1.296	1.098	-.075	-1.180	.241
Education	.224	.471	.030	.476	.635
Source of land	.566	.931	.038	.608	.545
Organizational member	1.428	.909	.106	1.571	.119
Contact with extension workers	.680	.788	.056	.863	.390
Labor sources	-.588	.656	.059	-.897	.372
Farm income	9.841E-6	.000	.011	.156	.876
Year of farming	-.093	.061	-.124	-1.531	.129
Household size	.343	.231	.113	1.487	.140
Farm size	.001	.004	.020	.294	.769
Knowledge	.544	.084	.429	6.464	<b>.000</b>
Awareness	.671	.096	.483	6.963	<b>.000</b>
F	2.009				
Sig	.024 <sup>a</sup>				
R	.461 <sup>a</sup>				
R Square	.231				

In Figure 1, farmers indicated that they are aware of agricultural market information and they use radio with 68.9% and newspaper with 68.1% respectively. Radio and newspaper are more likely to have provided high awareness due to low cost, as well as being an appropriate tools that fulfills the farmers' needs and it is because these sources are easily accessible at anytime and everywhere especially radio offers preference of different languages for different audiences. Previously, Akullo et al., (2007) found that radio programmes were the major ICT channels used by farmers to acquire agricultural information knowledge in Uganda. Farmers also prove that they are not aware of internet as their reliable source of information, this shows that rural small scale farmers are still backward with technology. This could be because In rural communities use of computer is rare not everyone has the knowledge on how to use computers, this brings the issue of low literacy level and some might know how to use these machines but another constraint could be the accessibility to them since internet café are not popular amongst rural communities.

The findings from the study revealed that a large number of farmers (67.5%) use radio as the most appropriate technology to access agricultural market information. Radio is one of the most widespread and popular tools of communication in Africa. Chapman et al. (2003) argue that the strength of radio as an extension tool is widely praised for its ability to reach illiterate farmers and provide them

with information related to all aspects of agricultural production in a language they understand. They further maintain that in an era of rapidly developing information and communication technologies, rural radio is a powerful mechanism for linking old and new technologies, providing information resources cheaply to those who need to improve their livelihoods, while at the same time strengthening existing resource of knowledge, enterprise and cultural identity. Furthermore, Okwu et al. (2007) also show that radio is one broadcast medium which almost all experts have found to be the most appropriate medium of mass communication in the rural population. He further maintains that radio is favored as a medium of communication in rural communities because of the advantage of demanding less intellectual effort than the print media messages and also because it is able to reach remote areas, even where there are no extension agents, as long as there is a good reception. Ozowa (2011) that majority of the farmers use radio as the most common information channel but agrees with him that the second most used channel is television. A study by Narula (2010) corroborates that information on commodity prices and agricultural markets is extremely important.

The results of multiple regression analysis of relationships between demographic characteristics and use of agricultural market information amongst small scale farmers in Table 3. The independent variables were significantly related to use of agricultural market information with an F value of

2.009. Also an R value of 0.461 showed that there was strong correlation between the independent variable and use of agricultural market information. Significant determinants were knowledge ( $t= 6.464$ ), awareness ( $t= 6.963$ ). These findings imply that as knowledge and awareness of agricultural market information among small scale farmers increases the use of such information also increase.

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## A review of selected theories and their applications to information seeking behavior and adoption of organic agricultural practices by farmers

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**Abstract:** This paper discusses the application of selected theories to explain information seeking behavior and adoption of organic agricultural practices by farmers. The paper begins with a brief description of these selected theories which are the theory of Planned Behavior, diffusion theory and innovation theory. Following the brief description, the application of each of these theories to information seeking behavior and adoption of organic agricultural practices were discussed. Therefore, the paper recommends that in order to ensure adequate dissemination and the adoption of organic agricultural practices, extension officers, change agents, NGOs and the policy makers should adopt the principles in each of these theories to enhance farmers acceptance and implementation on the innovation.

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**Key words:** Theories, application, information seeking, adoption, organic, farmers

### Introduction

Van Es, (1984) reported farmers as voluntaristic decision makers i.e farmers have virtually full control over the decision to invest in agricultural technology. Lynne et al, (1995) observed that persuasion to encourage changes in beliefs and evaluations may also be the only way to technology compliance. Farmers may need to perceive at least some control in order for them to move forward with technology decisions: with more (internal) control, farmers are more likely to take action, and to invest more intensely. This paper examines selected theories dealing with human behavior and technological innovation in agriculture, reviewing the attributes and principles of each of them for adoption of innovation. The main theories examined are theory of planned behavior, innovation theory and diffusion theory. All of which are associated with technological change. This paper has significant policy implications, especially for advocacy and adoption of organic agricultural practices by farmers for sustainable agricultural development.

Theory of Planned Behavior (ToPB): The Theory of Planned Behavior (ToPB) (Ajzen, 1985) is a general theory of every kind of social behavior. It encompasses three theoretical constructs (Figure 1), which influence the intention to perform a given behaviour, viz. the attitude towards behaviour, the subjective norm, and the perceived behavioural control. These constructs are formed by three different kinds of beliefs, namely consequence beliefs, normative beliefs and control beliefs. Consequence beliefs influence the attitudes towards the behavior. These attitudes are subjective

evaluations of the consequences of performing the given behaviour. Normative beliefs cause the subjective norms with regard to the given behaviour. The subjective norm shows the perceived social pressure to perform the behaviour. Control beliefs, in comparison, form the perceived behavioural control. Perceived behavioural control contains the subjective assessment about a person's ability to control the behaviour in question.

The more favourable the attitude toward a given behaviour and the subjective norm, and the greater the perceived behavioural control, the stronger should be the person's intention to perform the behaviour in question. Once an intention is formed, people are expected to carry out their intentions when the opportunity arises. After performing a behaviour people can revise and change their beliefs, because personal experience is seen as one of the important factors for changing attitudes. Therefore, there is a feedback between the performance of the behaviour and the three different kinds of beliefs. When beliefs are changed, a change in attitude, subjective norm and perceived behavioral control will also follow. The foundation of the ToPB is the subjective expected utility theory (SEU) and, like the main assumption of the SEU, persons are assumed to behave in a rational way. It means that persons are systematic information processors and they behave in accordance with their subjective expected or perceived utility (Fishbein and Ajzen, 1975). According to Bamberg and Schmidt (1993), ToPB is one of the prominent theories in the social psychology domain. This theory has proved to be useful in explaining many cases such as recycling

behaviour (Bamberg and Lüdemann, 1996), choice of public transport (Bamberg and Schmidt, 1997), use of tobacco and alcohol (Higgins and Conner, 2003),

blood donation behaviour (Giles and Cairns, 1995) and exercise behaviour (Arnscheid and Schomers, 1996).

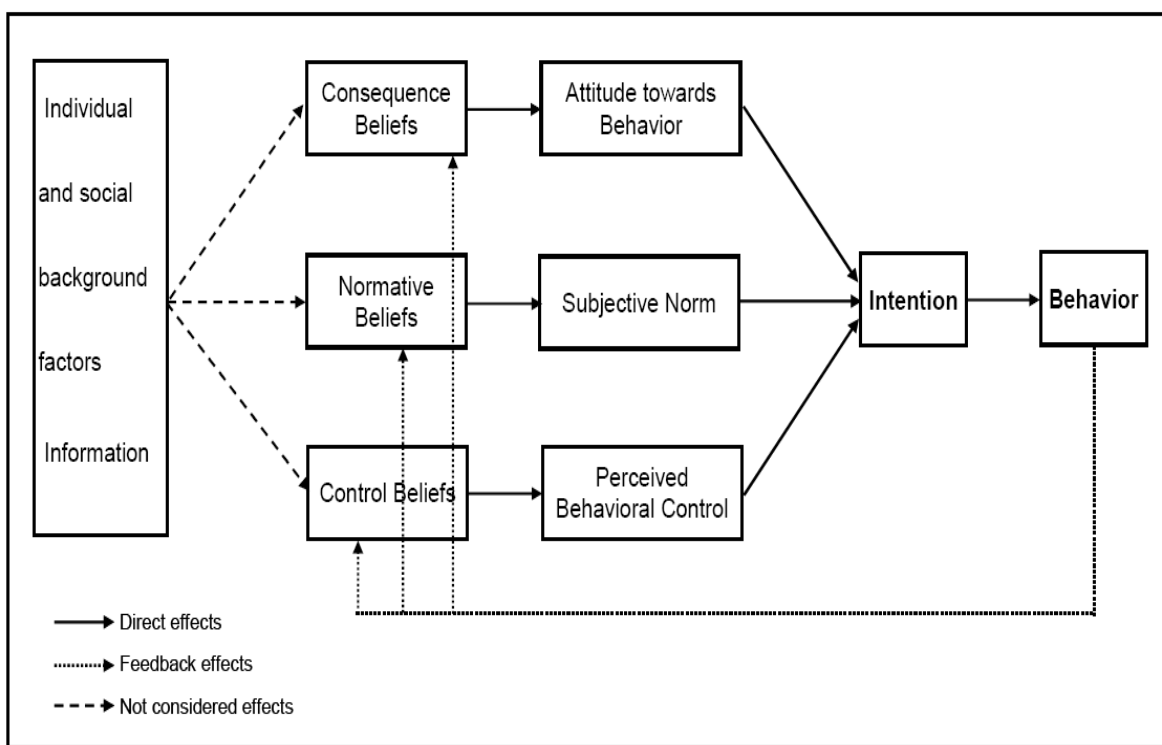


Figure 1: Theory of Planned Behaviour (ToPB), Source: Adapted from Ajzen and Fishbein, 2005

### Application of ToPB to Information Seeking Behaviour and Adoption of Organic Agriculture Practices among farmers.

This theory is related to farmers' information seeking behavior and adoption of organic farming in the sense that adoption of organic farming depends on the attitude and the knowledge that farmers have about organic farming and it is only through relevant information that knowledge can be built up. According to this theory the more favourable the attitude toward a given behaviour and the subjective norm and the greater the perceived behavioural control, the stronger should be the person's intention to perform the behaviour in question. This implies that favourable attitude of farmers towards organic agriculture will enhance its adoption. After adoption, and farmers realize a better result, the farmers can revise and change their beliefs about organic agriculture based on their experience.

### Diffusion Theory (DT)

The Diffusion Theory is used as another theoretical background to extend the ToPB. The Diffusion Theory has been mainly developed to explain the farmers' adoption of innovations

(Leeuwis, 2004). The adoption of an innovation is seen as a process and follows five main phases (Rogers, 1995, 2003):

1) Knowledge about the innovation, to become aware of the innovation; in this phase, mass media plays an important role as a source of information;

2) Persuasion, evaluation of the attributes of an innovation, i.e. formation of attitudes regarding the innovation, comparing its advantages and disadvantages; and friends and neighbours are the most important sources of information at this stage;

3) Decision to adopt the innovation or not; this stage is described as an active information seeking and processing phase, the aim is to reduce uncertainty about the advantages and disadvantages of the innovation; important sources of information again are friends and neighbours;

4) Implementation of the innovation; sometimes an adaptation of the innovation to the own farm environment may be needed and personal experience is very important at this stage of the adoption process;

5) Confirmation, i.e. the individual seeks reinforcement for the innovation-decision already made.

### **Application of the Diffusion theory to information seeking behaviour and adoption of organic farming practices among farmers**

According to Diffusion theory, adoption of an innovation is seen as a process and follows five main phases. This theory can be used to explain the information seeking behavior and adoption of organic practices among farmers. This is because farmers can either adopt organic agriculture fully or partially. The relationship is explained as follows:

**Knowledge:** Farmers can only adopt what they are aware of, besides, they can only have the knowledge by obtaining relevant information through appropriate source. Therefore, adoption of organic farming practices can be enhanced when the farmers are aware of the innovation through appropriate channels. Mass media being one of the sources of information plays an important role at this stage because it helps to stimulate farmers to wanting to know more in detail about organic agriculture.

**Persuasion;** At this phase of adoption, farmers will adopt organic agriculture fully, partially or will not adopt at all depending on his attitude regards the innovation and having compared the advantages and disadvantages. Friends and neighbours are the most important information sources at this stage.

**Decision:** At this information seeking and processing phase, farmers will want to obtain more concrete information before adopting the practice of organic agriculture. Friends and neighbours are sources of information and they have greater influence on the decision of the farmers either positively or negatively. This is because they are able to persuade and convince the farmers whether to adopt or not to adopt.

**Implementation:** Farmers may want to try these organic agriculture practices on a small portion of his farm i.e. an adaptation of the innovation. This will enable him to have a personal experience of the practice before he adopts the innovation fully.

**Confirmation:** Having tried the innovation and seen the result, the farmers will not only adopt the innovation but also seek reinforcement for the innovation decision already made.

### **Innovation Theory**

According to the Innovation Theory (Albrecht, 1992; Rogers, 2003; van den Ban and Hawkins, 1996), the adoption of an innovation depends on the attributes of the innovation, social norms and communication channels which are used as information sources to reduce uncertainty about the innovation. The attributes of the innovation are the relative advantages, the compatibility, the complexity, the trialability and the observability.

Social norms are established behaviour patterns within a social system. Not to behave like the norms will cause some kind of consequences. Uncertainty about an innovation exists because not all persons have the same information or understanding of the innovation. Information sought through different communication channels can reduce uncertainty. Mass media channels are relatively more effective in creating general knowledge about the innovation and can therefore reduce uncertainty. However, interpersonal channels are relatively more effective in forming and changing attitudes toward the innovation and thus influence the decision to adopt or reject the innovation.

### **Application of the innovation theory to information seeking behaviour and adoption of organic agriculture practices among farmers**

This theory also relates to information seeking behaviour and adoption of organic farming practices because farmers will adopt an innovation when they know the relative advantages, compatibility, complexity, triability etc., particularly when the relative advantages are more than those of the practices they have been using. Social norms within a social system can affect the adoption of organic farming practices particularly when the elders in a community are not convinced about the new innovation. The whole community may end up rejecting the innovation as a result of their strong influence on the entire community. Communication channels used to get the people informed about the organic farming practices will determine whether they will reject or adopt. This is because mass media channels create general knowledge, however, interpersonal channels are relatively more effective in forming and changing attitudes and influence the decision to adopt or reject the innovation. The paper has revealed the application of theory of planned behavior, diffusion and adoption of organic agricultural practices by the farmers. It is therefore recommended that extension officers, change agents, NGOs and the policy makers should adopt the principles in each of these theories to enhance farmers' acceptance and implementation of the innovation.

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**Growth and mineral constituents of proso millet (*Pennisetum glaucum*) irrigated with sea water**

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**Abstract:** The effect of different Sea water salinity levels (0, 25, 50, 75 and 100%) on seed germination, seedling growth and mineral ion concentration (K, Ca, Mg, Na, Cl, Fe, Cu, Mn and Mo) of proso millet (*Pennisetum glaucum*) were studied. Germination of proso millet seed was affected by high salinity levels, the germination percentages were 97, 84, 80, 77 and 57 % for the treatments used 0, 25, 50, 75 and 100% sea water, respectively. Plant growth was also affected by salinity; shoot lengths were more pronounced than on root and leaf. The shoot, root and leaf lengths reached their maximum at 50% seawater salinity before they encounter reduction with increasing salinity. The shoot length was reduced 7.2% at 50% and 24.6% at 100% seawater salinity, root length increased 13.9% at 50% and reduced about 4.6% at 100%, while leaf length was only affected by salinity at 100% seawater salinity compared to plants irrigated with freshwater (control). Seawater salinity had a significant effect on mineral ion concentration in proso millet plants. Concentration of K, Ca, Mg and Fe was reduced in the shoot with increasing salinity levels to 39.9- 83.1%, 49.0-92.2%, 9.9-13.8%, 10.2-33.0%, while Na, Cl, Mn and Mo concentration showed increase in concentration with increasing seawater salinity to 62.3-58.8%, 337.5-4.4%, 0-80%, 22.1-8.1% at 50% and 100% respectively. Also in the root, the concentrations of K, Ca, Mg, Fe, Cl, were significantly reduced to values of 56.3-895.3%, 59.4-95.8%, 0-58.5%, 18.5-13.6%, 5.6-85.2%, and that of Na, Mn and Mo increased to values of 64-64%, 25.7-46.6%, 92.7-117.6% at 50% and 100% increase in seawater salinity respectively.

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**Key words:** Salinity; Seawater; growth; Chemical Constituents; Proso millet

**1. Introduction**

Although saline soils occur in humid regions in areas affected by sea water, the most extensive occurrence is in arid and semi arid regions, where rainfall is not sufficient to wash and transport salts away from the plant root zone. In such saline regions, cultivation of crop plants could mainly be achieved either after washing of excess salts by repeated flooding with fresh water or by introducing plants adapted to such saline conditions (Heikal *et al.*, 1982; ajar *et al.*, 1993 and Howladar, 2010). Since sufficient amounts of fresh water are not always available the second alternative seems to be more applicable.

The ionic composition of seawater is dominated by Na and Cl ions; it also contains in abundance ions essential to plant growth, i.e. K, Mg, Ca, SO<sub>4</sub>; and it is buffered towards alkalinity (pH 8.5) and also usually contains trace elements (Fe, Mn, Zn...) and organic matter: the latter contains a certain amount of the total nitrogen where nitrogen fixation in the saline soil is at a low level (Al-Zahrani, 1990). The presence of the ions has consequences, however, for the free energy of water. As the salt concentration increases in any solution, the water becomes less and less accessible to plants (Harvey, 1966); therefore, a plant must produce an osmotic potential lower than that of the soil solution to take up water to its tissues (Larcher, 1980).

The plant adaptation to salinity started from seed germination. The germination ability of seeds under

salinity condition was recorded to be sometimes suppressed under salinity condition (Khan and Panda, 2002). Extensive studies have been made concerning the effect of water stress on seed germination using different crop species and different osmotic substances (Munns and Tester, 2008; Maiti *et al.*, 2010 and Yakubu *et al.*, 2010). Generally it was recorded that salinity stress may slow down the rate of germination or may completely inhibit it (Heikal and Shaded, 1982). Salinity may also affect concentration of mineral nutrients in plants. Salinity disrupts mineral nutrient acquisition by plants in two ways, the ionic strength of the substrate can influence nutrient uptake and translocation, and by reduction of nutrient availability by competition by major ions (i.e. Na<sup>+</sup> and Cl<sup>-</sup>) in the substrate (Chartzoulakis *et al.*, 2002).

A considerable number of studies were conducted to investigate the effects of salinity on growth of plants. Morphologically the most typical symptoms of saline injury to plants are stunted growth (Parida and Das, 2010). A reduction in growth was also recorded by number of researchers. Abdel Azim and Ahmed (2009) indicated that salinity concentration of 4000 ppm significantly depressed plant height fresh and dry weights, plant fresh and dry yield /fed, crude protein, total ash, proline, phenol, essential oil and potassium percentage of *Achillea fragrastissima*. Mozafar and Oertil (1990) recorded that increasing of NaCl in the nutrient solution increased the concentration of Na, Cl, total P, PO<sub>4</sub> and Zn and reduced the concentration of

K, Ca, total N, NO<sub>3</sub> and SO<sub>4</sub>, but did not affect the concentration of total S in the barley tops. Also (Carmer *et al.*, 1985, Lynch and Lauchli, 1988) reported that NaCl salinity displace Ca from the membranes and make them leakier for Rb and Ca but Zn concentration increased. Koyro (2006) demonstrated a high concentration of Ca ions in the root of halophyte *Plantago coronopus* (L). Salinity may disrupt nutrient acquisition by plants by reduction in nutrient availability by competition with major ions (i.e. Na<sup>+</sup> and Cl<sup>-</sup>) in the substrate (Flowers and Colmer, 2008).

The aim of the present study is to investigate the effect of different concentrations of sea water on germination, growth and some mineral ion concentrations of proso millet (*Pennisetum glaucum*).

## 2. Materials and Methods

### Germination experiment:

The germination experiment of proso millet (*Pennisetum glaucum*) seeds was performed, continued for one week. The following seawater salinity levels (0, 25, 50, 75 and 100 % of sea water) corresponding to (0, 8250, 17500, 26250 and 35000 ppm) were used together with tap water. Seawater percentages were prepared by mixing seawater with fresh non saline tap water at different ratios (v/v) under room temperature.

Thirty seeds of proso millet were placed on absorbent pads in each Petri dish to which 30 ml of the experimental solution were added (3 Petri dishes in each treatment), and seeds were considered to be germinated after radical emergence from the tests.

### Growth experiment:

Seeds of proso millet were sown in perforated plastic pots, each containing 10 kg of mixed, sieved, acid washed sand and peat moss soil (3:1 by volume). The pots were irrigated with fresh water till complete germination and seedling emergence. The pots were divided into five groups, three pots for each treatment. Five seedlings per pot were left to grow in open field at about 40°C and at soil water potential near field capacity, then watered with half Strength Hoagland nutrient solution prior to seawater irrigation with the corresponding salinity levels (0, 8250, 17500, 26250 and 35000 ppm). Plants were irrigated at two days intervals. In order to prevent accumulation of salts, the soil in each pot was leached every ten days with fresh water.

At the end of experimental period (one month from imposing salinity), shoot, leave and root lengths were measured in each pot. Shoot and root were then dried in aerated oven at 70°C and the dry samples were ground into fine powder for determination of mineral ions (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>2+</sup>, Cu<sup>2+</sup>, Mn<sup>2+</sup>, Mo<sup>2-</sup> and Cl<sup>-</sup>) concentrations using wet digestion method of Hamphries (1956) In every case three replicates were

used and data were statistically analyzed to calculate the least significant differences.

## 3. Results and Discussion

### Effect of seawater on germination:

Germination is a critical period in the plant life cycle, and inhibition of seeds germination of crop plants by any environmental factors leads to reduction in the yield; so germination and seedlings characteristic are the most useful criteria used for selecting salt tolerance species. From Tables (1 and 2) it can be noticed that the highest salinity levels (75% and 100%) effected the germination which resulted a high decrease in seed germination rates of proso millet compared to the lower sea water salinity levels (0.25 and 50%). There was a delay in the beginning of germination and a reduction in germination percentage with increase in salinity levels. Percentage of germination decreased with increasing salinity concentrations. The highest germination rate was observed in the seeds exposed to fresh water (43.3 % after 24 hours and 97% after 72 hours). While the lowest germination rates were for those exposed to 75 and 100% seawater (0% after 24 hours and 23.3 and 13.3% after 72 hours, respectively). Salinity up to 50% level delayed germination but did not significantly reduce the final percentage to the critical phase. It was reduced significantly at 100% salinity level.

This reduction in germination percentage with increasing salt stress is in agreement with results obtained for another plant species by Hajar *et al.* (1993); Chiroma *et al.* (2007) and Yakubu *et al.*, (2010). This means that seed germination of proso millet was not affected by relatively low and moderate salinization levels used (0, 25 and 50%). The reduction in germination percentage may be attributed to the combined effect of osmotic stress and specific ion toxicity (Haung and Redmann, 1995). High salt concentration is also known reducing water potential in the medium which hinders absorption of water by seeds of plants and thus reduces germination (Maas and Grieve, 1987 and Yakubu *et al.*, 2010).

### Effect of seawater on plant height and leaf area:

Shoot length and leave area increased with increasing seawater salinity from zero to 25 % and then significantly reduced as seawater salinity increased (Table 3). The results also showed that the maximum shoot and root lengths and leaf area were recorded at the 25% seawater salinity level, then the results showing gradual reduction with increasing salinity in all plant parts. But the decreasing in root lengths still higher with comparison with plants irrigated with fresh water, except the last treatment (100 % seawater). On the other hand leaf lengths did not show any significant reductions compared to control, except the last treatment (100 % seawater). This results were in agreement with those obtained by (Afifi *et al.*, 2010;

Hakim *et al.*, 2010; Maiti *et al.*, 2010 and Yakubu *et al.*, 2010) whom explained that the increase in root lengths may be due migration of carbohydrates to the lowest plant parts in salinity, where is the reduction in shoot lengths may be due to many reasons such as increase in the osmotic pressure of soil solution which reduce water availability, interference of nutrient

uptake, or due to toxic effect of the NaCl used in the irrigation and finally, unbalanced nutrient uptake by seedlings. Chartzoulakin *et al.*, (2002) postulated the same result working on the effect of seawater salinity on growth of 6 olive cultivars, and found reduction in shoot height.

**Table (1): Effect of sea water salinity on daily germination of proso millet seeds**

Salinity levels %	Days of germination						
	1	2	3	4	5	6	7
0	13	26	29	29	29	29	29
25	0	16	24	25	25	25	25
50	0	8	16	17	21	24	24
75	0	3	7	14	18	23	23
100	0	1	4	10	12	14	16

**Table (2): Effect of sea water salinity on the final germination of proso millet seeds, deviation from the mean**

Salinity levels %	No. of seed germination	Germination %
0	29±1.2	97
25	25±1.6	84
50	24±1.1	80
75	23±3.2	77
100	17±2.5	57

**Table (3): Effect of sea water salinity on shoot, root and leaf lengths (cm) of proso millet, deviation from the mean**

Salinity levels %	Lengths (cm)		
	Shoot	Root	Leaf
0	62.7±7.8	43±8.3	41±6.9
25	64.7±4.2	52±2.9	43±5.8
50	58.2±4.4	49±3.5	42±2.6
75	57.0±2.6	47±6.5	41±3.8
100	47.3±4.1	41±1.3	31±2.4

#### Effect of seawater on ion concentration:

Seawater had a significant effect on the ion concentrations in proso millet plant parts (shoot and root), the results are shown in Tables (4 and 6). Regarding plant shoot ion concentrations there were increase in Na<sup>+</sup> to ( 62.3 and 58.8% ), Cl<sup>-</sup> to (337.5 and 58.8%), Mo to (22.1 and 8.1%) at 50% and 100% seawater salinity respectively, compared to plants irrigated with fresh water. Mn ion concentrations increased with the very high seawater levels (75 and 100%) to an 80% increase compared to plants irrigated with fresh water. On the other hand there were significant reductions in metal ion concentrations in plant shoot as regard K, Ca, Mg, Na. K<sup>+</sup> was reduced by (39.9 and 83.1%), Ca<sup>2+</sup> by (49 and 92.2%) , Mg<sup>2+</sup> by (9.9 and 13.8%) and Fe<sup>2+</sup> by (10.2 and 33.0%) at 50% and 100% seawater level respectively. While Cu ion concentrations in the plant shoot remained unaffected by seawater salinity, the increase in Na<sup>+</sup> and reduction in K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> concentrations in the plant shoot is in agreement with the findings of Ismail

(1998) who found the same trend in *Atriplex* species irrigated with seawater.

As for root content of nutrients Na<sup>+</sup>, Mn<sup>2+</sup> and Mo<sup>2+</sup> showed increased concentration with increase in seawater salinity, while K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup> concentrations were reduced in roots with increase in seawater salinity compared to plants irrigated with fresh water. Na<sup>+</sup> increased in root with increase in all seawater salinity levels without any significant differences between its concentrations. On the other hand Fe<sup>2+</sup> concentration showed an increase in root up to 75% salinity level before it drops down at 100% seawater level compared to control. Mg<sup>2+</sup> in the root was not affected by seawater salinity only at the highest levels (75 and 100%).

Na<sup>+</sup>, Mn<sup>2+</sup>, Mo<sup>2+</sup> showed following concentration increases in plant root with increase in seawater salinity levels (64 and 64%; 25.5 and 46.6%; 92.7 and 117.6%) at 25 and 100% salinity level respectively. And the following metals showed reduction in root concentration, Ca<sup>2+</sup> (59.4 and 95.8%), K<sup>+</sup> (56.3 and 895.3%), Mg<sup>2+</sup> (zero and 58.5% ), Cl<sup>-</sup> (5.6 and 85.2%),

at 50% and 100% seawater salinity level respectively compared to plants irrigated with fresh water.

Fe<sup>2+</sup> gave an increase in root with increase in seawater salinity up to 75% before it dropped to 13.6% at 100% salinity level. These results are in agreement with results obtained by **Yakubu et al., (2010)**; **Hajar et al., (1993)** and **Abdel Azim and Ahmed, (2009)** whom reported that extent of elements accumulation with saline solution vary among shoots and roots and the highest estimated in the roots may be due to many glycophytes being retained in the roots.

The relation between the ions accumulated in shoot and root of *Pennisetum glaucum* are shown in Table (5) all ion ratios are very small and most of the ratio less than one which give indication that *Pennisetum glaucum* have the ability to prevent Na to accumulate at the plant shoot or root. Cl was slightly higher than Na but Na/Cl varied little between treatments.

The increase in some elements and decrease in others in the tissues of prose millet may be due to the effect of salinity on the physiological phenomenon. The Na and Cl accumulated in the shoot showed no big change between zero and 35000 ppm NaCl external concentration. Excess of Na<sup>+</sup> to an even greater extent and excess Cl<sup>-</sup> in the protoplasm leads to disturbances in ionic balance (k<sup>+</sup> and Ca<sup>++</sup> to Na<sup>+</sup>) as ion specific effects on enzyme proteins and membrane as a result, too little energy is produced by photophosphorylation

and phosphorylation in the respiratory chain (**Flowers, 1990**). Therefore, the above discussion permits to some extent to the relatively low and moderate salinity levels.

Different plant species have different mechanisms for preventing excessive accumulation of Na and Cl in the leaves: for example, in *Aegialitis annulata* and *Tamarix aphylla* the leaf concentration of Na and Cl remained unchanged due to an equivalent excretion (mainly of NaCl) from the salt glands (**Berry and Thomson, 1967**). Some other plants absorb a sufficient amount of water, during growth, to prevent the increase of salt concentration in their leaves. This dilution of the cell sap has been found, for example, in the halophyte *Rhizophora mucronata* (**Levitt, 1980**) and in some of the non-halophytes when grown under salt conditions- such as tobacco (**Flowers et al., 1986**).

The concentrations of Na accumulated in the shoot and root of this crop plant are relatively constant over a period of one month following salinization to an external salinity which is optimal for growth and in agreement with those reported by **Yakubu et al. (2010)**. The decline in plant growth (and early plant death) under high external salinities may be due to: (a) NaCl toxicity (b) insufficient uptake of other ions in the presence of high NaCl concentrations (c) osmotic effect of salts or (d) any combination of these; so, the plant dehydrated earlier at higher salinity.

**Table (4): Effect of sea water salinity on the shoot and root ion content (mg/g dw) of prose millet, deviation from the mean**

Salinity levels %		K	Ca	Mg	Na	Cl
0	Shoot	161±26	7.7±1.2	10.1±1.6	0.51±0.13	0.48±0.04
	Root	93.5±6.9	9.6±1.5	8.2±1.3	0.50±0.13	0.88±0.11
25	Shoot	115±17	2.6±0.8	9.4±2.1	0.80±0.10	1.35±0.26
	Root	63.2±6.4	1.8±0.2	8.5±1.4	0.82±0.10	1.83±0.12
50	Shoot	96.7±8.8	3.9±0.5	9.1±0.7	0.83±0.08	2.1±0.14
	Root	41.1±3.8	5.3±0.4	8.2±0.8	0.82±0.07	0.83±0.09
75	Shoot	41.6±3.7	4.7±0.9	9.2±1.7	0.82±0.07	0.50±0.03
	Root	23.2±2.1	2.2±0.9	3.1±0.3	0.83±0.05	0.75±0.03
100	Shoot	27.2±2.4	0.6±0.1	8.7±0.8	0.81±0.03	0.50±0.13
	Root	4.5±0.4	0.4±0.1	3.4±0.3	0.82±0.07	0.13±0.08

**Table (5): Effect of sea water salinity on ion ratios accumulated in the shoot and root of prose millet**

Salinity levels %		Na/K	Na/Ca	Na/Mg	Na/Cl
0	Shoot	0.0032	0.066	0.051	1.06
	Root	0.0053	0.052	0.061	0.57
25	Shoot	0.007	0.308	0.085	0.59
	Root	0.013	0.456	0.097	0.45
50	Shoot	0.0068	0.213	0.091	0.40
	Root	0.02	0.155	0.100	0.99
75	Shoot	0.02	0.175	0.089	1.64
	Root	0.036	0.377	0.259	1.11
100	Shoot	0.03	1.35	0.093	1.62
	Root	0.18	2.05	0.241	6.31



**Table (6) Effect of sea water salinity on the shoot and root ion content ( $\mu\text{g/g dw}$ ) of prose millet, deviation from the mean**

Salinity levels %		Fe	Cu	Mn	Mo
0	Shoot	45.1 $\pm$ 1.9	45.5 $\pm$ 1.9	11.5 $\pm$ 4.5	8.6 $\pm$ 3.4
	Root	18.3 $\pm$ 3.4	84.5 $\pm$ 1.3	10.5 $\pm$ 1.6	5.1 $\pm$ 0.8
25	Shoot	42.8 $\pm$ 4.4	44.6 $\pm$ 1.8	11.2 $\pm$ 3.5	12.5 $\pm$ 2.5
	Root	22.6 $\pm$ 3.1	42.9 $\pm$ 1.5	10.5 $\pm$ 1.6	9.6 $\pm$ 1.5
50	Shoot	40.5 $\pm$ 2.3	44.5 $\pm$ 9.1	11.1 $\pm$ 4.4	10.5 $\pm$ 4.2
	Root	21.7 $\pm$ 2.9	44.1 $\pm$ 7.2	13.2 $\pm$ 2.1	10.6 $\pm$ 1.6
75	Shoot	30.3 $\pm$ 3.1	46.3 $\pm$ 2.6	13.3 $\pm$ 5.3	8.4 $\pm$ 3.6
	Root	18.5 $\pm$ 1.1	89.1 $\pm$ 9.2	14.3 $\pm$ 2.2	16.6 $\pm$ 2.5
100	Shoot	30.2 $\pm$ 3.3	43.6 $\pm$ 1.2	20.7 $\pm$ 8.3	9.3 $\pm$ 3.7
	Root	15.8 $\pm$ 3.6	32.7 $\pm$ 5.2	15.4 $\pm$ 2.3	11.1 $\pm$ 1.7

### Conclusion

The most important results obtain from this study and would be emphasis that is this crop species (*Pennisetum glaucum*) not only grow in a high salinity concentration (which irrigated directly with seawater) but also did not accumulate either Na nor Cl in their parts (shoot and root) more than that in the control plants, which need to do more studies in this direction to variety of this species can be irrigated with seawater to face the lack of fresh water in arid and semi arid regions.

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## Benchmarking of patient satisfaction with physical rehabilitation services in various hospitals of Jeddah

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**Abstract: Objective:** To study patients' satisfaction in rehabilitation services and its relation to staff job satisfaction in public, private and teaching hospitals in Jeddah. **Method:** A cross sectional survey was used to measure patients' satisfaction with rehabilitation services in ten different hospitals in Jeddah (Kingdom of Saudi Arabia). A validated Monnin and Perneger's questionnaire with a 5-points Likert scale was utilized. At the same time, staff job satisfaction was measured by the Effort-Reward Imbalance model in the same health care facilities. **Results:** On 725 patients who responded to the survey, 80.6% were overall satisfied with the rehabilitation services they received. Patient satisfaction was significantly different ( $p=0.001$ ) between hospital types. In average, 88.5% of the patients of the teaching hospitals were satisfied with the rehabilitation services, compared to 77% of the patients in the public hospitals and 75.7% of the patients in the private hospitals. Moreover there was no statistically significant correlation between staff job satisfaction and patients' satisfaction. **Conclusion:** Patients' satisfaction with rehabilitation services was the highest in the teaching hospitals in comparison with the other types of health care facilities. Patients' satisfaction was however not significantly correlated with the staff job satisfaction.

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**Keywords:** Rehabilitation services, patient satisfaction, job satisfaction, Effort-Reward Imbalance, quality management

### 1. Introduction

Patients' satisfaction is considered an important quality indicator and a basic element of any quality monitoring program in health care services<sup>1</sup>. From the same perspective, one might suppose that if health care providers are satisfied in their work, it could reflect on improved patients' satisfaction and outcomes. It is thought that bringing more satisfaction to the staff in their work could subsequently increase patients' satisfaction though this statement can differ in the rehabilitation services and might be related to other factors of care<sup>2</sup>. The assessment of patient's satisfaction with the treatment outcomes in physical rehabilitation presents underlying difficulties. It is based on the patient's perception of the treatment effectiveness and could be subjective as in severe or pathologies or multiple disabilities where therapeutic outcomes are often related to the patient's well being, relief of pain or functional skills improvement<sup>3,4</sup>. The intrinsic aspect of the rehabilitation outcomes can be analyzed in a single hospital however, it is not evident when several facilities are compared with various degrees of patient's pathologies.

Several patients' satisfaction evaluation tools have been recently designed for out-patients of physical therapy services such as the Physical Therapy Outpatients Survey (PTOPS)<sup>5</sup> or the Medrisk instrument<sup>6</sup>. They are considered as valid tools for

measuring patient satisfaction with physical therapy and measure the variables of satisfaction based on the interactions with the therapists, allocated time and explanations or instructions during treatment. The aspect of satisfaction of both in- and outpatients has been evaluated by<sup>7</sup> in their research using a validated tool suiting both patients' categories. It has considered the fact that, in the healing process, the patients are following a continuum of care from the inpatients to the outpatients services of rehabilitation. The motivation behind using this questionnaire is based on its utilization in studies for inpatients and outpatients without distinction, in addition to its internal validity and the fact of being short and easy to be understood by all patients independently from their educational level. In the current research, the financial aspects were excluded and were not considered as the hospitals participating in the survey were not disclosing their financial data or providing services free of charge.

### Correlates of patients' satisfaction

a. *Patients' characteristics* as socio-demographic characteristics of the patients including the age, gender, level of education may be considered in addition to the patient's pathology and if the patient him/herself answers the survey questionnaire or a patient's relative. Age has been

found to be the most consistent factor, with older patients being more satisfied with their care. However, in several researches, most correlations of patients' satisfaction with socio-demographic variables were extremely small and have then been considered as a minor predictor of satisfaction<sup>8</sup>.

- b. *Health related patient behavior:* Compliance to the treatment and utilization of the services are positively related to satisfaction of the patients. As often observed, a patient that becomes unsatisfied with the rehabilitation services will intent to switch services to another care provider or would show a decreased willingness to return. Additionally, patients who are more satisfied are more apt to comply by appearing on time and following treatment instructions<sup>8</sup>.
- c. *Provider-patient interaction:* Perceptions of the quality of care are most heavily influenced by the attitude of the therapist and the manifestations of concern and care with positive communication in addition to the evidence of technical competence. Professionals who are seen as warm, friendly are related to higher levels of patient's satisfaction. Both humaneness and informativeness have been studied in several researches and are the baselines for a good therapist-patient relationship. Sensitivity to patients needs based on clear and sufficient communication are also contributing factors to satisfaction. Heinemann and colleagues<sup>9</sup> concluded that the same items of communication, effectiveness of care and environmental factors were perceived as important component of the patients' satisfaction. Patients who complete their course of physical therapy reported that the professional interaction between the therapist and patient, especially the meaningful exchange of relevant information, was critical for patient satisfaction with healthcare<sup>6,10</sup>.

The study aimed at studying the patients' satisfaction with rehabilitation services and its relation to staff job satisfaction in public, private and teaching hospitals in Jeddah.

## 2. Subjects and Methods

### Subjects

A population of 724 patients attending physical, occupational and respiratory therapy services was from public (n= 341), private (n= 250) and educational health care facilities (n= 134) in Jeddah area participated in the survey. Patients received the survey questionnaire after attending their therapy sessions in University hospitals or public hospitals related to the Ministry of Health as well as in private health care facilities and large outpatient clinics of the town of Jeddah. The hospitals' sizes varied in average from 700 in-patients beds to smaller dimensions of

250 beds. By random selection, several healthcare facilities that only treat out-patients were also included in the survey. Exclusive criteria were patients suffering from degenerative pathologies and cognitive abnormalities.

In addition to the patients, a number of 166 therapists and assistant therapists of the same health care facilities received a survey questionnaire evaluating their job satisfaction level using the Effort-Reward Imbalance model<sup>11</sup>.

### Survey Design

#### Patients' Satisfaction Measure

A survey prospective design by self administered questionnaires to the patient population was used by a standardized and validated survey questionnaire<sup>7</sup> for both in-patients and out-patients where the aspects of accessibility, communication, attitude in addition to socio-demographic variables have been outlined. As the present research was also targeting the study of both occupational and respiratory therapists in the rehabilitation services, the term physical therapist is replaced by therapist in the questionnaire. The patients' opinions in each domain were measured using 5-point Likert scale that ranges from "strongly disagree" to "strongly agree".

The patients' satisfaction questionnaire has been translated into Arabic (specific to the local Saudi dialect) by Arabic language teachers and bilingual staff members of physical therapy department at King Abdul-Aziz University (appendix). It was validated by physical and occupational therapy patients in a pre-test protocol and was re-translated from Arabic to English. Corrections were made to one question and the term secretary or receptionist was replaced by the term "therapist" as in many hospitals, the therapists commonly handle their patients' appointments by themselves without a secretary or a clerk. Questionnaires were distributed to each patient attending for therapy during one month and collected by an independent person from the departments.

#### Staffs' Job Satisfaction Measure

In parallel, another questionnaire was distributed to 222 therapists and assistant therapists working in the same health care facilities using the Effort-Reward Imbalance model to evaluate their job satisfaction and received a response rate of 74.9 % with 166 returned questionnaires after two weeks. The questionnaires were specifically adapted for the current research based on three sections; the first one was concerned with the socio-demographic information related to the therapists and assistants, the second section was evaluating the Effort- Reward Imbalance Ratio and over-commitment of the staff<sup>12</sup>. A third section was a questionnaire including specific questions related to the practice of work in

rehabilitation such as the schedule and average number of patients seen on daily basis.

### Statistical Analysis

The data was encoded and analyzed in a SPSS software and descriptive and correlation analysis were made using the Spearman- rho test , and a mean score of satisfaction was calculated per hospital category and compared by Kruskal Wallis test.

### 3. Results

Three thousand nine hundred sixty (3960) survey questionnaires were randomly distributed in ten rehabilitation services of the Jeddah area and obtained a response rate of 18.9%. Out of 725 patients, 61.9 % males responded to the survey with a mean age ( $\pm$ SD) of  $34.9 \pm 20.7$  yrs and 70.9 % of them were attending the outpatients' rehabilitation services. Moreover, 60 % of the patients completed the questionnaires by themselves while the remaining ones had completed the survey by their close relatives.

From a global aspect in the 10 surveyed hospitals, it was revealed that the average percentages of satisfied patients with rehabilitation services were 88.5%, 75.7% and 77.4% in the educational, private and public hospitals respectively.

Table (1) shows the number and percentages of satisfied staff and their comparison by hospital category. The mean number of satisfied patients showed significant differences between groups for variables of satisfaction related to the therapists' input. While there was a higher mean percentage of satisfaction in the teaching hospitals compared to that in the other two categories regarding the therapists' ability to reassure, the difference between groups showed however no significant difference. The patients' satisfaction with the quality of information and given explanations on the treatment plan as well as the feeling of security are significantly higher in the teaching hospitals. Concerning the patients' satisfaction with the effect of the treatment (if the treatment was adapted to the problem) a significantly higher percentage is seen in the teaching hospitals. In addition, the satisfaction with the information related to the treatment plan is also higher in the non-profit hospitals compared to the profit making hospitals. As to the overall treatment satisfaction, it appears significantly different between groups and higher in the teaching hospitals compared to the non- profit hospitals.

**Table (1): Percentage of satisfied patients per hospital categories**

Patients Satisfaction Variables	Satisfied Patients per Hospital Category			Total Satisfied Patients N = 725	p-value	p-value within groups
	Teaching N=134	Profit Making N=250	Non Profit N=341			
Reassurance in therapy	119 90.2%	211 84.4%	277 82.0%	84.3%	.090	NS
Explanations in therapy	116 87.9%	181 73.6%	273 80.3%	79.4%	.004	$P1 \leq .001$
Information on treatment plan	106 80.3%	147 59.8%	243 71.7%	69.2%	.001	$P1 \leq .001$ $P3 \leq .003$
Feeling of security	125 93.3%	197 79.4%	268 78.6%	81.6%	.001	$P1 \leq .001$ $P2 \leq .001$
Adapted treatment to problem	124 92.5%	190 76.6%	254 75.1%	78.9%	.001	$P1 \leq .001$ $P2 \leq .001$
Overall treatment satisfaction	115 87.1%	200 80.6%	260 76.5%	79.9%	.033	$P2 \leq .001$

$P1$  = Teaching and Profit Making Hospitals     $P2$  = Teaching and Non Profit Hospitals     $P3$  = Profit and Non Profit Hospitals

Considering the staff job satisfaction working in the same rehabilitation services, the results identified the Effort-Reward Imbalance ratio for each therapist during the same period of time. However a Pearson analysis did not conclude into a significant correlation between staff and patients satisfaction.

### 4. Discussion

Patient satisfaction is increasingly used to monitor patient perceptions of the quality of health

care services<sup>13,14</sup>. The findings of the current study has identified significant differences between the hospital types that the patients expressed a comparatively high level of satisfaction in the educational hospitals and a lesser degree in the private and public facilities.

Some patient's surveys were also completed by their close relative who may contribute to the obtained results of higher level of satisfaction. Our findings show that the patient's relative is commonly more

critical towards the provided services than the patient himself. In accordance, Quintana *et al.*<sup>15</sup> found that the person who completed the questionnaire has an important effect on the results, in that a more negative satisfaction level was recorded on those surveys answered by someone other than the patient. While previous research found that younger patients are more critical about their therapy which is similar in our results<sup>7,15,16</sup>.

A client centered approach based on the importance of giving attention, information and the feeling of security in treatment was high in the educational hospitals where explanations and detailed information about the treatment plan was significantly correlated to the patient satisfaction. This is confirmed by other studies<sup>10,17,18</sup>. Teaching hospitals have a strong educational role in their mission to the patients, students and staff and the health care professional's expertise can be reflected by the nature and extend of the explanations given during the treatment. Additionally, as Hills *et al.*<sup>19</sup> outlined the importance to establish the patients' needs in therapy, particularly the extent to which these might be psychosocial rather than physical. In the same line, the description of the patients' overall evaluation of their physiotherapy care in terms of satisfaction has been made in two dimensions and based not only on the clinical outcome but also related to the "Therapeutic Encounter" and interpersonal attributes of the therapist<sup>4,20</sup>. This appears also for the aspects of medical care in general as Cleary already identified that more "personal" care is associated with higher levels of satisfaction<sup>21</sup>.

While acute cases are more numerous in the teaching hospitals, in agreement with other research we have also identified that acute cases are in general more satisfied with their treatment compared to the chronic patients<sup>19</sup>.

On the other hand, the lowest level of satisfaction presented by patients treated in the private hospitals may be related to the lack of explanations at the end of the treatment sessions regarding future planning. This could be explained by the economic mission of such hospitals reflected on an increased productivity. Therapists in private hospitals have a limited time to discuss or clarify the treatment plan to their patients as they concentrate more on the effective treatment delivery and the procedure than on explanations and patient education. In some surveyed hospitals, therapists reported that the treatment plan is discussed within the rehabilitation team or left to be explained by the referring physician. An additional factor is related to the staff expertise to give explanations and it was identified that a larger proportion of staff have a higher educational degree in the teaching hospitals compared to the public hospitals where more numerous assistants therapists are working with a diploma level certification.

In reference to the job satisfaction of the staff, it appears that the therapists' feelings and satisfaction about their work are not directly reflected on their patients and thus independent of the patients satisfaction with the rehabilitation process.

Many therapists in the survey have expressed in written open questions that the most rewarding aspect about their work is related to patients' progress and outcome improvements. The humanistic aspect of the health care profession is fundamental and motivates the therapist without projecting possible negative personal emotions in work. In the same line, one could also expect that the written policies and procedures related to health care practice maintain appropriate standards of care and make the patients receive the quality of treatment and attention independently from the level of staffs' job satisfaction. The high degree of over-commitment in the work that was also identified is an additional indication that therapists are committed about their work in all health care facilities.

### Conclusion

The patients' level of satisfaction with rehabilitation services was found to vary between the different hospital types. The highest patients' satisfaction was found in the teaching hospitals, followed by the non-profit making and finally by the profit making healthcare facilities. Patients' satisfaction seemed to be related to the hospitals' mission and to the perception of the process of care and the therapists' input, such as the ability to reassure or the quality of information given. Rehabilitation staffs' job satisfaction was however not correlated with the patients' satisfaction about their treatment and the rehabilitation process.

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**Appendix****إستبيان عن مدى رضا المريض عن الخدمات التأهيلية**

يختص هذا الاستبيان بالعلاج الطبيعي أو الوظيفي أو التنفسي الذي يتلقاه المريض بالمستشفى، إجاباتك سوف تساهم للنهوض بخدماتنا، لا يوجد إجابة صحيحة ولا إجابة خاطئة، كما أن الإجابات سوف يتم تحليلها بمنتهى السرية.

طبقا للجملة اختر مستوي الإجابة التي تعكس رأيك.

1 = ضعيف

2 = مقبول

3 = جي

4 = جيد جدا

5 = ممتاز

ممتاز	جيد جدا	جيد	مقبول	ضعيف	
5	4	3	2	1	1 سهولة إجراءات الدخول.
5	4	3	2	1	2 إهتمام ومساعدة السكرتارية.
5	4	3	2	1	3 سهولة الحصول على أول موعد.
5	4	3	2	1	4 قدرة الأخصائي علي طمانتك ومساعدتك.
5	4	3	2	1	5 توضيح ما سيتم عمله أثناء العلاج.
5	4	3	2	1	6 نوعية المعلومات التي حصلت عليها في نهاية العلاج كخطة مستقبلية.
5	4	3	2	1	7 مدى الإحساس بالأمان خلال فترة العلاج.
5	4	3	2	1	8 الإحساس بالأمان خلال فترة العلاج.
5	4	3	2	1	9 مدى ملاءمة العلاج لحالتك المرضية.
5	4	3	2	1	10 سهولة الوصول للوسائل المستخدمة في العلاج.
5	4	3	2	1	11 وجود علامات ارشادية للوصول لأماكن المستشفى المختلفة.
5	4	3	2	1	12 مدى ملاءمة غرفة العلاج.
5	4	3	2	1	13 هدوء وراحة مناخ غرفة العلاج.
5	4	3	2	1	14 تقييمك الشامل للعلاج
					15 هل تنصح المقربين لك بالعلاج في نفس المكان.
				1	لا بالتأكيد
				2	إحتمال لا
				3	غير متأكد
				4	احتمال نعم
				5	نعم بالتأكيد

شكرا لمساعدتكم



## The Influence of Number of Filaments on Physical and Mechanical characteristics of Polyester Woven Fabrics

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**Abstract:** In this paper different polyester woven fabrics having different number of filaments in its filling yarns were woven. The effect of the number of filaments on the fabric properties was analyzed. The experimental results were assessed using analysis of variance and regression methods via SPSS statistical package. The statistical analysis revealed that properties of polyester fabrics have affected significantly by the number of filaments. As the number of filaments in the cross section of filling yarns increases, fabric thickness, tensile strength, fabric elongation and crease recovery increases. By the contrast, increasing number of filaments leads to a reduction in fabric air permeability, weight loss due to abrasion and fabric tearing strength.

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**Key words:** polyester fabrics, denier per filaments, fabric tensile strength, air permeability, tearing strength, abrasion resistance, crease recovery.

### 1. Introduction

Polyester fiber (polyethylene terephthalate) becomes more popular due to its unique properties such as excellent mechanical characteristics, good resiliency, chemical inertness and heat resistance. The micro polyesters have been more focal point in research during the past decade because of its aesthetic and highly soft touch [1, 2]. Microfibers are the fibers with fineness below 0.1 tex. This is the general definition of microfibers, although the Japanese have already made a fiber with fineness below 0.01 tex [3, 4]. The development of microfibers with fineness below 0.1 tex began in the seventies in Japan, and in the eighties and nineties in Europe and America. In 1992, about 10000 tons microfibers, mostly as microfilaments, were spent in USA [5]. PES microfilament yam, PA microfilament yam, microstaple fibers, acryle microfibers, viscose microfibers and polypropylene microfibers are mainly produced nowadays. In 1992, about 35000 t microfibers were produced all over the world [6]. Asahi, Kanebo, Kuraray, Mitsubishi, Rayon, Teijin, Toray, Toyobo and Unitika are the Japanese companies that produce microfibers, filament and staple fibers [7].

Besides having a luxurious body and drape, microfiber fabrics are also lightweight resilient. They can retain their shape and resist pilling. Compared to other fabrics of similar weight, they are relatively strong and durable. Since fine yarns can be packed tightly together, microfiber fabrics have good wind resistance and water repellency. As the number of filaments in a yarn of given linear density increases, the surface area of all the fibers increases and the spaces between the fibers get smaller. Liquid water is prevented by surface tension from penetrating the fabric, which will have a degree of water repellency.

On the other hand, the spaces between the yarns are porous enough to breathe and wick body moisture way from the body [8-11].

Some examples for microfiber application are sports wear, medical, and protective wear, different filter material upholstery etc. It can be assumed that their uses will increase due to their outstanding aesthetic, physical and textile properties [12-15].

In the present study, the effect of the number of filaments on physical and mechanical properties of polyester woven fabrics was investigated. The physical and mechanical characteristics of five different 150 denier polyester filament yarns containing different number of filaments were studied with respect to fabric thickness, fabric strength, breaking elongation, air permeability, tearing strength, crease recovery and abrasion resistance.

### 2. Materials

In this study five different polyester continuous filament yarns of 150 denier supplied by the same manufacturer from the same lot. It contained 34 filaments (4.4 denier per filament), 48 filaments (3.13 denier per filament), 108 filaments (1.39 denier per filament), 144 filaments (1.04 denier per filament), and 208 filaments (0.72 denier per filament) were selected. The fabric sample having 208 filaments is considered a micro polyester fabric, but the other samples represent normal polyester fabrics.

The polyester filament yarns were woven on Water-Jet weaving machine with the following particulars:

Warp density: 156 ends / inch

Weft density: 60 picks / inch

Fabric width: 165 cm

No. of harness frames: 4

Warp yarn count: 150 denier

Weave structure: 1/1 plain.

After weaving, all the polyester fabric samples were hot washed, thermal fixed, bleached and dyed.

### Laboratory Testing

Physical and mechanical tests were carried out in weft direction after conditioning of the fabrics for 24 hours under the standard atmospheric conditions ( $20 \pm 2$  °C temperature,  $65 \pm 2\%$  relative humidity). Ten individual readings were averaged for each fabric property. The fabrics were tested for the following characteristics; Fabric thickness, fabric strength, breaking elongation, air permeability, tearing strength, crease recovery and abrasion resistance.

Thickness is the distance between one surface to its opposite in textiles, the distance between the upper and lower surface of the material, measured under a specified pressure. The specimens were tested as directed in ASTM test method D. 1777. Tensile strength and elongation measurements of the plain fabrics in weft direction were performed on an Instron 4411 Tester (Instron Inc., USA) according to TS EN ISO 13934-1; and the air permeability tests were conducted on Shirley Air Permeability tester in accordance with ASTM D737.

For tearing test, an Intensity tearing tester (Elmendorf type) was used according to ASTM D1424. The abrasion resistance measurements were carried out with the help of a Martindale abrasion tester. At the end of 2000 rubs, the abrasion cycle was ended. The abrasion resistance of the fabrics was evaluated according to their weight loss (%) after 2000 rubs. For each fabric sample, the abrasion tests were carried out 3 times, and the average weight loss was calculated. The abrasion resistance was measured according to the relevant standard BS 5690. Crease recovery of polyester fabric samples having different levels of continuous filaments was evaluated using the measurement of crease angle. Crease angle of the woven samples was measured via Wrinkle Recovery tester according to AATCC Test method 66-1975.

### Statistical Analysis

To explore the effect of the number of filaments in yarn cross section on the physical and mechanical properties of the woven polyester fabrics, One – Way ANOVA statistical analysis was performed using SPSS statistical package. All test results were assessed at significance level  $0.05 \leq \alpha \leq 0.01$ . To predict the mechanical property of each woven fabric sample at different levels of number of filaments, a linear or non-linear regression models was executed via a regression analysis.

The regression non- linear models which correlate the number of filament in the yarn cross section to the woven fabric properties has the following form:

$$Y = a X^2 + b X + c$$

Where,

Y= Fabric properties such as (tensile strength, elongation, air permeability, -- etc.)

X= Number of filaments in the yarn cross section.

C= constant

a, b = Regression coefficients.

In the case of linear models, the value of regression coefficient, a – value, equals zero. The validation of the regression models was performed using the coefficient of determination,  $R^2$ , R-square (the coefficient of determination), measures the reduction in the total variation of the dependent variable (fabric properties) due to the independent variable (total number of filaments in the yarn cross section).

### 3. Results and Discussion

In order to study the effect of filament fineness on physical and mechanical properties of woven polyester fabrics such as thickness, tensile strength, breaking elongation, air permeability, tearing strength, crease recovery and abrasion resistance, five different fabrics containing 150 denier polyester yarns were used and the effect of the number of filaments in the yarn cross section was studied.

#### Fabric Thickness

Some properties of fabrics like keeping warm and bulkiness, are depends on the thickness of the fabrics. Since thick and porous fabrics contain more air, form a thicker layer between human body and surrounding and make the heat transfer difficult, they keep warmer.

The values of fabric thickness versus the levels of the number of filaments in the yarn cross section were demonstrated in figure 1. An increasing trend is detected assuring that as the number of filaments increases the fabric thickness follows the same trend. It is also noticed that the thickness of microfiber polyester fabric is more than those woven from normal polyester fibers. This means that micro polyester fabrics is more efficient in thermal insulation and give warmth to the man's body. Increasing fabric thickness value with the increase in number of filaments may be due to compactness of the finer filament yarn in the fabric.

The regression model which correlates the fabric thickness to the number of filaments has a linear model with the following form:

$$\text{Thickness (mm)} = 0.0002 X + 0.274$$

The coefficient of determination for this model equals 0.94, which means that this model fits the data very well.

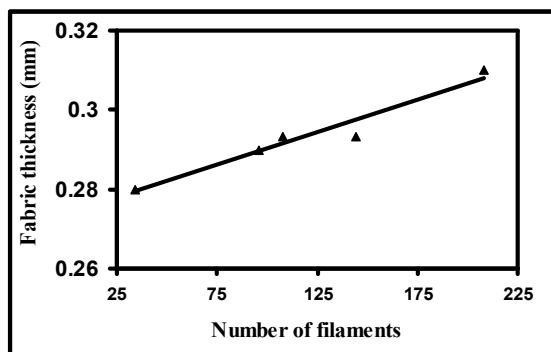


Figure 1: Effect of number of filaments on fabric thickness

### Tensile Strength

Fabric tensile strength is the force in Newton which is used to break the fabric sample. The results of the analysis of variance and comparison of individual means for fabric strength revealed that the effect of number of filaments is highly significant. From figure 2 it is seen that as the number of filaments in the yarn cross section increases the tensile strength of the woven polyester fabrics also increases. This means that the tensile strength of micro polyester fabric is more than those woven from normal polyester fibers.

The relation between the number of filaments in the yarn cross section and the fabric tensile strength has the following form:

$$\text{Tensile strength (Newton)} = 0.012 X^2 - 0.85 X + 413.8$$

The statistical analysis proved that the  $R^2$  value for this model equals 0.99, which means that this model fits the data very well.

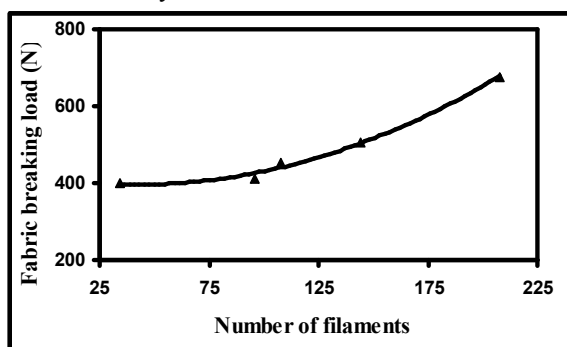


Figure 2: Effect of number of filaments on fabric tensile strength

### Breaking Elongation

Breaking elongation of the polyester woven fabrics at the different levels of the number of filaments in the yarn cross section was illustrated in figure 3. The same trend was detected as the above figure, assuring that as the number of filaments increases the fabric breaking elongation follows the same trend. It also noticed that the micro polyester fabric has higher breaking elongation compared to the

normal polyester fabrics. This is because the higher number of filaments in their cross section.

The relation between the number of filaments in the yarn cross section and the fabric breaking elongation is as follows:

$$\text{Breaking elongation (\%)} = 0.005 X^2 - 0.019 X + 16$$

The coefficient of determination of this regression model is 0.97, which means this model fits the data very well.

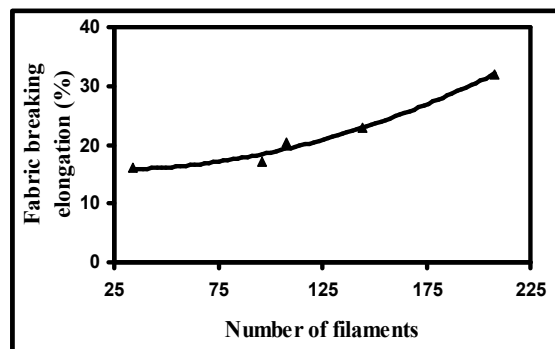


Figure 3: Effect of number of filaments on fabric breaking elongation

### Air Permeability

The impact of the number of filaments in the yarn cross section on the air permeability of the polyester woven fabrics was depicted in figure 4. The statistical analysis proved that fabric air permeability has significantly affected with the number of filaments. A decreasing trend is detected confirming that as the number of filaments increased the air permeability of the polyester woven fabrics decreased. It is also shown that the air permeability of micro polyester woven fabrics is lower than those woven from normal polyester fibers. This means that micro polyester fabric is suitable for making wind resistant fabrics and filter fabrics

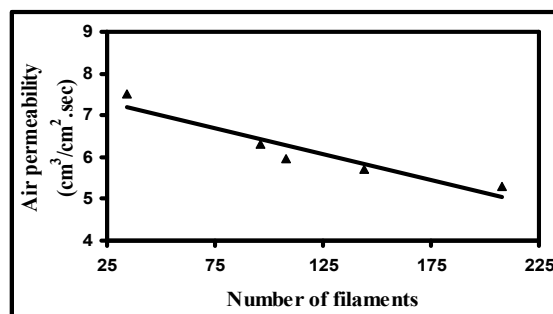


Figure 4: Effect of number of filaments on fabric air permeability.

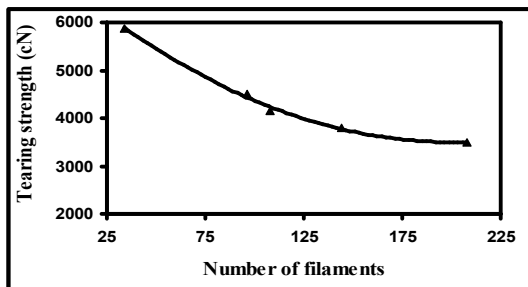
The relation between air permeability and the number of filaments in the cross section of the weft yarns has the following linear form:

$$\text{Air permeability (cm}^3\text{/cm}^2\text{.sec)} = -0.012 X + 7.6$$

The  $R^2$  value for this model equals 0.87.

### Tearing strength

Fabric tearing strength is related to its serviceability and depends on its weave structure and the strength of the constituent warp and weft yarns. The fabric tearing strength versus the number of filaments in the weft yarn cross section was shown in figure 5. It is noticed that the number of filaments has a profound effect on fabric tearing strength. A decreasing trend is detected assuring that as the number of filaments increases the fabric tearing strength decreases. This means that the tearing strength of micro polyester fabric had lower tearing strength compared to other woven fabrics. This is because the higher number of filaments in the yarn cross section. Under tearing force, the higher number of filaments in yarn cross section behaves as single filament. On the contrary, for the fabrics having lower number of filaments, these filaments withstand the tearing load individually.



**Figure 5: Effect of number of filaments on fabric tearing strength.**

To predict the fabric tearing strength at the different levels of the number of filaments, the following regression model can be used:

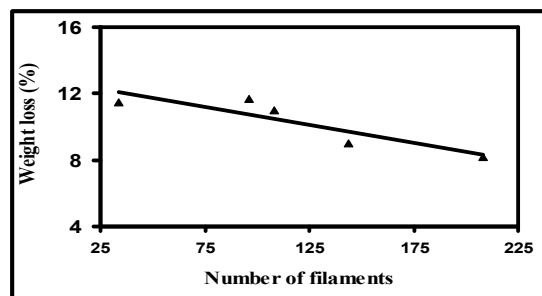
$$\text{Tearing strength (cN)} = 0.086 X^2 - 34.6 X + 6964.$$

This regression models fits the experimental data very well with a higher value of  $R^2$ , 0.99.

### Abrasion resistance

In this study, abrasion resistance of the woven fabric samples was evaluated by the percentage of fabric weight loss. As the weight loss decreases the abrasion resistance of the woven fabrics increases. The weight loss of the woven fabric sample according to different number of filaments was plotted in figure 6. The statistical analysis showed the huge influence of the number of filaments on the weight loss. As seen from this figure, the amount of weight loss decreased as the number of filaments in the yarn cross section increased. The highest improvement in terms of weight loss was observed on microfiber polyester fabrics. This can be attributed to the higher frictional forces between fibers in the yarn cross section in the fabrics having micro polyester fibers. As a result, it became more

difficult for the fibers to be removed from the fabric structure.



**Figure 6: Effect of number of filaments on fabric abrasion resistance.**

The regression model which correlates weight loss of woven fabrics due to abrasion to the number of filaments has the following linear form:

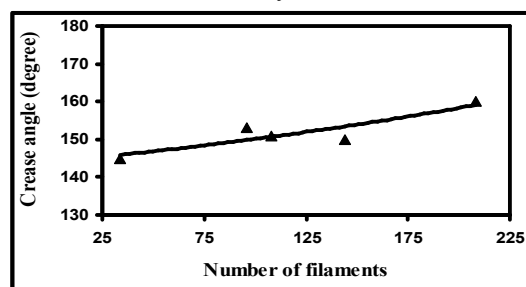
$$\text{Weight loss (\%)} = -0.0219 X + 11.843$$

The  $R^2$  value for this model is 0.75.

### Crease Recovery

The crease recovery is one of the fundamental properties of fabrics which affects product performance. Crease recovery refers to the ability of the fabric to return to its original shape after removing the folding deformations. The crease recovery of fabrics is determined by measuring the crease recovery angle. As the crease angle increases the fabric crease recovery increases.

Crease angle of polyester woven fabrics at different levels of number of filaments was plotted in figure 7. It is shown that number of filaments in the weft yarn cross section has a significant influence on the crease angle. An increasing trend is detected, conforming that as the number of filaments increases the fabric crease angle increases. This means that fabric crease recovery enhanced with the higher number of filaments in the yarn cross section.



**Figure 7: Effect of number of filaments on fabric crease recovery.**

To predict the crease angle of polyester fabric at different levels of number of filaments, the following linear model can be used:

$$\text{Crease angle (degree)} = 0.076 X + 142.8$$

The  $R^2$  for this model is 0.79.

**Conclusion**

Thickness, tensile strength, breaking elongation, air permeability, crease recovery, abrasion resistance, and tearing strength of polyester woven fabrics at different number of filaments have been investigated. The statistical analysis showed that increasing the number of filaments enhanced the fabric tensile strength and its elongation, crease recover and abrasion resistance. But polyester woven fabrics having higher number of filaments had lower air permeability, which means that these fabrics suitable for fabric filters and wind proof garments.

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## Anatomical, Histological and Histochemical Adaptations of the Reptilian Alimentary Canal to Their Food Habits: I. *Uromastix aegyptiaca*

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**Abstract:** A series of studies was carried out to elucidate the relationship between the microscopic anatomy of the alimentary canal and the food habits in reptiles. Three reptiles were chosen according to different feeding habits, *Uromastix* is a herbivorous, *Chameleon* is an insectivorous, while *Crocodilys* is a carnivorous reptile. So, it is obvious that the anatomy as well as the histology of the alimentary tract of reptiles demonstrate certain specific characteristics of functional adaptations as a reflection of the herbivorous, carnivorous and insectivorous mode of feeding. The anatomical and histological study of the alimentary canal of *Uromastix aegyptiaca* was carried out. A comparison between the different histological structures found and those known in other reptiles was done. The straight oesophagus is lined with ciliated epithelium and goblet cells, leading to the stomach which consists of two portions, fundic or oxyntic and pyloric or mucous. The small intestine is comparatively short although the animal is purely herbivorous. It consists of the duodenum and ileum. The duodenal mucosa is in the form of leaf-like villi provided with shallow branched Lieberkühn crypts at their bases. The ileum is devoid of glands. The large intestine is formed of a well developed large caecum, colon and rectum. At the posterior edge of the caecum there is a small blind sac which is considered as the appendix. The caecum which is devoid of glands is lined with simple columnar cells of a special type. While the ileo-caecal valve is in the form of a characteristic well developed protrusion, the caeco-colic valve is formed of a flap arising from one side. The mucosa of the colon is folded and lined with goblet and columnar cells, while that of the rectum is, more or less, straight and is rich in goblet cells and lymph spaces. The distribution and localization of different carbohydrate categories (PAS-positive material, mucopolysaccharides) were studied in the mucosal epithelium of the alimentary canal of *Uromastix aegyptiaca*. The goblet cells of the oesophagus are rich in acid mucopolysaccharides, those of the small and large intestine contained smaller amounts. Neutral mucopolysaccharides were found in small to moderate amounts, being most obvious in the gastric mucosa. Mode of feeding as well as habitat, show, more or less a close similarity in the histochemical pattern of their gut mucosa as regards to the distribution and localization of proteins and nucleic acids.

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**Key words:** Anatomy - Histology – Histochemistry – Alimentary canal - reptiles.

### 1. Introduction

The present study deals with the morphological adaptations in the reptilian digestive system in relation to their food nature. In general, most reptiles feed chiefly on animals and insects, but land tortoises, some turtles and few lizards eat vegetations. Lizards and small snakes eat insects and other small invertebrates. Small turtles feed on aquatic invertebrates. Large lizards, turtles, snakes and crocodiles eat various vertebrates from fishes to mammals (Saber, 1989; Sadek, 1992 and Saleh, 1993). Reptiles include as many as 7500 different species, most known are: alligators, turtles, tortoises, lizards and snakes (Elliott, 2007). The total intake of food is small in amount if compared with that necessary for birds and mammals. Reptiles eat more, and digestion is faster at higher temperatures (Booolotian, 1979 and Karasov *et al.*, 1986). In general, the digestive system of reptiles consists of the buccal cavity with its associated structures, the

oesophagus, the stomach, the small and large intestine in addition to the accessory digestive glands represented by the liver and the pancreas (Ibrahim, 1991). The anatomical and histological structure of the alimentary tract of the agamid *Uromastix aegyptiaca* and its relation to the herbivorous type of feeding will be discussed in detail.

Reptiles have been suggested to be useful models for the study of the regulatory mechanism of the gastrointestinal system for several reasons (Secor and Diamond, 1998). First, their regulatory responses are easier to examine experimentally than those of the typical mammalian models because they have extreme responses to feeding. Many reptiles consume huge meals (up to 160% of their own body mass) at infrequent intervals (sometimes fasting for 18 months). By contrast, most the mammalian model species (i.e. rats, mice, rabbits, pigs, etc.) that eat small meals have correspondingly larger regulatory responses to feeding than do mammals. Second, studying the

digestive responses of reptiles improves our understanding of the evolution of the regulatory mechanisms of the gastrointestinal tract. Although previous research on the digestive responses of reptiles has examined regulatory phenomena such as hormone release, the growth and atrophy of organs, and acid–base homeostasis (Secor and Diamond, 1995&1998; Starck, 1999; Busk *et al.*, 2000; Secor *et al.*, 2001; Starck and Beese, 2001, 2002; Pennisi, 2003; Andrade *et al.*, 2004; Starck *et al.*, 2004).

Reptiles have been suggested to be a future useful model for studying the physiological regulation of the digestive process as they have well responses to feeding even more than other commonly used experimental mammals such as mice, rats, rabbit and pigs (Secor and Diamond, 1998).

The digestive system of the reptiles contains all the structures present in other higher vertebrates, from the oral cavity to the cloaca. The oral cavity is lined by mucous membrane made of non-keratinized stratified squamous epithelium with salivary glands distributed in the submucosa (Putterill and Soley, 2003).

The alimentary tract of reptiles is similar to higher vertebrates with some exceptions. The oesophagus shows adaptive modifications from group to group. In turtles, the oesophagus has heavily keratinized papillae that protect the mucosa from abrasive diet such as speculated sponges and jellyfish, and also may act as filtering devices. In lizards, it is formed of folds lined by ciliated columnar epithelium with goblet cells. Some snakes have mucous glands along their submucosa (Elliott, 2007). The muscularis mucosa of the oesophagus is absent in many species of reptiles but may be found in some species of turtles (Elliott, 2007).

Oppel (1896, 1897& 1900) described the alimentary canal of reptiles; Greschik (1917) studied the anatomy and histology of the alimentary canal of both *Ableparus pannonicus* and *Anguis fragilis*. Langley (1881) gave an account on the histology and physiology of pepsin-forming glands in some reptiles. Beguin (1904 a & b) studied the oesophageal glands of reptiles. Staley (1925) gave a brief account on the structure of gastric glands of *Alligator mississippiensis*. Beattie (1926) described the ileo-caecal region of *Tupinambis teguixin*.

Compared with mammals, reptiles possess a number of peculiarities that could be of specific advantages for some histological aspects in general and histochemical ones in particular. A considerable bulk of literature analyzing histochemically the lacertilian gastrointestinal tract has come to light (Chou, 1977; Odehode *et al.*, 1979; El-Taib and Jarra, 1983; Taib, 1984; Dehlawi *et al.*, 1987c and 1988 a & b; Zaher *et al.*, 1987 a & b).

The macroscopic structure of the reptilian alimentary tract has been subjected to extensive studies since the work of Beguin (1904 a & b), Kahlie (1913) and Krause (1922). Reports on this subject were presented by Abo-Taira *et al.* (1988a & 1988c) on *Acanthodactylus boskianus* and *Tarentola annularis*, respectively. Zaher *et al.* (1989b, 1990a & 1991a) studied the anatomy of the alimentary tract in *Stenodactylus slevini*, *Mabuya quinquetaeniata* and *Echis carinatus*, respectively.

Efforts have been exerted in the study of the microscopic structure of the reptilian alimentary tract organs. Of these, the histological studies of the alimentary tract of some lizards were carried-out by several authors including the work of El-Toubi and Bishai (1958) on *Uromastix aegyptiaca*; Bishai (1959) on *Varanus griseus*; Anwar & Mahmoud (1975) on two egyptian lizards *Mabuya quinquetaeniata* and *Chalcides ocellatus*; Amer and Ismail (1976) on *Agama stellio*; Farag (1982) and Dehlawi *et al.* (1988a) on *Uromastix philbyi*, Dehlawi and Zaher (1985a) on *Acanthodactylus boskianus*; Zaher *et al.* (1989a & 1990b) on *Mabuya brevicollis* and *Chalcides sepioides*, respectively.

The histology of the tract organs of the gecko *Gekko japonicus* was studied by Oidumi & Ishihara (1964). Chou (1977) and Dehlawi and Zaher (1985b) studied the histology of the alimentary tract organs in the geckos *Ghyra mutilata* and *Pristurus rupestris*, respectively.

The microscopic structure of the tract organs of some snakes was also investigated by Heyder (1974) on *Typhlops vermicularis* and Abdeen *et al.* (1994) on *Malpolon monspessulanus*, *Coluber florulentus* and *Tarpophis obtusus*. Ballmer (1949) studied the histology of the digestive tract organs of some american turtles; Thiruvathukal and Kuriakosa (1965) studied the digestive tract of the fresh water turtle *Chrysemys picta*.

Extensive histochemical studies have been carried-out on the alimentary tract organs of reptiles. Dehlawi and Zaher (1985b & 1987a) studied the histochemical distribution and localization of carbohydrates in the alimentary tract of the gecko *Pristurus rupestris* and the lizard *Agama adramitana*, respectively.

A comparative histochemical study on the distribution of carbohydrates in the gut mucosa of the lizard *Uromastix philbyi* and the snake *Naja nigricolis* was carried-out by Badr El-Din (1991).

The histochemical distribution of lipids and mucopolysaccharides in the alimentary tract mucosa of the gecko *Tarentola annularis* was studied by Amer *et al.* (1987a). The mucopolysaccharides, lipids and proteins in the tract mucosa of the lizard *Acanthodactylus scutellatus* was subjected to such study by Amer *et al.* (1988).

The localization of lipids, proteins and nucleic acids in the mucosal epithelium of the alimentary tract was investigated in the gecko *Pristurus rupestris* by **Zaher et al. (1987a)**, the lizard *Uromastyx philbyi* and the snake *Naja nigricolis* by **El-Dawoody et al. (1992)**.

The localization and distribution of carbohydrates, proteins, lipids, and nucleic acids in the mucosal coat of the reptilian gut has been extensively described (**El-Taib and Jarrar 1983; Dehlawi and Zaher, 1985 a & b; Amer et al., 1987b ; Abo Taira et al., 1988 a & b; Zaher et al., 1989 a & b; Abdeen, et al., 1990 a ; El-Dawoody, 1992 and Zaher et al., 1995**). Also, **Berrin (2005)** made an immunohistochemical study on the endocrine cells in the gastrointestinal tract of the freshwater turtle *Mauremys caspica caspica*, while **Giovanni et al. (2008)** worked on the histochemical and the immunohistochemical characterization of exocrine cells in the foregut of the red eared slider turtle, *Trachemys scripta*. **Banan Khojasteh et al. (2009)** showed that the intestinal goblet cells of *Oncorhynchus mykiss* have both acidic and neutral mucosubstances. **Perez-Tomas et al. (1990)** suggested that mucins can play different roles among regions of the digestive tract of the Greek tortoise, *Testudo graeca*.

The distribution of carbohydrates, proteins, nucleic acids, and lipids in the alimentary tract was studied by **Amer et al. (1987b)** in the snake *Echis carinatus*. Reports on this subject was presented by **Abdeen et al. (1990 a & b)** on the skink *Eumeces schneideri* and the snake *Cerastes vipera*, **Amer et al. (1990)** on the gecko *Pristurus flavipunctatus*, and **Zaher et al. (1990b)** on the lizard *Chalcides sepidoides*.

**Uriona et al. (2005)** studied the structure and function of the oesophagus of *Alligator mississippiensis*; **Strarck et al. (2007)** studied the physiological and morphological responses to feeding in *Caiman latirostis*; **Ahmed et al. (2009)** studied the histological and histochemical of the gut of *Varanus niloticus*; **Biomy (2010)** studied the ultrastructural and histochemical characterization of the alimentary tract of the insectivorous reptile, *Scincus scincus*. **Khamas and Reeves (2011)** studied the morphology of the oesophagus and stomach of the gopher snake *Pituophis catenifer*.

In this respect, the present work is designed to add a new speculation about the anatomical, histological and histochemical variations of the alimentary canal in the herbivorous species *Uromastyx aegyptiaca*. The main goal of the present study is to cast light on the relationship between the anatomical, histological and histochemical adaptations of the alimentary canal of the investigated species to its herbivorous mode of feeding.

## 2. Material and Methods

*Uromastyx aegyptiaca* (family: Agamidae) which lives in the desert, is purely herbivorous. It is one of the very few forms among lizards having this feeding habit. Ten animals caught from Gabal Al-Maghara, south of El-Arish city, northern Sinai, Egypt were used as a model of herbivorous reptiles (Fig.1).

The specimens were anaesthetized by chloroform and then dissected carefully by making a longitudinal incision at the midventral surface.

For gross anatomy, photographs were taken for the digestive system within the body of the animal and also for the alimentary canal taken out of the body. In addition, in two specimens, the alimentary canal was cut longitudinally to describe the structure of the internal surface as the folds, the villi and valves.

For the general histological studies, the contents of the gastrointestinal tract were drained by saline solution, small pieces of the various segments were fixed in aqueous Bouin solution, after fixation, parts of the gastrointestinal tract were dehydrated, embedded in paraffin wax and then transversely sectioned 6µ thick. Sections were stained with differential double stained Mayer's haemotoxylin and eosin (**Castro and Camargo, 1951**).

For the histochemical studies, the following techniques were implemented:

- 1- General carbohydrates were illustrated using the periodic acid –Schiff (PAS) technique (Pearse, 1968). In this procedure, sections were placed in 0.5% periodic acid for the liberation of aldehydes, and then treated with Schiff's reagent for 2 minutes. A positive reaction is indicated by the appearance of magenta colouration resulting from the reaction between aldehydes and the decolourized solution (leucofuchsin) of Schiff's reagent.
- 2- Acid and neutral mucopolysaccharides were demonstrated by the Alcian blue-PAS method (**Mowry, 1956**). By this method, acid mucins exhibit blue stainabilities whereas neutral mucins take a reddish colouration, and the mixtures of both mucins acquire a purple stainability.
- 3- For displaying the total proteins, the mercuric bromophenol blue method (**Mazia et al., 1953**) was employed. The existence of a dark blue stainability denotes the occurrence of total proteins.
- 4- Nucleic acids (DNA and RNA) were demonstrated by the methyl green pyronin method (**Kurnick, 1955**), while the application of Feulgen reaction was used for demonstration of DNA only (**Stowel, 1945**).

Photomicrographs were taken to illustrate the histological structures of the various organs of the alimentary canal.

## 3. Results

### Gross anatomy

The buccal cavity leads to the funnel-shaped pharynx which opens into the oesophagus. The latter



structure is a long tube with its anterior end wide, then it becomes narrow till the stomach region. Its narrow cavity is lined by numerous high longitudinal folds. The oesophagus leads to the stomach, the transition between the two is not clear.

The stomach is a wide curved tube with lesser and greater curvatures. It is placed at the left side of the body cavity. It is a long tube which ends in the pylorus near which it becomes gradually narrow. The pylorus is guarded by a strong sphincter muscle which partly protrudes into the duodenum.

The intestine is differentiated into small, and large intestines.

The small intestine begins from the pylorus till the caecum. The large intestine includes the well-developed caecum, colon and rectum.

The small intestine is a coiled tube which is wide near the pylorus, and gradually narrows towards the caecum. There is no external or internal indication of the transition between the duodenum and the ileum as they pass indistinguishably in one another.

The small intestine is comparatively short, although this animal is purely herbivorous. Its length is about 3/4 the length of the large intestine. The ileum protrudes into the caecum for about 6-10mm. This protruding part is covered internally and externally by mucous membranes, which are thrown into longitudinal folds parallel to its longitudinal axis. The mucous membrane around the ileo-caecal opening is thrown into radiating folds, which are continuous with both the external and internal longitudinal folds of the protruding part. This protruding part of the ileum is well-developed and provided with a strong sphincter muscle; its cavity is distinctly narrow. Thus this part can be considered as a valve, and is called the ileo-caecal valve.

The caecum is extremely large possessing a thin wall. Its mucosa has no folds, but it possesses numerous shallow pits, which are only seen when examined with low power of the microscope and they are evenly distributed on its inner surface. The caecum occupies a great part of the body. It lies on the right side opposite to the stomach. The caecum possesses at its posterior surface, near the colon, a small flattened blind sac (the appendix). This sac is found in all animals dissected but it differs in size and shape. It is strongly attached to the wall of the caecum by connective tissue. The caecum leads to a wide colon. The two being separated by a narrow curved part, which is provided by a strong muscle arising from one side. The colon is a wide, short tube provided internally with well-developed longitudinal folds. It leads to the rectum which is also short, but provided with less distinct longitudinal folds. The outer wall of both the colon and the rectum is provided with longitudinal striations. The rectum opens into the cloaca which is of small diameter. Its opening to the

exterior is guarded by a sphincter muscle. A membranous, bask-shaped urinary bladder is connected to the ventral wall of the cloaca.

The liver is a large triangular gland and it is composed of two lobes. They are completely fused together anteriorly. These fused parts are connected to the dorsal body wall by a strong falciform ligament. Posteriorly, the two lobes of the liver diverge from each other. Each lobe is more or less triangular, and is notched anteriorly. (Figs. 2,3)

There are two long bile ducts, which run parallel to each other, and open posteriorly in the duodenum close to the pylorus.

The gall-bladder is a large, membranous sac which lies in the notch present on the inner surface of the right elongated lobe of the liver.

The pancreas is a large, yellowish organ. It is formed of a thin layer spread out in the mesentery between the duodenum and stomach. A single short pancreatic duct arises from the edge and opens in the duodenum near the openings of the bile ducts.

### **The Histological studies of the Alimentary Canal**

#### **The oesophagus**

The serosa is in the form of a thin, folded membrane covering the musculosa, which is narrow, and formed of smooth muscles. Two layers can be seen, an outer longitudinal muscle layer and an inner circular one. The former is narrow and folded externally. Its muscles are in the form of patches separated by little connective tissue. It is not uniform in thickness, but is more developed than the inner circular muscle layer. The latter layer is also not uniform in thickness, and it is not well developed. It contains more connective tissue. It is not in the form of a continuous layer. Some of its fibers lie in an oblique manner. The two layers of musculosa are separated by intermuscular connective tissue layer.

The submucosa is narrow and contains mainly collagenous fibers, and some reticular fibers.

The muscularis mucosa appears as scattered patches of longitudinal muscle fibers entering in the formation of the oesophageal folds. Near the posterior end of those, the muscularis mucosa is in the form of a continuous layer of longitudinal muscles, and there are no circular muscles.

The oesophageal mucosa is in the form of longitudinal folds, which are highly extending in the lumen of the oesophagus. It is in the form of simple epithelium. Two types of cells that can be observed which are, the ciliated cells, and the mucous secreting cells or goblet cells. (Fig. 4A)

The goblet cells are cylindrical cells composed of two portions, an upper large hyaline portion, and a lower small protoplasmic one. The upper part is stained by the specific mucous stains. These cells have wide openings. (Fig. 4B)

The ciliated cells are scattered irregularly between the goblet cells. Their bodies are elongated and thin. The nucleus is elongated, rod-shaped and located in the middle of the cells. (Fig. 4B)

#### **The stomach:**

In a transverse section through the stomach, the serosa appears as a thin membranous layer.

The outer longitudinal layer of the musculosa is narrow and composed of fine fibres. It is in the form of elongated patches separated by plenty of connective tissue. The inner layer is thicker than the outer one and is formed of compact circular muscles. The two layers of the musculosa are separated by a thin intermuscular connective tissue layer.

The submucosa is more developed than that of the oesophagus (Fig.5A).

The muscularis mucosa is represented by a continuous layer composed of an outer longitudinal muscle layer and inner circular muscle layer, both being well developed. In the anterior portion of the stomach, the two layers are more or less equal in thickness. Towards the middle region, the inner circular muscle layer is more developed. Near the pylorus, the inner circular muscle layer of the muscularis mucosa begins to disappear and it is in the form of few fibers which lie beneath a more developed outer longitudinal muscle layer. The two layers, however, are less developed in the pyloric region but they form a continuous layer (Fig.5A).

The mucosa is thick and contains gastric glands which open into gastric pits. The latter are continuous with the surface epithelium. The surface epithelial cells, as well as those of the gland pits and necks, have the same structure. There is no sharp line of demarcation between the neck cells and the surface epithelium. These cells (cells lining gland necks, pits and gastric mucosa) secrete a substance related to mucin (Fig.5B).

The gastric glands in *Uromastix aegyptiaca* are of two types, fundic and pyloric glands.

The fundic glands are tubular with narrow cavities. A gland may open by a separate neck, but two or more glands may have one common neck. The body of the fundic gland is formed of polyhedral granular cells with central rounded nuclei. Their granules react in the same way as do oxyntic cells. This shows that the glands are oxyntic, and comparable to oxyntic glands described in the stomach of other reptiles.

The pyloric glands extend from the middle region of the stomach to the pylorus where they disappear. Near the middle region of the stomach, the pyloric glands are simple tubular or branched tubular with long necks; while near the pylorus they are alveolar with longer necks. The pyloric glands are numerous near the middle region, where near the pylorus they are few with much connective tissue in between them. The body of these glands is formed only of one type of cells.

The cytoplasm of these cells is pale and appears clear, as it contains indistinct granules.

#### **The Small intestine:**

The small intestine extends from the pylorus to the caecum as a narrow tube. Near the pylorus it is in the form of a wide tube which narrows gradually towards the caecum. The first loop after the pylorus, receiving the pancreatic and bile ducts, is considered as the duodenum, the rest is the ileum. There is no external indication to differentiate between the duodenum and the ileum. Examining the mucosa, it is noticed that the duodenal mucosa is much folded, the folds are high and numerous. Low folds occur between the high ones. Thus the intestine in the duodenal region has a wide cavity full of folds. On the other hand, the ileal region has a narrow cavity lined with few longitudinal folds. The folds of the small intestine run in a "zigzag" manner parallel to each other.

The serosa is a very thin membrane continuous with the mesenteries.

The musculosa is formed of two layers, an outer longitudinal and an inner circular. They are much less developed than those of the musculosa of both the oesophagus and the stomach. The outer longitudinal muscle layer, in the region after the pylorus, is weakly developed and formed of fine scattered fibres; while the inner circular muscle layer is formed of scattered oblique muscles, with much connective tissue in between. Towards the middle region of the intestine the two layers of the musculosa are well developed and separated by a well developed intermuscular layer of connective tissue. The outer longitudinal muscle layer is in the form of patches covered with the folded serosa.

The submucosa is narrow near the pylorus and wider towards the posterior region near the caecum where it is well developed.

The muscularis mucosa appears only near the pylorus, as a continuation of the muscularis mucosa of the stomach. Here, it is in the form of scattered longitudinal muscle fibres which enter in the formation of the folds (Fig.6A). A short distance from the pylorus the muscularis mucosa disappears and the greater part of the small intestine is devoid of muscularis mucosa. It makes its appearance again at the posterior region of the intestine, near the caecum. In this region the muscularis mucosa is in the form of patches of longitudinal fibres which are, more or less, in the form of a layer. These muscle fibres enter in the formation of the folds, where they are more developed at the top (near the lumen). Also it enters in the formation of the ileo-caecal valve.

The mucosa shows a marked difference in the nature and shape of the folds according to the region. Thus in the region near the pylorus, the mucosa is in the form of numerous long villi, between the bases of which the intestinal glands or Lieberkühn crypts are

found, while near the caecum, the mucosa is in the form of few thick folds (Fig. 6B).

The epithelial lining of both regions is formed of the same elements, *i.e.* columnar and goblet cells. Thus, histologically, one can differentiate between the duodenum and the ileum, by the presence of Lieberkühn crypts in the former.

#### **The Large intestine:**

It is composed of a very conspicuous caecum, colon and rectum.

#### **The caecum**

In *Uromastix aegyptiaca*, the caecum is an exceedingly large sac which is attached to the ileum from one side and to the colon from the opposite side, *i.e.* lying between the ileum and the colon. The ileum protrudes into the caecum forming a well developed ileo-caecal protrusion which acts as a valve, as it is provided with strong-muscles. On the other hand, the opening between the caecum and the colon is guarded by a caeco-colic valve.

The ileo-caecal protrusion in *Uromastix aegyptiaca* is well developed. It extends inside the caecum and is provided with a narrow cavity, while its wall is provided with strong muscles continuous with the circular muscle layer of the musculosa of both the ileum and caecum. It is covered by caecal mucosa and lined by ileal mucosa.

The caecum is a thin-walled sac. Its mucosa is in the form of concentric shallow pits, which are small and uniformly arranged. In a transverse section through the caecal wall, it is composed of the following layers (Fig. 7A).

The serosa is a thin membrane followed by a thin layer of connective tissue.

The outer longitudinal layer of the musculosa is narrow and composed of fine fibres. It is in the form of elongated patches separated by plenty of connective tissue. The inner layer is thicker than the outer one and is formed of compact circular muscles. The two layers of the musculosa are separated by a thin intermuscular connective tissue layer.

The submucosa is comparatively narrow and well supplied by blood vessels.

The muscularis mucosa is a continuous layer directly in contact with the mucosa. It is formed of two layers, an outer longitudinal and an inner circular. Both layers are well developed and formed of fine fibres.

The mucosa is formed of one type of simple columnar epithelial cells. These cells are very much elongated. The nuclei are small, oval, and located in the center.

The mucosal cells may contain pigment granules which, when present, are numerous near the lumen. It is noticed also that in some regions, goblet cells exist between the tall epithelial cells; however, they are very rare. The mucosa of the caecum is invaded by a large number of lymphocytes which are numerous near the

basement membrane. The great number of lymphocytes is noticed only to exist in both the mucosa and the lamina propria of the caecum (Fig. 7B). They exist in small amounts in the mucosa of the colon and rectum. The lymphocytes, which are present in the caecum, are small and provided with small darkly stained rounded nuclei. Their cytoplasm is clear and scanty.

A blind sac with narrow lumen arises from the posterior surface of the caecum near the colon. This sac is considered to be the appendix it is small, flattened and connected by compact connective tissue to the caecal wall. It widely opens into the caecum. Its wall is covered by the thin-walled serosa which is continuous with that of the caecum. The serosa is followed by a wide well developed subserosa.

The opening between the caecum and the colon is guarded by a caeco-colic valve, which is provided with a strong muscle arising from one side.

The longitudinal muscle layer of the caecum is continuous with that of the colon. It does not enter in the formation of the caeco-colic valve. The inner circular muscle layer of the caecum and that of the colon form at their junction the strong, well developed muscle of the caeco-colic valve. This caeco-colic muscle may contain few longitudinal fibres at its periphery.

The muscularis mucosa of the caecum in this region is continuous with that of the colon. This caeco-colic muscle is covered at the caecal side with caecal epithelium, and at the colonic side with colonic epithelium. When the valve is relaxed, a constriction is seen between the caecum and the colon.

#### **The Colon**

The colon is widely attached to the caecum. At the junction of the two there is the caeco-colic valve. The large intestine, after the caecum, is in the form of a wide short tube. Its anterior portion is the colon, and the posterior portion is the rectum. There is neither an internal nor an external morphological difference between the colon and the rectum, as the two are widely connected to each other. But, histologically, they are different in the nature of their mucosal folds and blood supply.

In a transverse section through the colon the following layers are observed.

The serosa is formed of flattened cells with flat nuclei. The serosa as well as the outer longitudinal muscle layer of musculosa are in the form of low longitudinal folds. Thus these folds appear externally as white longitudinal striations on the wall of the colon. The serosa is followed by a very thin layer of connective tissue which extends between the patches of the longitudinal muscle layer of the musculosa.

The musculosa is composed of two layers. The outer longitudinal muscle layer is thin, formed of wavy bundles of fine muscle fibres. The inner circular

muscle layer is thick formed of compact fibres. The two layers of the muscosa are separated by a layer of connective tissue containing small lymph spaces (Fig. 8A).

The submucosa is well developed and well supplied with blood vessels. The muscularis mucosa is a well developed continuous layer. It is composed of an outer longitudinal and an inner circular muscle layer. The two layers are, more or less, equal. The muscularis mucosa enters in the formation of the large folds of the mucosa, where it is well developed at the top of these folds.

The mucosa is in the form of shallow concentric depressions, it is thick, and composed of two types of cells; columnar, and goblet cells. Below the epithelial layer there is one or more layers of cells, which do not reach the surface; these are considered as replacing cells. Thus the mucosa appears, more or less, stratified.

The columnar cells are few especially at the base of the shallow folds. Their nuclei are oval and lie near the base.

Between the columnar cells, the goblet cells exist and they are abundant in such a way that they predominate in between the columnar cells. The mucosa lies on a basement membrane, which is formed as a condensation of the reticular fibers of the lamina propria. It is invaded by numerous leucocytes, which are usually near the basement membrane (Fig. 8A).

The lamina propria is not well developed, especially at the bases of the shallow folds, where the muscularis mucosa lies in close contact with the mucosa. At the top of the folds, the lamina propria is more developed. It is provided with numerous blood capillaries directly underneath the mucosa, but still fewer than those in the caecum.

### **The Rectum**

The serosa is thin with shallow longitudinal folds continuous with those of the colon. Underneath the serosa, there is a thin layer of subserosa. It extends between the patches of the longitudinal muscle layer of the muscosa.

The muscosa is more developed than that of the colon. The outer longitudinal muscle layer is in the form of folded bundles. The inner circular muscle layer is well developed, and penetrated by large blood vessels. The two layers of the muscosa are separated by intermus-culosal connective tissue, which is wider than that of the colon. It is noticed that this layer is richly supplied with large blood vessels; also it contains numerous lymph spaces. The last two characters of the intermusculosal connective tissue layer are not noticed in the corresponding narrow layer of the colon. The submucosa is well supplied with blood vessels, and small lymph spaces.

The muscularis mucosa is more developed than that of the colon. The outer longitudinal muscle layer is thicker than the inner circular layer. The muscularis

mucosa enters in the formation of the folds where it is well developed at the top of these folds (Fig.9A).

The mucosa is thicker than that of the colon. There are few large folds which project in the lumen. But the mucous membrane is straight, and not in the form of shallow pits, as in the case of the colon. The shallow pits of the mucosa are only found at the bases of the large folds. The mucosa is formed of two types of epithelial cells, columnar and goblet cells. These columnar cells are few and become fewer near the cloacal region, where the mucosa is formed of goblet cells (Fig.9B).

The goblet cells are provided with an upper goblet part, which is larger than that of the goblet cells of the colon. It occupies the greater part of the cells and widely opens in the lumen. Underneath the epithelial cells there is another layer of replacing cells which do not reach the surface, and are wedged between the epithelial cells. The mucosa lies on a well developed basement membrane. It is invaded by numerous leucocytes, which are usually present near the basement membrane. These leucocytes are similar to those present in the mucosa of the colon, as previously described.

The lamina propria is more developed than that of the colon. It is formed of reticular fibres. The lamina propria is richly supplied with blood vessels and contains numerous large lymph spaces, compared to that of the colon.

### **The histochemical studies of the alimentary canal**

#### **1-Carbohydrates (PAS-positive material) :**

##### **Oesophagus:**

Application of the PAS-technique indicated that the goblet cells were loaded with positively stained material (Fig.10), indicating that it is rich in carbohydrates.

##### **Stomach:**

The mucosal cells lining the gastric lumen appeared strongly PAS-positive (Fig .11). The PAS-positive material was generally found in the luminal portions of the epithelial cells, the rest of the cell (middle and basal parts) displayed very weak reactivity.

##### **Small intestine:**

In the small intestine, the positive reaction was moderate in the goblet cells and at the luminal surface lining the intestinal villi. The reaction was very weak in the cytoplasm of the columnar epithelial cells (Fig.12).

##### **Large intestine:**

As in the small intestine, the goblet cells as well as the luminal surface of the columnar epithelial cells displayed a moderate PAS- positive reactivity (Fig .13). The cytoplasm of the columnar mucosal cells was very lightly stained, which indicates the presence of only traces of carbohydrates.

#### **2-Mucopolysaccharides:**

**Oesophagus:**

The Alcian blue–PAS method showed that most of the carbohydrates present in the mucosal epithelium of *Uromastix aegyptiaca*, oesophagus are in the form of acid mucopolysaccharides (Fig .14, the blue colour ). The figure indicates that most cells contain only acid mucosubstances , a few contain a mixture of both, acid and neutral mucopolysubstances .

**Stomach:**

Neutral mucopolysaccharides were abundantly found in the luminal poles of the mucosal epithelium (Fig .15).

A positive stained border lining the luminal surface of the stomach was clearly observed.

**Small intestine:**

As shown in figure 16, a mixture of acid and neutral mucopolysaccharides appears in the goblet cells of the small intestine. The PAS reaction is much less than that at the oesophageal goblet cells. The acid mucin was found towards the lumen (in the upper parts of the goblet cells).

In some cells, the neutral mucopolysaccharides were predominating. The reaction at the luminal surfaces of the cells was also in the form of a mixture of both types of mucopolysaccharides. The cytoplasm of the columnar epithelial cells contained very few neutral mucopolysaccharides.

**Large intestine:**

Moderate amounts of acid mucopolysaccharides were detected in the luminal poles of mucosal epithelial cells making a continuous positive sheet along the luminal border of the colon. The rest of the cells displayed a weak neutral mucopolysaccharides reactivity. In the rectum (Fig.17) a mixture of moderate amounts of both polysaccharides was found in the goblet cells and along the luminal surface of the columnar epithelial cells .

**3-Total proteins:****Oesophagus :**

Application of mercuric bromophenol blue method on the oesophagus of *Uromastix aegyptiaca* proved an exaggerated amount of proteonic elements situated in the cytoplasm of the columnar epithelial cells. On the other hand their goblet cells showed a weak response to the above mentioned method (Fig. 18). This means that a small amount of proteins was only present in these cells.

**Stomach:**

In the stomach of *Uromastix aegyptiaca*, the cytoplasm of its superficial columnar cells contains a moderate amount of proteins. However low proteonic content was detected in the cytoplasm of the cells constituting the bodies and the necks of the gastric glands. On the other hand, the cells of the gastric glands showed a strong response to the bromophenol blue method. This proves the presence of a large amount of proteonic elements. (Fig.19)

**Small and large intestines:**

In the small and large intestines of the *Uromastix aegyptiaca*, comparatively less amounts of proteins was scored in the cytoplasm of the columnar cells, while a weak reaction was noticed in the cytoplasm of the goblet cells. (Fig .20).

**4-Nucleic acids:**

Histochemical demonstration of DNA revealed the appearance of a dense product in the nuclei of the oesophageal, gastric and intestinal mucosal columnar cells. Such a positive staining product is present in the place of the chromatin substances containing DNA, (Figs. 21&22). Application of methyl green pyronin method proved the existence of a considerable amount of RNA inside the cytoplasm of the columnar epithelial cells in the different gut regions of *Uromastix aegyptiaca*. (Figs. 23- 25).

**4. Discussion**

The anatomical observations of the alimentary canal of *Uromastix aegyptiaca* detect the absence of a constriction between the oesophagus and the stomach. The absence of such a constriction seems to be a common feature for the lacertilian species, since it characterizes also all the described lacertilian insectivorous members (Bishai, 1960; Al-Nassar, 1976; Chou, 1977; Zaher *et al.*, 1987c and 1990 a &b and Abo-Taira *et al.*, 1988a). Such a character was also detected in the carnivorous ophidian species (Luppa, 1977, Abo-Taira *et al.*, 1988b; Afifi *et al.*, 1990 and Zaher *et al.*, 1990 c). On the contrary, the presence of a constriction between the oesophagus and the stomach was referred in turtles (Luppa, 1977). Obviously, the absence of a constriction between the oesophagus and stomach is, thus, closely related to the type of food, where it permits easier passage of food to the stomach. The alimentary canal mucosa of the examined species as indicated from the present study, confirms to a great extent the basic reptilian pattern. The oesophagus is represented by a well developed muscular wall which may help in performing the function of mechanical conveyance of ingested food and in food swallowing.

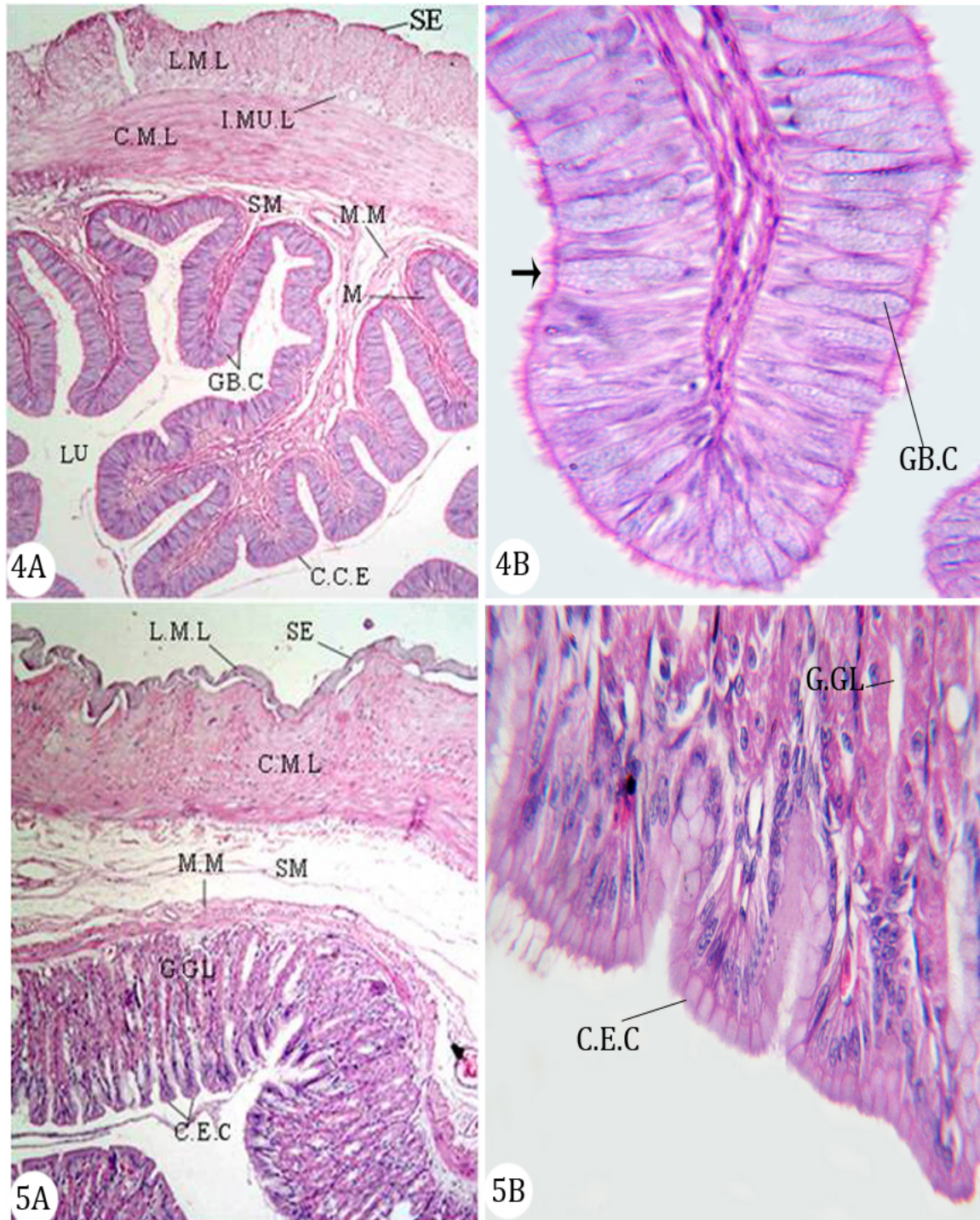
In the present investigation, the mucosal epithelium of the oesophagus is represented by simple ciliated columnar epithelial cells and goblet cells. Such structural observation of the oesophageal mucosa was reported in other reptiles studied by El-Toubi and Bishai (1958), Andrew and Hickman (1974), Amer and Ismail (1976), Przystalski (1980), Farag and Al-Robai (1986) and Mohallal and Rahmy (1992). However, in *Ablephorus pannonicus* (Greschik, 1917), *Chamaeleon vulgaris* (Bishai, 1960) and *Uromastix philibiyi* (Farag, 1982), the mucosal membrane of only the anterior region of the oesophagus consists of simple epithelial cells, while that of the posterior region is formed of a stratified one.



**Fig. (1):** Photograph of *Uromastix aegyptiaca*.

**Fig. (2):** Photograph of the dissection of the alimentary canal of *Uromastix aegyptiaca*.

**Fig. (3):** Photograph of a fresh isolated alimentary canal of *Uromastix aegyptiaca* showing (the oesophagus, stomach, small and large intestine). The liver is shifted to the left.

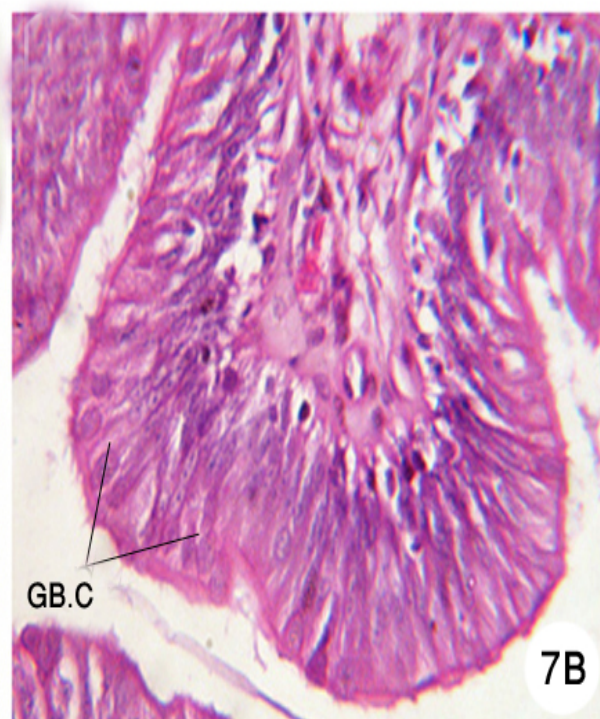
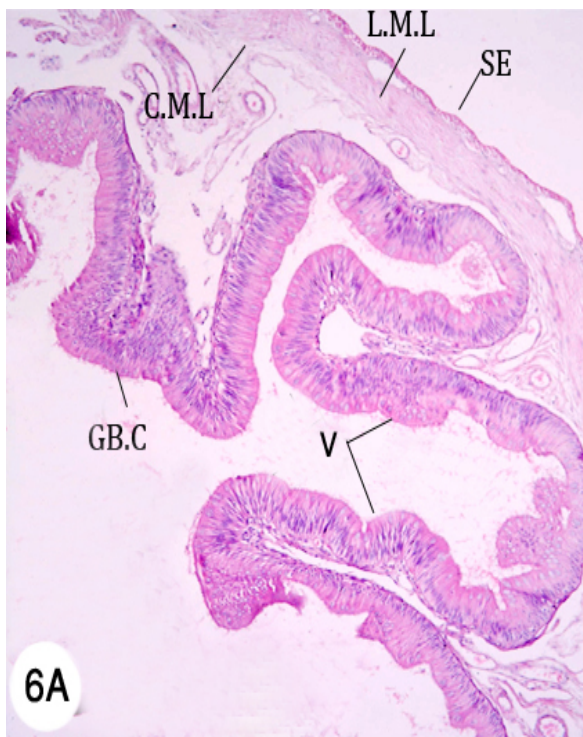


**Fig. (4A):** Photomicrograph of a transverse section of the oesophagus of *Uromastix aegyptiaca* showing the mucosa(M), submucosa (SM), muscularis mucosa(M.M), muscularis ( circular and longitudinal layers C.M.L&L.M.L) and serosa (SE). H&E stain, X100.

**Fig. (4B):** Photomicrograph of enlarged portion of the oesophagus of *Uromastix aegyptiaca* showing the mucosa(M). H&E stain, X600.

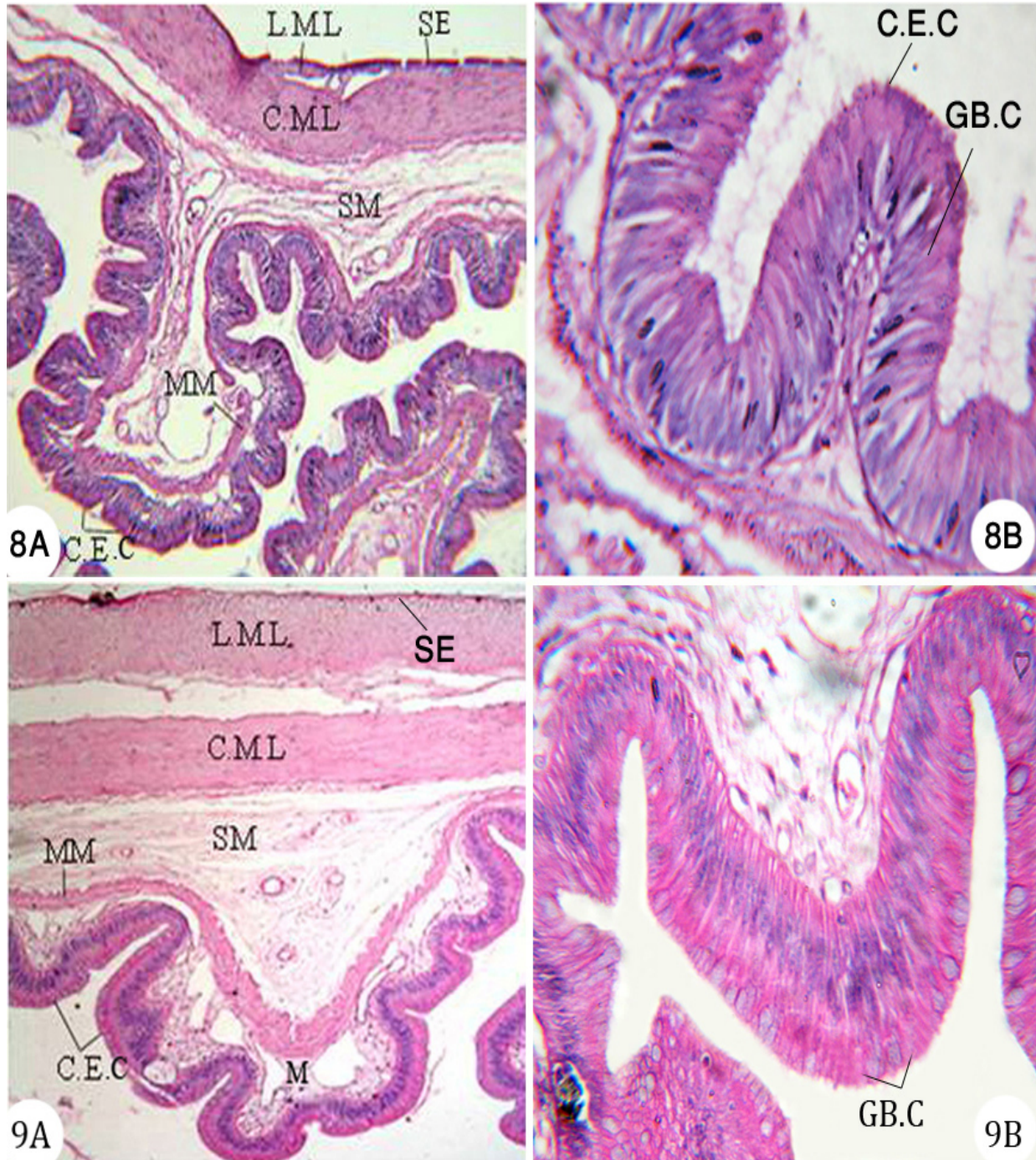
**Fig. (5A):** Photomicrograph of a transverse section of the stomach of *Uromastix aegyptiaca* showing the mucosa(M), submucosa(SM), muscularis mucosa (M.M), muscularis (circular and longitudinal layers C.M.L&L.M.L) and serosa(SE). H&E stain, X100.

**Fig. (5B):** Photomicrograph of enlarged portion of the stomach of *Uromastix aegyptiaca* showing the mucosa(M). H&E stain, X600.



- Fig. (6A):** Photomicrograph of a transverse section of the small intestine of *Uromastix aegyptiaca* showing the mucosa(M), muscularis mucosa(M.M), muscularis (circular and longitudinal layers C.M.L.&L.M.L) and serosa(SE). H&E stain, X100.
- Fig. (6B):** Photomicrograph of enlarged portion of the small intestine of *Uromastix aegyptiaca* showing the mucosa(M). H&E stain, X600.
- Fig. (7A):** Photomicrograph of a transverse section of the large intestine (Caecum) of *Uromastix aegyptiaca* showing the mucosa(M), submucosa(SM), muscularis mucosa(M.M), muscularis (circular and longitudinal layers C.M.L.&L.M.L) and serosa(SE), H&E stain, X60.
- Fig. (7B):** Photomicrograph of enlarged portion of the large intestine (Caecum) of *Uromastix aegyptiaca* showing the mucosa(M). H&E stain, X600.



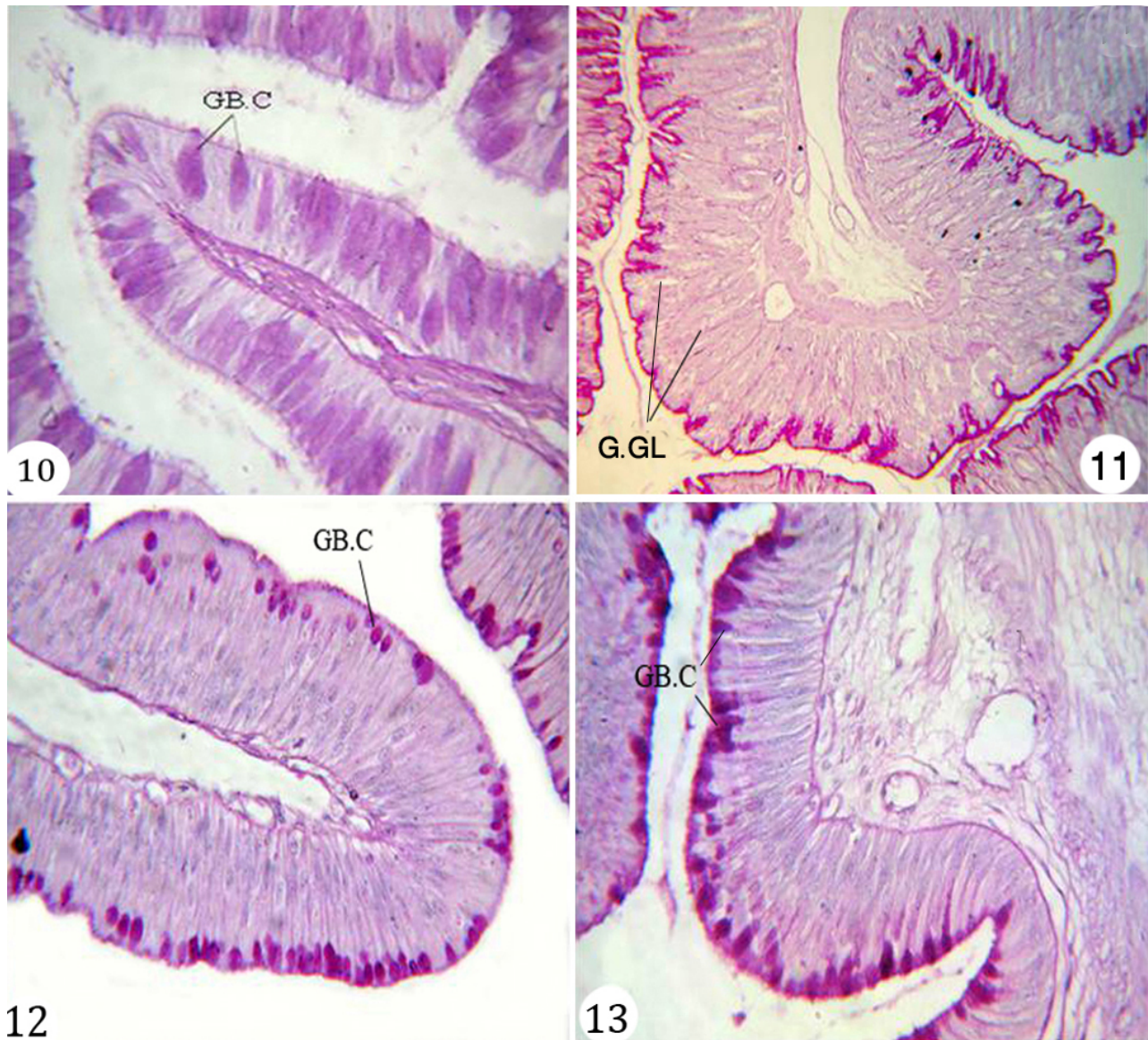


**Fig. (8A):** Photomicrograph of a transverse section of the large intestine ( Colon ) of *Uromastix aegyptiaca* showing the mucosa(M), submucosa(SM), muscularis mucosa(M.M) ,muscularis (circular and longitudinal layers C.M.L&L.M.L) and serosa(SE), H&E stain, X100.

**Fig. (8B):** Photomicrograph of enlarged portion of the large intestine ( Colon ) of *Uromastix aegyptiaca* showing the mucosa(M). H&E stain, X600.

**Fig. (9A):** Photomicrograph of a transverse section of the large intestine (Rectum) of *Uromastix aegyptiaca* showing the mucosa(M), submucosa(SM), muscularis mucosa(M.M) ,muscularis ( circular and longitudinal layers C.M.L&L.M.L) and serosa(SE), H&E stain, X100.

**Fig. (9B):** Photomicrograph of enlarged portion of the large intestine(Rectum) of *Uromastix aegyptiaca* showing the mucosa(M). H&E stain, X600.

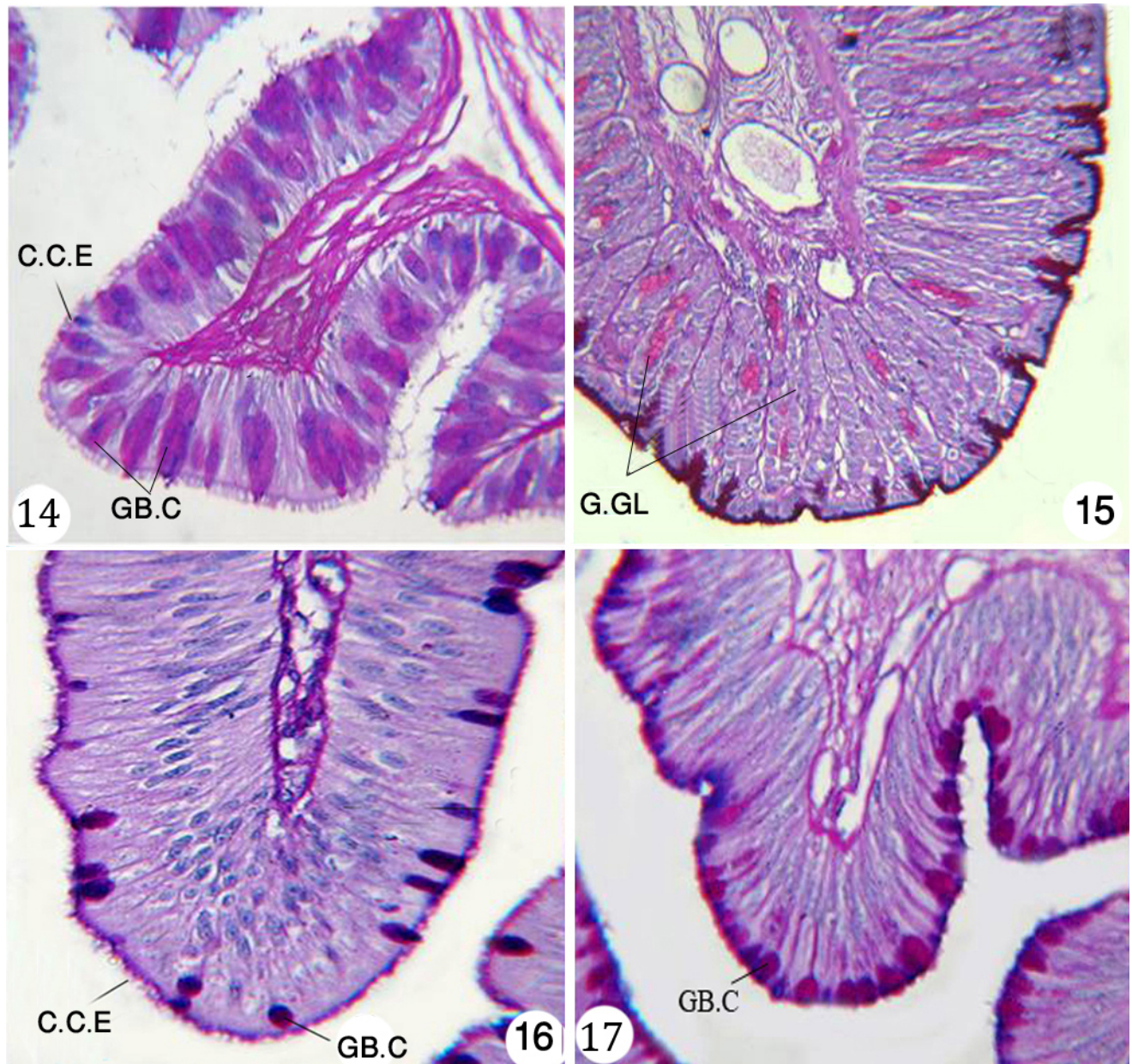


**Fig. (10):** Photomicrograph of a transverse section of the oesophagus of *Uromastix aegeptiaca* showing the carbohydrate content: (PAS positive stain) X 560.

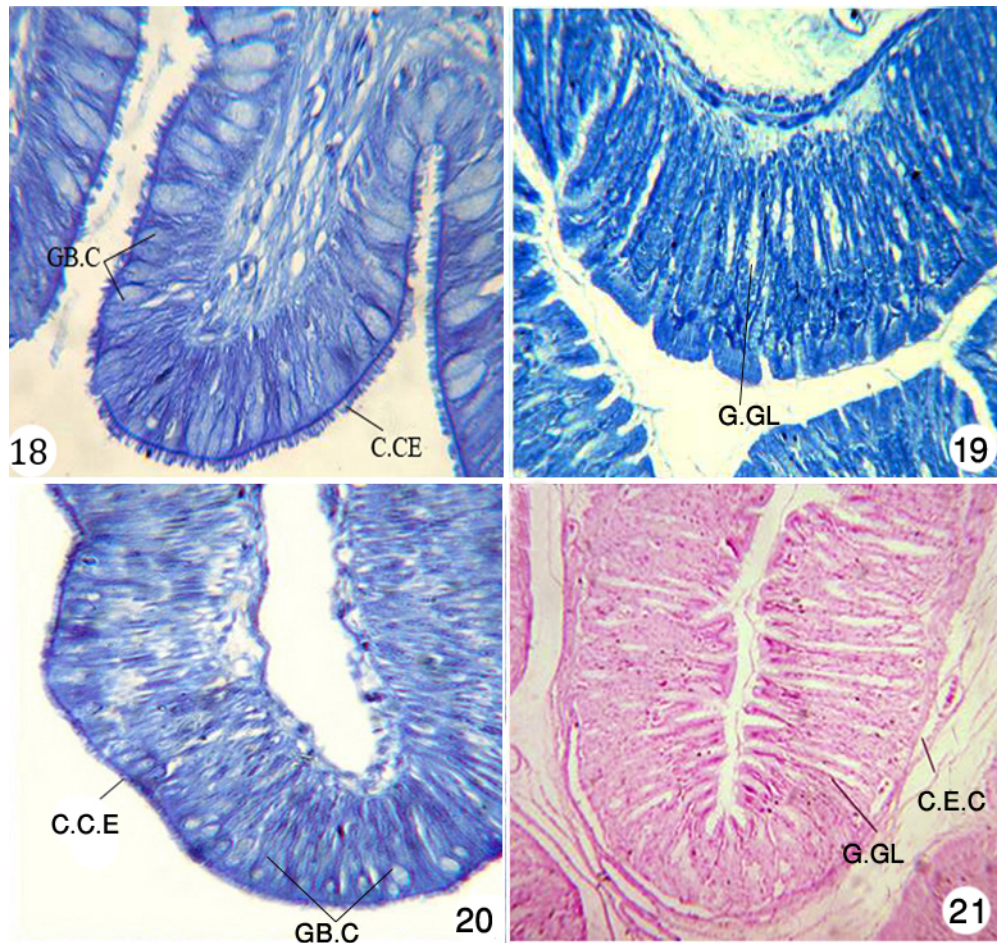
**Fig. (11):** Photomicrograph of a transverse section of the stomach of *Uromastix aegeptiaca* showing the carbohydrate content: (PAS positive stain) X 560.

**Fig. (12):** Photomicrograph of a transverse section of the small intestine of *Uromastix aegeptiaca* showing the carbohydrate content: (PAS positive stain) X 656.

**Fig. (13):** Photomicrograph of a transverse section of the large intestine (Rectum) of *Uromastix aegeptiaca* showing the carbohydrate content: (PAS positive stain) X400.



- Fig. (14):** Photomicrograph of a transverse section of the oesophagus of *Uromastix aegeptiaca* showing the mucopolysaccharide content. (Alcian blue stain) X656.
- Fig. (15):** Photomicrograph of a transverse section of the stomach of *Uromastix aegeptiaca* showing the mucopolysaccharide content. (Alcian blue stain) X164.
- Fig. (16):** Photomicrograph of a transverse section of the small intestine of *Uromastix aegeptiaca* showing the mucopolysaccharide content. (Alcian blue stain) X 656.
- Fig. (17):** Photomicrograph of a transverse section of the large intestine (Rectum) of *Uromastix aegeptiaca* showing the mucopolysaccharide content: (Alcian blue stain) X600.

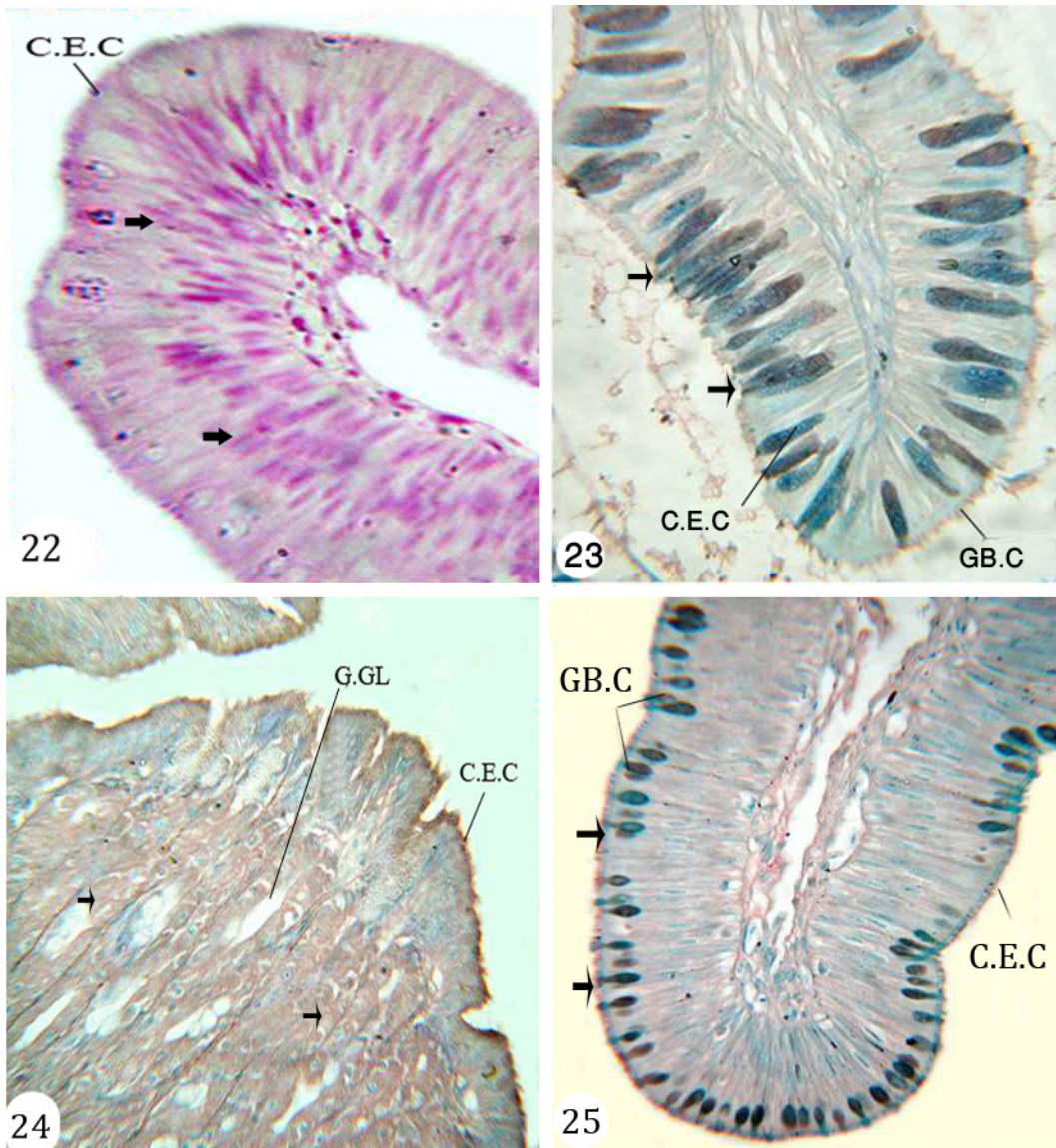


**Fig. (18):** Photomicrograph of a transverse section of the oesophagus of *Uromastix aegeptiaca* showing the protein content (Bromophenol blue stain) X 600.

**Fig. (19):** Photomicrograph of a transverse section of the stomach of *Uromastix aegeptiaca* showing the protein content (Bromophenol blue stain) X 164.

**Fig. (20):** Photomicrograph of a transverse section of the small intestine of *Uromastix aegeptiaca* showing the protein content (Bromophenol blue stain) X 600.

**Fig. (21):** Photomicrograph of a transverse section of the stomach of *Uromastix aegeptiaca* showing the DNA content (small arrow). (Feulgen technique;) X 140.



**Fig. (22) :** Photomicrograph of a transverse section of the small intestine of *Uromastix aegeptiaca* showing the DNA content (small arrow). ( Feulgen technique; ) X 560.

**Fig. (23):** Photomicrograph of a transverse section of the oesophagus of *Uromastix aegeptiaca* indicating the DNA (small arrow) and RNA (large narrow) contents (Methyl green-pyronine stain) X560.

**Fig. (24):** Photomicrograph of a transverse section of the stomach of *Uromastix aegeptiaca* indicating the DNA (small arrow) and RNA (large narrow) contents (Methyl green-pyronine stain) X 560.

**Fig. (25):** Photomicrograph of a transverse section of the small intestine of *Uromastix aegeptiaca* indicating the DNA (small arrow) and RNA ( large narrow) contents (Methyl green-pyronine stain) X400.

In the gecko *Ghyra mutilata* (Chou, 1977) this membrane is free from goblet cells, while in the gecko *Pristurus rupestris* (Dehlawi and Zaher, 1985b) it is evident, that the oesophageal mucosal membrane does not show a constant histological configuration among the so far investigated reptilian species related to different families. This membrane assumes different histological patterns in *Ablephorus pannonicus* (Greschik, 1917), *Chamaeleon vulgaris* (Bishai, 1960), *Uromastix philbyi* (Farang, 1982), *Chalcides*

*levitoni* (El-Taib *et al.*, 1982), *Mauremyes caspica* (El-Taib and Jarrar, 1983) and *Acanthodactylus boskianus* (Dehlawi and Zaher, 1985a) where the mucosal membrane of the anterior region of the oesophagus declares the present configuration, while it is composed of non-ciliated simple columnar and goblet cells in its posterior region.

The oesophageal mucosa of the studied species is characterized by the absence of glands. This finding confirms that found in *Agama stellio* (Amer and

Ismail, 1976), *Lacerta agilis*, *Natrix matrix* and *Vipera berus* (Pryzystaliski, 1980), *Agama adramitana* (Farang and El-Robai, 1986), *Natrix tessellata* (Abo-Taira et al., 1988b), *Coluber florlentus* (Dehlawi and Zaher, 1989; Afifi et al., 1990).

However, Jeksajewa and Koloss, (1964) and Dilmuhamidov(1975) confirmed the presence of tubuloalveolar mucous glands in the oesophagus of *Testudo graeca* and *Testudo horsfieldii*, respectively. Moreover, Farang and El-Robai (1986) revealed the presence of two types of glands in the oesophagus of *Scincus hemprichi*, one is probably mucous secreting and the other has cells with acidophilic granules. This may confirm that the presence or absence of oesophageal glands is independent of the mode of feeding. Additionally, there is no link between the animal's systematic position and the presence or absence of such glands.

The present study reveals that the oesophageal mucosa is thrown into several longitudinal folds. This feature is common for all the described reptiles e.g. *Uromastix philiby* (Farang, 1982) and *Pristurus rupestris* (Dehlawi and Zaher, 1985b).

The stomach of *Uromastix aegyptiaca* is differentiated into two clearly visible portions, the fundic and pyloric portions. This result agrees with that of Abo-Taira et al. (1988a) in *Acanthodactylus boskianus*, Zaher et al. (1989a) in *Mabuya brevicollis*, and Liquori et al. (2000) in *Podarcis sicula campestris*.

The muscularis layer is composed of a thin outer longitudinal layer and a well-developed inner circular one. This observation agrees with that of Zaher et al. (1989a) in *Mabuya brevicollis*.

The stomach of the examined species is characterized by the presence of a relatively thick gastric muscularis layer which is obviously a good adaptation for breaking up food to small pieces through strong muscular contractions.

The gastric mucosa of the fundic and pyloric portions of *Uromastix aegyptiaca* is thrown into prominent folds, which are lined by simple columnar epithelial cells. These columnar cells possess a homogeneous faintly stained cytoplasm and oval basally located nuclei. These findings are in accordance with those of Giraud et al. (1979) in *Tiliqua scincoides*, and Dehlawi and Zaher (1985b) in *Pristurus rupestris*. However, these findings disagree with those of Chou (1977) who stated that the gastric mucosa of *Ghyra mutilate* contains goblet cells. Moreover, the apical cytoplasm of these cells contains condensed secretory granules which have a positive PAS reaction. This result is in agreement with that of Abdeen et al. (1990b) in *Cerastes vipera*, and Zaher et al. (1990c) in *Eryx colubrinus*.

The gastric mucosa of *Uromastix aegyptiaca* is characterized by the presence of fundic and pyloric glands. This observation is in accordance with that of Amer and Ismail (1976) in *Agama stellio*, Farang (1982) in *Uromastix philbi* and Dehlawi and Zaher (1985b) in *Pristurus rupestris*. These glands are extensively coiled in the fundic portion, while they are simple and straight in the pyloric portion. This result agrees with that of Giraud et al. (1979) in *Tiliqua scincoides* and Zaher et al. (1989a) in *Mabuya brevicollis*.

The simple columnar cells and the glandular cells of the fundic portions of the stomach show moderate amounts of protein content, while their nuclei are deeply stained. This finding is in accordance with that with that of Abdeen et al. (1990b) in *Cerastes vipera*, and Zaher et al. (1990c) in *Eryx colubrinus*.

The pyloric glands of *Uromastix aegyptiaca* are lined by the glandular cells which possess a positive PAS reaction. These results indicate that the pyloric glands may be involved in the mucous secretion to facilitate the passage of food material, as previously reported by Abo-Taira et al. (1988a and 1988c) in *Acanthodactylus boskianus* and *Tarentola annularis annularis*, respectively, and Zaher et al. (1989a) in *Mabuya brevicollis*.

Examination of the mucosal epithelium of the small intestine, (duodenum and ileum) of the studied species revealed the absence of the intestinal glands. Such a condition was recorded in the intestinal mucosa of *Mabuya quinquetaeniata*, *Chalcides ocellatus* (Anwar and Mahmoud, 1975), *Agama stellio* (Amer and Ismail, 1976), and *Pristurus rupestris* (Dehlawi and Zaher, 1985b). On the contrary, the presence of the intestinal glands was recorded in the intestinal mucosa by Toro (1930) and Farang (1982) in crocodylians, and *Uromastix philipyi*, respectively.

Microscopic examination of the intestinal mucosa indicated the presence of extremely long and coiled villi to compensate the shortness of the small intestine. Such a histological feature may allow efficient absorption of the digested food.

The Lieberkühn crypts are described in reptiles by many authors. In *Uromastix aegyptiaca*, these crypts are found at the bases of the duodenal villi. They are branched and composed of columnar cells with thin outer borders. Goblet cells are also present. The columnar cells are provided with oval central nuclei, and their protoplasm is granular and darkly stained.

Similar structures were described in several reptiles and birds, as Jacobshagen (1915) gives a similar diagram in *Lotta lota*.

The villi of the duodenum, which are numerous, wavy and leaflike, contain central lymph spaces (i.e. lacteals). Fine fibres of the muscularis mucosa enter in the formation of these villi. The present investigation detects the absence of dudeno-ileac constriction which

is considered as a significant squamatic merit (Afifi *et al.*, 1990).

The present study revealed the complete absence of glandular crypts in the mucosa of the large intestine of the studied species. Such a condition is concordant to what were recorded by Farag (1982) and Dehlawi and Zaher, (1985b) in *Uromastix philipyi* and *Pristurus rupestris*, respectively.

In *Uromastix aegyptiaca*, the large intestine is composed of a large caecum, colon and rectum. The caecum is a large thin walled sac with a blind end. It is attached to the ileum from one side and to the colon from the opposite side. The caecal mucosa is thrown up into a limited number of longitudinal shallow pits. The simple tall columnar epithelium lining the mucosal surface is composed of absorptive cells which are provided with finely granular cytoplasm with elongated darkly stained nuclei situated at the middle or near the base. In between these cells, there are few goblet cells which are mucus-secreting in nature. The striated border is completely absent (Naguib, 1988 and 1991).

The mucosa of the colon is in the form of few large folds. It is thick and composed of two types of cells; tall columnar and few goblet cells. The columnar cells have finely granular cytoplasm and oval basally located nuclei. Below the epithelial layer there are one or more layers of cells that do not reach the surface. These are considered as replacing cells. Thus, the mucosa appears more or less stratified (El-Toubi and Bishai, 1958 ; Naguib, 1988).

The rectal mucosa has a series of small longitudinal folds, which increase gradually in length towards the posterior direction. These folds are large and broad. The mucous membrane is lined with simple columnar epithelium which contains numerous goblet cells. The relative length of the small and large intestine is obviously greater in herbivorous than in carnivorous and insectivorous reptiles, since the plant-origin food is more resistant to digestion than the animalized diet (Groombridge 1982; Hamlyn, 1989).

The present study revealed that a strong PAS-positive reaction was given by the mucosal epithelium of both the oesophagus and the stomach of *Uromastix aegyptiaca*, and the mucosa of the small and large intestine showed a moderate reaction. These observations are similar to those of Mousa *et al.* (1985) on the lizard and Dehlawi *et al.* (1987c), on the gecko *Pristurus rupestris*.

The present findings are similar to those in mammals (Amer, 1983 and El-Beih *et al.*, 1987), in that the goblet cells are the source of acid mucopolysaccharides, and that gastric mucosa is devoid of these substances, containing only neutral mucopolysaccharides. Similarly, Anwar and Mahmoud (1975) found that the goblet cell in the alimentary tract of the Egyptian lizards contains mucoid secretions of an acid mucoprotein nature.

These being more abundant in the rectum than in the ileum.

The present study reveals that the goblet cells show a strong acid mucopolysaccharide reactivity in the oesophagus and moderate reactivity in the small and large intestines. Mousa *et al.* (1985) also reported that the goblet cells of small and large intestine of the lizard gave a strong reaction for acid mucopolysaccharides. However, in contrast with the present results, Mousa and his co-workers claimed that the gastric glands of the lizard stomach showed a strong acid mucopolysaccharide reactivity.

The present data showed that in *Uromastix aegyptiaca*, the distribution of proteins in the cytoplasm of their gut mucosal cells is more or less identical. It is of interest to mention that the histochemical pattern of the proteins in the gut mucosa of the described animals is closely similar to the previously investigated reptiles (Anwar and Mahmoud, 1975; El-Taib and Jarrar, 1983; Zaher *et al.*, 1987 a, b and 1991 a, b; Zaher and Abdeen, 1991).

The present work showed also a proportional correlation between the RNA content and the proteonic amount of the cytoplasm of the mucosal epithelial cells in the different gut regions. This feature confirms the findings of Amer *et al.* (1987b), Zaher *et al.* (1987a), Zaher *et al.* (1990c, 1991a, b) and Zaher and Abdeen (1991).

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**Study of the Monocyte Chemoattractant Protein-1 (MCP-1) as a Biomarker of Lupus Nephritis Clinical Status**Samia MA Ramadan<sup>1</sup>, Yasser A Abdel-Hamid<sup>1</sup>, Hala A Agina<sup>2</sup>, Khaled M Belal<sup>3</sup> and Eman A Baraka<sup>1</sup><sup>1</sup>Rheumatology & Rehabilitation, <sup>2</sup>Pathology and <sup>3</sup>Clinical Pathology Departments. Benha Faculty of Medicine, Benha University  
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**Abstract: Background:** SLE is an unpredictable multi-systemic autoimmune life-long disease whose etiology and pathogenesis are incompletely understood. The monocyte chemoattractant protein-1(MCP-1) is a chemokine thought to be responsible for monocyte and T lymphocytes recruitment in acute inflammatory conditions and may be an important mediator in chronic inflammation. **Objective:** Aimed to measure the serum and urinary levels of MCP-1 in patients with SLE, to find out its relation to clinical disease activity and to explore its role in LN. We also aimed to investigate correlations of these levels with histopathological changes of renal biopsies in order to evaluate their reliability as biomarkers for LN clinical status. **Patients and Methods:** After an ethical approval, this correlative study was conducted on thirty SLE female patients and 20 apparently healthy volunteers- age and sex matched. Serum and urinary levels of MCP-1 were determined by the ELISA technique, while a transcutaneous renal biopsy was obtained from all patients. **Results:** There were significant differences in the mean levels of serum MCP-1 ( $P<0.05$ ) and urinary MCP-1 ( $P<0.001$ ) between SLE patients and controls. The mean level of the urinary MCP-1 was significantly higher ( $P<0.001$ ) in patients with active LN versus patients with inactive LN. There were insignificant differences ( $P>0.05$ ) between the mean levels of C3, C4, ANA, anti-dsDNA and serum MCP-1 regarding the WHO classification system of LN, meanwhile, the mean urinary MCP-1 level showed a significant difference ( $P<0.05$ ) among the histopathological groups. The diagnostic sensitivity and specificity of the urinary MCP-1 biomarker was greatest when it was combined with anti ds-DNA testing, being 88.8% sensitive and 100% specific. **Conclusion:** Urinary MCP-1 is a valuable, sensitive and non-invasive biomarker for the assessment of renal disease in SLE patients; it is well correlated with the clinical, laboratory and pathological parameters of LN activity. MCP-1 would be a potential therapeutic target in SLE. [Samia M. A. Ramadan, Yasser A Abdel-Hamid, Hala A Agina, Khaled M Belal and Eman A Baraka. **Study of the Monocyte Chemoattractant Protein-1 (MCP-1) as a Biomarker of Lupus Nephritis Clinical Status.** Life Sci J 2012;9(3):105-113]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 14

**Keywords:** lupus nephritis, LN, monocyte-chemoattractantprotein-1, MCP-1, systemic lupus erythematosus, SLE.**1. Introduction**

SLE is an unpredictable multi-systemic autoimmune life-long disease whose etiology and pathogenesis are incompletely understood. Clinical features are diverse and episodic with differing immunological manifestations. Both adults and children with SLE have a significant morbidity and mortality, which is closely related to renal involvement<sup>(1)</sup>. Renal disease affects the great majority of patients with SLE (~ 66-90%) sometime at the evolution with lupus nephritis (LN) occurring in more than one-third of patients<sup>(2)</sup>. Production of nephritogenic autoantibodies, glomerular immune complex deposition and cytokine overproduction has been postulated to contribute to the pathogenesis of LN<sup>(3)</sup>. The presence of subendothelial deposits in glomerular capillaries is crucial for the induction of severe damage and it correlates with end-capillary proliferation, necrosis, karyorrhexis and crescent formation<sup>(2)</sup>.

The monocyte chemoattractant protein-1(MCP-1) is a chemokine thought to be responsible for monocyte and T lymphocytes recruitment in acute inflammatory conditions and may be an important mediator in chronic inflammation<sup>(4)</sup>. The TNF superfamily cytokines-weak inducers of apoptosis (TWEAK) provoke the mesangial

cells, podocytes and endothelial cells to secrete pro-inflammatory chemokines including MCP-1, interleukin-10 which are crucial in the pathogenesis of LN<sup>(5)</sup>. Experimental data and studies on human renal tissue in patients with glomerulonephropathies indicated that MCP-1 plays a central role in progression of inflammatory processes in kidney diseases<sup>(6)</sup>.

As LN is a major cause of morbidity and mortality in SLE, it is important to identify reliable, noninvasive methods to repeatedly assess the condition of the kidneys in these patients<sup>(5)</sup>. Moreover, treatment of SLE nephritis is associated with side effects that could be mitigated if the onset, severity and response of renal flare could be predicted, and therapy modified accordingly<sup>(7)</sup>.

**2- Patients and Methods**

Thirty patients fulfilling at least four of the updated ACR revised criteria for the classification of SLE<sup>(8)</sup> were enrolled into this study. These patients were selected from the in-patients' and out-patients' clinics of the Rheumatology and Rehabilitation department of Benha University Hospitals through 2010. Twenty apparently healthy volunteers, age and sex matched to SLE patients, were carefully chosen as

a control group. This study was approved by the research ethical committee of our institution. Patients and controls were well educated about the study. They were clarified about objectives, importance, deliberate nature and procedures (including sequences and time they have to go through during the study). All patients were subjected to:

### 2.1-History taking:

Personal history, patients' complaint/s, history of present illness with stress on symptoms of renal disease, history of previous and present medications, past history and family history.

### 2.2-Clinical examination:

General examination, chest, cardiovascular, abdominal, neurological and musculoskeletal examinations.

### 2.3-Assessment of SLE disease activity:

According to Systemic Lupus Erythematosus Disease Activity Index [SLEDAI] score<sup>(9)</sup>. The renal SLEDAI (r SLEDAI) score consisted of 4 items of the total SLEDAI: hematuria, pyuria, proteinuria and urinary casts/HPF. The presence of each one of the 4 parameters takes a score of 4 points, thus the rSLEDAI score ranged from 0 to a maximum score of 16<sup>(9)</sup>. Patients should have a negative urine bacterial culture and/or a negative CRP testing in the absence of antibiotic treatment to exclude the active urinary sediment of urinary tract infection. Patients were also excluded from the study, if they had diabetes or other inflammatory diseases.

### 2.4- Laboratory investigations:

Five centimeters of venous blood were obtained for the measurement of the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete blood picture (CBC), renal function tests (serum creatinine, blood urea and creatinine clearance), antinuclear antibodies (ANA) titer, anti-dsDNA antibodies titer, complements (C3 and C4) levels and serum MCP-1. A fresh urine sample was obtained for complete urine analysis (urinary casts, hematuria, pyuria), urine protein: creatinine ratio and measurement of urinary MCP-1. Kits for serum and urinary of MCP-1 were obtained from **Bender Med systems GmbH, campus Vienna Biocenter 2, Austria, Europe** and levels were determined for all patients and control subjects by ELISA using a specific human monoclonal anti-human MCP-1 antibody according to Proost et al.<sup>(10)</sup>.

### 2.5-Obtaining, staining and scoring the renal biopsy:

A renal biopsy from each patient was obtained on the same day of blood sampling under computed tomography "CT" guidance using a true cut needle biopsy. Specimens were fixed in 10% formalin solution, embedded in paraffin, sliced into 4  $\mu$ m sections then stained with hematoxylin – eosin, for

examination under light microscopy using the criteria of the World Health Organization (WHO) classification system for grading of LN<sup>(11)</sup>.

The predominant histopathological class was reported for each sample. Activity and chronicity indices (AIS and CIS) respectively were used for biopsy assessment according to the standards of the National Institute of Health (NIH) for LN<sup>(12)</sup>.

### 2.6-Ethical considerations:

Our study followed the principles of Helsinki Declaration developed by the World Medical Association (WMA). An informed consent was obtained from all participants. Only individuals who accepted to participate were included in the study. The consent included data about objectives of the work. Participants were informed that all data are collected for scientific research only and will be confidential. No harmful techniques or interventions will be applied.

### 2.7-Statistical analysis:

Results of this study were tabulated and statistically analyzed by descriptive and analytic statistics on IBM personal computer using the SPSS version 6. For groups having quantitative variables with different variance, statistical results were further analyzed by Dunns' post hoc testing.

## 3-Results

This study included 30 patients with SLE. They were all females (100%) with a mean age of 27.7 $\pm$ 8.2 years and disease duration of 4.2  $\pm$  3 years. Patients and controls were matched for age ( $P>0.05$ ) and sex ( $P>0.05$ ). After proper examinations, SLE patients were stratified to one of 2 groups:

**Group I:** Included 6 female SLE patients (20%), who had never shown any past or present clinical and/or laboratory evidence of major renal manifestation attributable to SLE as well as having normal renal biopsies. Their mean age was 29.67  $\pm$  8.3 years while, their mean disease duration was 2  $\pm$  1.8 years.

**Group II:** Included 24 female SLE patients (80%) with renal disease based on the results of the renal biopsy. Their mean age was 28.7 $\pm$  8.7 years while; their mean disease duration was 4.8  $\pm$  3.1 years.

There was a significant difference between group I and II regarding their disease duration ( $P<0.05$ ). **Group II was subdivided into 2 subgroups:**

**Group II A:** including 18 female patients (75%) with active LN (rSLEDAI score  $\geq$  4) and **Group II B:** including 6 female patients (25%) with inactive renal disease (rSLEDAI score = 0).

Renal SLE disease affected 80% (24/30) of our patients, with 75% of them (18/24) having *active* LN, while 25% (6/24) of them had *inactive* LN. The prevalence of *active LN* in all SLE patients was 60% (18/30).

**Table (1):** Comparisons of MCP-1 levels among the studied groups

Group Variable	Serum MCP-1 (pg/ml) Mean $\pm$ SD	Urinary MCP-1 (pg/ml) Mean $\pm$ SD
**All SLE patients	192.7 $\pm$ 54.5	1790.5 $\pm$ 874.2
Group I (n = 6)	187.7 $\pm$ 33.2	700 $\pm$ 89.4
Group II (n = 24)	194 $\pm$ 59.1	2063.2 $\pm$ 757.6
Controls	150.8 $\pm$ 68.2	399.3 $\pm$ 85.6
F	2.3	56.5
P	< 0.05*	< 0.001*

\*Significant if ( $P < 0.05$ ).

\*\* There were statistically significant differences between the mean levels of either *serum* MCP-1 ( $P < 0.05$ ) or *urinary* MCP-1 ( $P < 0.001$ ) of SLE patients and the control group.

- **Post-hoc testing**, revealed that there was statistically significant difference in the mean level of the *serum* MCP-1 between group II and the control group ( $P < 0.05$ ), while there were insignificant differences between group I and group II as well as group I and the control group ( $P > 0.05$ ).

- The mean level of *urinary* MCP-1 was statistically significantly higher in group II than group I and the control group ( $P < 0.001$ ), while there was insignificant difference in the mean level of *urinary* MCP-1 between group I and the control group ( $P > 0.05$ ).

**Table (2):** Comparisons of laboratory parameters among the studied SLE patients' groups

Group Variable	Group I (n=6) Mean $\pm$ SD	Group IIA (n=18) Mean $\pm$ SD	Group IIB (n=6) Mean $\pm$ SD	F	P
ESR mm / 1 <sup>st</sup> hr	42.3 $\pm$ 6.8	71.9 $\pm$ 18.01	50 $\pm$ 15.5	9.2	<0.05*
Creatinine clearance ml/min	110 $\pm$ 8.9	60.4 $\pm$ 10.01	75 $\pm$ 4.5	10.53	<0.05*
S. creatinine mg/dl	0.97 $\pm$ 0.19	1.4 $\pm$ 0.5	0.8 $\pm$ 0.2	4.9	<0.05*
S. urea mg/dl	11.7 $\pm$ 6.8	23.9 $\pm$ 7.3	16.3 $\pm$ 3.7	8.5	>0.05
Urine ptn. to creatinine ratio	0.133 $\pm$ 0.086	1.634 $\pm$ 1.081	0.358 $\pm$ 0.056	9.5	<0.05*
C3 mg/dl	130.7 $\pm$ 15.00	87.8 $\pm$ 7.9	100.7 $\pm$ 15.00	33.7	<0.05*
C4 mg/dl	31 $\pm$ 9.1	14.8 $\pm$ 4.7	25 $\pm$ 11.8	12.4	>0.05
ANA U/ml	104.7 $\pm$ 29.2	120 $\pm$ 86.5	94.7 $\pm$ 19.1	1.02	>0.05
Anti -ds DNA U/ml	22.7 $\pm$ 8.5	109 $\pm$ 25.5	97.4 $\pm$ 35.02	11.03	<0.05*
Serum MCP-1 pg/ml	187.7 $\pm$ 33.2	199.2 $\pm$ 64.6	178.5 $\pm$ 38.7	2.3	>0.05
Urinary MCP-1 pg/ml	700 $\pm$ 89.4	2409.8 $\pm$ 516.3	1023.3 $\pm$ 60.9	56.5	<0.001*

\*Significant if ( $P < 0.05$ ).

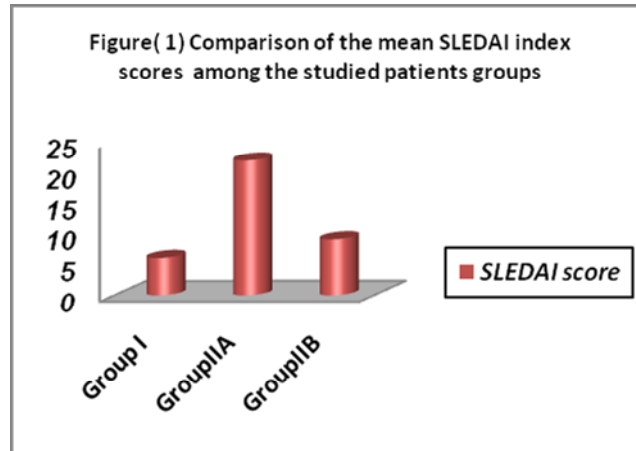
**Table (3):** Histopathological grading of renal biopsies of LN patients

Grade	II	III	IV
Frequency			
n = 24 (100%)	10(42%)	8 (33%)	6 (25%)

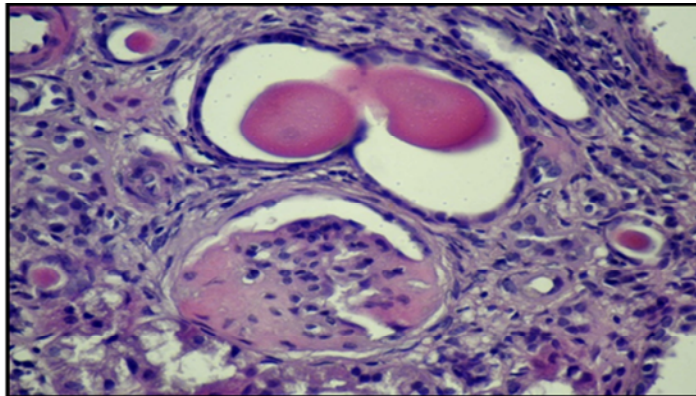
- **Post-hoc testing**, revealed statistically significant difference among SLE patients' groups as regards the mean value of ESR ( $P < 0.05$ ) being **higher** in group IIA, creatinine clearance ( $P < 0.05$ ) being **lower** in group IIA, urine protein to creatinine ratio ( $P < 0.05$ ) being **higher** in group IIA and the C3 level ( $P < 0.05$ ) being **lower** in group IIA.

- There was insignificant difference ( $P > 0.05$ ) between the mean level of *serum* MCP-1 in group IIA (active LN patients) and group IIB inactive LN patients.

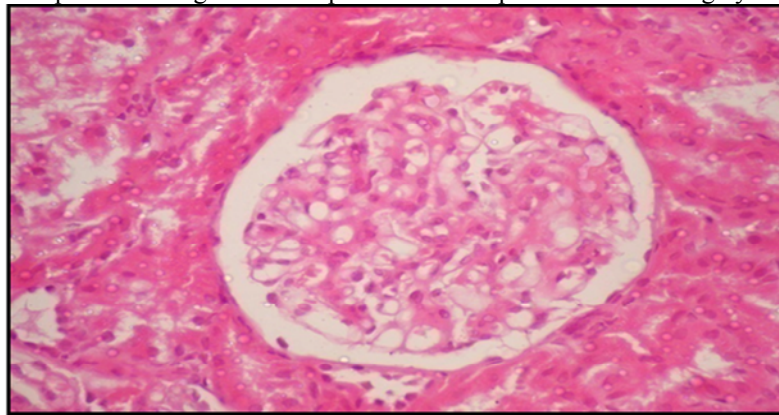
- There was highly statistically significant difference ( $P < 0.001$ ) between the mean value of *urinary* MCP-1 in group IIA (active LN patients) and group IIB (inactive LN patients).



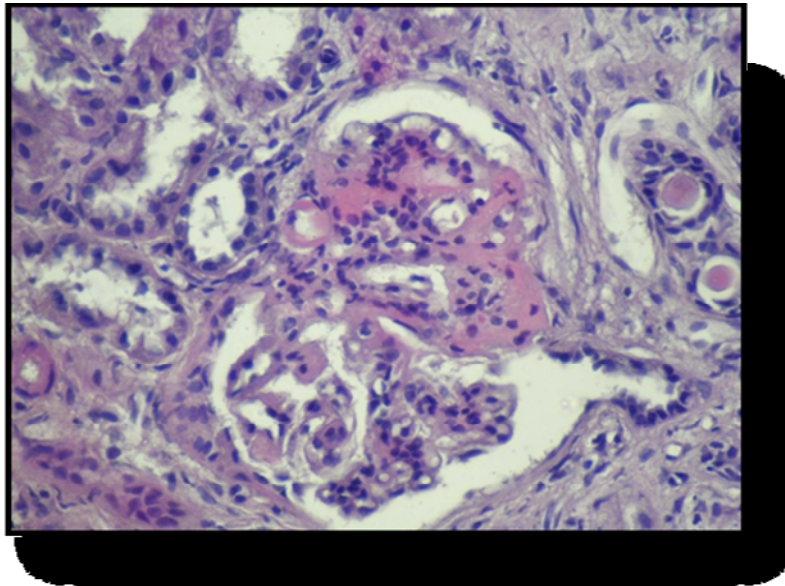
**Post-hoc testing**, revealed statistically significant increase in the mean SLEDAI score of group IIA versus group IIB and I ( $P < 0.05$ ), with an insignificant difference in the mean SLEDAI score between group IIB and group I ( $P > 0.05$ ).



**Figure (2):** Chronic proliferative glomerulonephritis with atrophic tubule showing hyaline casts (*H&E, X400*).



**Figure (3):** Glomerulonephritis class (III) with fibrinoid necrosis. The necrotizing lesions are associated with a clinical course of severe renal involvement and with greater probability of chronic glomerular changes. (*H&E, X400*).



**Figure (4):** Focal proliferative GN class III with segmental glomerular sclerosis (H&E X400).

**Table (4):** Pathological activity and chronicity indices scores in the LN patients' groups

Group Variable	Group IIA	Group IIB
Activity index score (max =24)		
Range	8-20	1-7
mean±SD	13.55 ± 4.36	3.66 ± 3.06
Chronicity index score (max =12)		
Range	1-7	3-5
mean±SD	3.67 ± 2.18	3.67 ± 3.33

**Table (5):** Comparison among laboratory parameters as regards histopathology

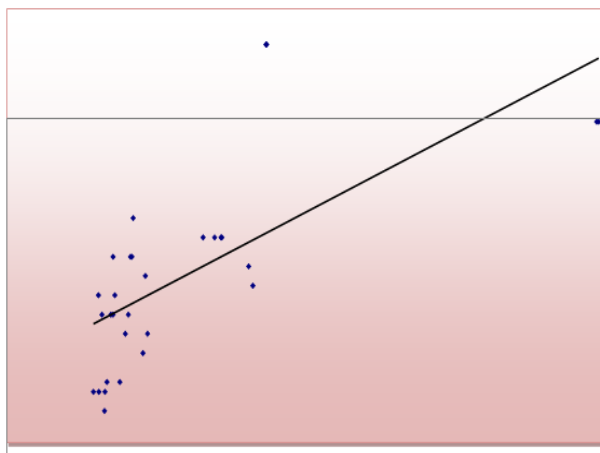
Class Variable	Class II n = 10 Mean ± SD	Class III n = 8 Mean ± SD	Class IV n = 6 Mean ± SD	F	P
C3 mg/dl	89.2 ± 1.92	95 ± 16.64	88.333 ± 16.07	3.05	>0.05
C4 mg/dl	17.8 ± 9.17	21 ± 9.7	11.66 ± 1.53	1.86	>0.05
ANA U/ml	101.8 ± 18.23	78.25 ± 29.84	181 ± 97.34	2.41	>0.05
Anti -ds DNA U/ml	85.8 ± 21.04	126.75 ± 21	89.33 ± 50.24	3.58	>0.05
Serum MCP-1 pg/ml	179.3 ± 48.1	189.5 ± 51.1	224.5 ± 81.8	1.1	> 0.05
Urinary MCP-1 Pg/ml	1517.6 ± 417.3	2042.5 ± 679.01	3000 ± 178.9	17.5	< 0.05*

\*Significant if ( $P < 0.05$ )

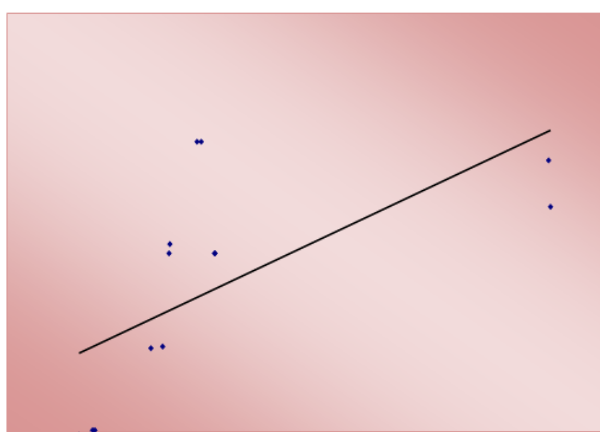
- Regarding **urinary** MCP-1 level, there was a statistically significant difference ( $P < 0.05$ )\* between groups, being **higher** in patients with class IV and **lower** in patients with class II.
- Regarding the clinical features in SLE patients, the mean **serum** MCP-1 level was significantly higher in the presence arthritis/arthralgia ( $P < 0.05$ ).
- The mean **urinary** MCP-1 level was significantly higher in patients with renal affection ( $P < 0.001$ ).
- There were statistically insignificant correlations ( $P > 0.05$ ) between **serum** MCP-1 levels and all

laboratory markers of SLE as well as SLEDAI score ( $r = 0.06$ ) or rSLEDAI score ( $r = 0.14$ ).

- There were statistically significant positive correlations between the **urinary** MCP-1 levels and ESR ( $r = 0.89$ ), anti-ds DNA titre ( $r = 0.44$ ), protein:creatinine ratio ( $r = 0.89$ ,  $P < 0.001$ ), SLEDAI score ( $r = 0.91$  rSLEDAI score and ( $r = 0.95$ ). In addition, there were statistically significant negative correlations with HB % ( $r = -0.68$ ), creatinine clearance ( $r = -0.89$ ) and C3 level ( $r = -0.28$ ).



**Figure (5):** Positive Correlation between the *urinary* MCP-1 levels and urine protein: creatinine ratio.



**Figure (6):** Positive Correlation of *urinary* MCP-1 levels with the rSLEDAI scores

**Table (6):** Correlations of laboratory parameters regarding activity and chronicity scores of renal biopsy in SLE patients

	Activity index scores		Chronicity index Scores	
	<i>r</i>	<i>P</i>	<i>R</i>	<i>P</i>
C3 mg/dl	0.15	> 0.05	2.98	> 0.05
C4 mg/dl	2.87	> 0.05	1.43	> 0.05
ANA u/ml	0.24	> 0.05	1.84	> 0.05
Anti-ds DNA u/ml	1.78	> 0.05	0.56	> 0.05
Serum MCP-1 pg/ml	0.14	> 0.05	0.18	> 0.05
Urinary MCP-1 Pg/ml	0.38	< 0.05*	0.02	> 0.05

\*Significant if ( $P < 0.05$ ).

$r$  = Spearman's correlation.

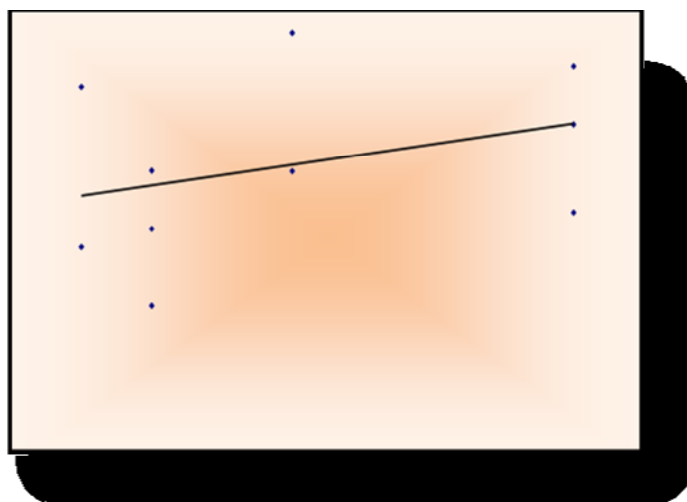
- There was only a statistically significant positive correlation ( $P < 0.05$ ) between *urinary* MCP-1 levels and

the activity scores ( $r = 0.38$ ) of the examined renal biopsies.

**Table (7):** Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPP) of anti-ds DNA antibody, serum and *urinary* MCP-1 in active LN

	Sensitivity	Specificity	PPV	NPV
Anti-ds DNA antibody	88.8%	83.3%	76.1%	33.3%
Serum MCP-1	33.3%	33.3%	60%	8.3%
Urinary MCP1	94.4%	83.3%	89.4%	80%
Urinary MCP-1& anti-ds DNA	88.8%	100%	88.8%	25%





**Figure (7):** Correlation between *urinary* MCP-1 levels and the activity scores of the renal biopsies in LN patients (Group II)

Sixteen patients in group IIA (88.8%) were found positive for both the anti-ds DNA antibody and the *urinary* MCP-1, while none of the patients in group IIB were found positive for both anti-ds DNA and the *urinary* MCP-1. This yielded a diagnostic sensitivity of 88.8%, specificity of 100%, PPV of 88.8% and NPV of 25% for both the anti-ds DNA antibody and the *urinary* MCP-1 in LN.

*Urinary* MCP-1 showed a higher diagnostic sensitivity of 94.4% and equal specificity of 83.3% to the anti-ds DNA antibody alone. The diagnostic sensitivity and specificity were greatest when both tests were found positive in combination being 88.8% for the diagnostic sensitivity and 100% for the diagnostic specificity

#### 4-Discussion

SLE is a chronic, relapsing inflammation and often febrile multi – systemic disorder of connective tissue, characterized principally by involvement of the skin, joints, kidney and serosal membranes. It is of unknown etiology, but is thought to represent a failure of the regulatory mechanisms of the autoimmune system<sup>(13)</sup>.

Conventional clinical parameters such as creatinine clearance, proteinuria, urine sediments, anti-dsDNA, and complement levels are not sensitive or specific enough for detecting ongoing disease activity in the lupus kidneys and early relapse of nephritis<sup>(14)</sup>

Renal biopsy is the gold standard for providing information on the histological classes of LN and the relative degree of activity and chronicity in the glomeruli. However, it is invasive and serial biopsies are impractical in the monitoring of LN. Thus, novel biomarkers that are able to discriminate lupus renal activity and its severity, predict renal flares, and monitor treatment response and disease progress are clearly necessary<sup>(15)</sup>.

It has been well established that proinflammatory chemokines play a critical role in the pathogenesis of experimental SLE nephritis (and the presence of chemokines in the urine of patients with SLE nephritis reflects intra-renal chemokine expression<sup>(16)</sup>).

In this study we found that renal affection (clinically and histopathologically) was present in 80% of patients with SLE, similar to *Kim et al.*<sup>(17)</sup>, who stated that renal involvement is clinically evident in 40% to 85% of SLE patients.

This was lower than the result of *Gigante et al.*<sup>(18)</sup>, who found that renal affection occurred in about 60% of their patients. *Galleli et al.*<sup>(19)</sup>, also reported that the incidence of LN was 50% in 60 patients.

The higher percentage in our study may be attributed to the diagnosis of LN based the results of the renal biopsy.

In our study, serum MCP-1 levels were significantly high ( $P < 0.05$ ) in SLE patients ( $192.7 \pm 54.5$  pg/ml) compared to the controls ( $150.8 \pm 68.2$  pg/ml). These levels were even significantly higher in patients with LN ( $194 \pm 59.1$  pg/ml) than the controls ( $P < 0.05$ ). Insignificant difference ( $P > 0.05$ ) was found between patients without nephritis ( $187.7 \pm 33.2$  pg/ml) and either those with nephritis or the controls. These results were also in agreement with *Alzawawy et al.*<sup>(20)</sup>.

*Lit et al.*<sup>(21)</sup>, found that the plasma concentration of inflammatory cytokines including MCP-1 were significantly higher in all SLE patients than healthy individuals. However, when their patient's cohort was stratified into renal involvement subgroups analysis, patients with renal involvement had a lower plasma concentration of MCP-1. They suggested that urine chemokines could serve as biomarkers for renal disease flare and that the lack

of significant increase in the circulating levels of serum MCP-1 in patients with nephritis was due to the possibility of excretion of locally produced MCP-1 into urine rather than circulating in the blood, and to the extremely short half life of MCP-1 in the serum.

Urinary MCP-1 levels, were significantly higher ( $P < 0.001$ ) in our SLE patients and in those with nephritis ( $P < 0.05$ ) than controls, while, they were insignificantly different between those without LN and the control group ( $P > 0.05$ ). This indicates that the difference between all SLE patients and controls was primarily due to patients who had LN. These results were in agreement with *Alzawawy et al.*<sup>(20)</sup> and *Tucci et al.*<sup>(3)</sup>.

This study revealed that there was insignificant statistical difference ( $P > 0.05$ ) in the mean serum MCP-1 level between patients with active nephritis and those with inactive nephritis. This difference was significantly high ( $P < 0.001$ ) regarding the mean urinary MCP-1 level.

*Rovin et al.*<sup>(22)</sup>, obtained serial measurement of urine chemokines in a cohort of prospectively followed SLE patients and found that urinary MCP-1 increases significantly at renal flare. They also reported changes of the urinary MCP-1 overtime paralleled the onset and the resolution of flare. Non-renal SLE flares were not accompanied by increase in urinary MCP-1 indicating that urine chemokines do not reflect general systemic SLE activity.

Our study showed that there were insignificant correlations of serum MCP-1 levels with all laboratory parameters of SLE.

As regards to urinary MCP-1 levels, we found them correlated positively with the anti-dsDNA antibody titer ( $r = 0.44$ ,  $P < 0.05$ ) and negatively with C3 ( $r = -0.81$ ,  $P < 0.05$ ). This finding was similar to those found by *Kiani et al.*<sup>(23)</sup>, *Tucci et al.*<sup>(3)</sup> and *Jason et al.*<sup>(24)</sup>.

Our study showed a highly significant positive correlation of urinary MCP-1 with rSLEDAI score ( $P < 0.001$ ) and insignificant correlation with the total SLEDAI score ( $P > 0.05$ ). This emphasized that elevations of urinary MCP-1 in SLE patients were specific to renal activity.

In addition, *Kiani et al.*<sup>(23)</sup>, showed that urinary MCP-1 was associated with measures of disease activity including patient's Global assessment (PGA), and the SLEDAI using SLEDAI renal descriptors.

*Chan et al.*<sup>(25)</sup>, stated that the urinary MCP-1 correlated with the degree of proteinuria ( $r = 0.73$ ,  $P < 0.001$ ) and serum creatinine level ( $r = 0.45$ ,  $P < 0.001$ ).

We found insignificant correlation of urinary MCP-1 with serum MCP-1 levels. However, *Tucci et al.*<sup>(3)</sup>, indicated that urinary MCP-1 levels reflect

predominantly local production of this chemokine rather than simple filtration.

Urinary MCP-1 levels, varied significantly ( $P < 0.05$ ) among the histopathological groups. *Seshan and Chales*<sup>(26)</sup>, explained the difference in levels of urinary MCP-1 among patients with WHO class IV, V and II by decreased cellularity in glomerular and tubulo-interstitial lesions in WHO class I and class II than WHO class IV.

On the other hand, there was statistically significant positive correlation ( $r = 0.38$ ;  $P < 0.05$ ) of urinary MCP-1 levels with the activity scores of the examined renal biopsies, while there was insignificant correlation ( $r = 0.02$ ;  $P > 0.05$ ) with the chronicity scores. This confirmed the findings of *Tucci et al.*<sup>(3)</sup>. These results further support the notion that MCP-1 may contribute to the development of renal lesions.

*Marks et al.*<sup>(27)</sup>, stated that genetic polymorphism of MCP-1 gene may predispose to the development of SLE and found that certain genotypes are at higher risk of developing LN through modulating MCP-1 expression. This would suggest its possible role in etiopathogenesis of LN with the useful monitoring of MCP-1 as a marker of disease activity.

## Conclusion

Urinary MCP-1 is a valuable, sensitive and non-invasive biomarker for the assessment of LN in SLE patients. It is well correlated with the clinical, laboratory and pathological parameters of LN activity. MCP-1 would be a potential therapeutic target in SLE.

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## Increased Serum Visfatin Levels in Patients with Type2 Diabetic Patients

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**Abstract: Background:** Obesity is highly associated with insulin resistance and the increased risk of type 2 diabetes and cardiovascular diseases. Visfatin is a cytokine that is highly expressed in visceral fat. Its expression in visceral fat is increased in obese subjects. Visfatin exerts insulin-mimetic effects and was as effective as insulin in reducing hyperglycemia in insulin-deficient diabetic mice. **Objective:** To study serum visfatin level in patients with type2 diabetes mellitus and to clarify its possible relationship with insulin resistance and glycemic control. **Patients and Methods:** Eighty cases (60 type2 diabetic patients and 20 apparently healthy subjects) were subjected for estimation of visfatin and fasting insulin by ELISA technique together with routine laboratory investigations including fasting blood glucose, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and HbA<sub>1C</sub>. **Results:** Fasting blood glucose, fasting insulin level, HOMA-IR, HbA<sub>1C</sub> and visfatin levels were significantly higher in diabetic than control group. HbA<sub>1C</sub>, fasting serum glucose and serum visfatin were significantly higher in uncontrolled than controlled diabetic subgroups. Weight, BMI and serum visfatin were significantly higher in overweight diabetic subgroup than normal BMI diabetic subgroup. A positive significant correlation was found between serum visfatin & HbA<sub>1C</sub> levels in the diabetic group. **Conclusion:** The increased serum visfatin concentration may be a compensatory mechanism aimed at ameliorating the functional consequences of insulin deficiency. The increased visfatin concentration may also promote insulin sensitivity by its stimulatory effects on insulin receptors.

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**Keywords:** Obesity, type2 diabetes mellitus, visfatin.

### 1. Introduction

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs<sup>(1)</sup>.

Type 2 is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically manifest. By definition, the specific reasons for the development of these abnormalities are not yet known<sup>(2)</sup>. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications<sup>(3)</sup>.

The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance<sup>(4)</sup>. Insulin resistance is often found in people with visceral adiposity (i.e. a high degree of fatty tissue underneath the abdominal muscle wall - as distinct from subcutaneous adiposity or fat between the skin and the muscle wall, especially elsewhere on the body, such as hips or thighs), hypertension, hyperglycemia and dyslipidemia involving elevated triglycerides, small dense low-density lipoprotein (sdLDL) particles, and decreased HDL cholesterol levels. With respect to visceral adiposity, a great deal

of evidence suggests two strong links with insulin resistance. First, unlike subcutaneous adipose tissue, visceral adipose cells produce significant amounts of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ), and Interleukins-1 and -6, etc<sup>(5)</sup>. Additionally, insulin resistance is found in hypertension, hyperlipidemia, and ischemic heart disease, entities commonly found in association with diabetes<sup>(6)</sup>, again raising the question as to whether insulin resistance results from different pathogenetic disease processes or is unique to the presence of type 2 diabetes<sup>(7)</sup>.

In numerous experimental models, these proinflammatory cytokines profoundly disrupt normal insulin action in fat and muscle cells, and may be a major factor in causing the whole-body insulin resistance observed in patients with visceral adiposity. A great deal of attention into the production of proinflammatory cytokines has focused on the IKK-beta/NF-kappa-B pathway, a protein network that enhances transcription of cytokine genes. Second, visceral adiposity is related to an accumulation of fat in the liver, a condition known as nonalcoholic fatty liver disease (NAFLD). The result of NAFLD is an excessive release of free fatty acids into the blood stream (due to increased lipolysis), and an increase in hepatic glucose production, both of which have the effect of exacerbating peripheral insulin resistance and increasing the likelihood of type 2 diabetes mellitus<sup>(5)</sup>.

Insulin resistance is also occasionally found in patients who use insulin. In this case, the production of antibodies against insulin leads to lower-than-expected glucose level reductions after a specific dose of insulin. With the development of human insulin and analogues in the 1980s and the decline in the use of animal insulin (e.g. pork, beef), this type of insulin resistance has become uncommon<sup>(8)</sup>.

Obesity is highly associated with insulin resistance and the increased risk of type 2 diabetes and cardiovascular diseases<sup>(9)</sup>. The accumulation of adipose tissue in the abdominal visceral depot is especially correlated with insulin resistance<sup>(10)</sup>. Visfatin, also known as pre-B cell colony-enhancing factor, is a cytokine that is highly expressed in visceral fat and was originally isolated as a secreted factor that synergizes with IL-7 and stem cell factors to promote the growth of B cell precursors<sup>(11)</sup>. Fukuhara *et al.*<sup>(12)</sup> found that visfatin expression in visceral fat is increased in obese subjects and that plasma concentration of visfatin correlated much more strongly with the amount of visceral fat than that of subcutaneous adipose tissue. Visfatin exerts insulin-mimetic effects that are dose dependent and quantitatively similar to those of insulin in stimulating muscle and adipocyte glucose transport and in inhibiting hepatocyte glucose production. In keeping with its insulin-mimetic effects, visfatin was as effective as insulin in reducing hyperglycemia in insulin-deficient diabetic mice. Visfatin was also found to be bound to and activate insulin receptor, causing receptor phosphorylation and the activation of downstream signaling molecule. However, visfatin and insulin did not compete for binding to the insulin receptor, indicating that the two proteins were recognized by different regions of the receptor<sup>(12)</sup>. The aim of the present work is to study serum visfatin level in patients with type2 diabetes mellitus and to clarify its possible relationship with insulin resistance and glycemic control.

## 2. Subjects and Methods:

The study was conducted on 80 subjects aged from 35-60 years; they were divided into two groups; group I (*patient group*) and group II (*control group*). Group I (*patient group*) included sixty type 2 diabetic patients (45 males & 15 females). They were recruited from internal medicine outpatient clinic of Benha University Hospital. This group was further subdivided according to body mass index (BMI), insulin resistance (IR) and glycemic control. Group II (*control group*) included twenty age and sex matched apparently healthy subjects (12 males & 8 females). Exclusion criteria were patients with diabetes mellitus on insulin treatment, patients with other endocrine disorders, patients with hepatic, renal, cardiac disease or chronic infections and pregnant females or those receiving oral contraceptives.

## The following was done for all subjects included in the study:

- A. Full clinical history.
- B. Anthropometric measurements:
  - Weight and height.
  - Body Mass Index (weight in kilograms divided by height in squared meters).
- C. Laboratory investigations:
  - Blood samples were drawn from all subjects after overnight fasting (10-16 hours) by veinpuncture:
    1. Two milliliters were anticoagulated using EDTA for determination of HbA<sub>1c</sub>.
    2. Two milliliters were anticoagulated using sodium fluoride for determination of fasting blood glucose level.
    3. Four milliliters were placed in plain test tubes and allowed to clot for 30 minutes in water bath at 37°C and then centrifuged for 15 minutes at 1000 xg. Serum was then subdivided into two aliquots:
      - a. The first aliquot was used for lipid profile assay.
      - b. The second aliquot was used for insulin and visfatin assays. This aliquot was kept at -70°C for subsequent assay.

## Methodology:

1. Fasting blood glucose, cholesterol, triglycerides, and HDL-cholesterol were performed by automated enzymatic methods (Cobas Integra 400 analyzer, Roche, Germany). LDL cholesterol were calculated according to Friedwald formula:  

$$\text{LDL cholesterol} = \text{Total cholesterol} - \text{HDL cholesterol} - \text{TG}/5.$$
2. Fasting insulin level was assessed through chemiluminescence immunoassay technique using Autobio, Zhengzhou, China kit. The insulin chemiluminescence test is a solid phase two-site immunoassay.
3. Glycated hemoglobin (HbA<sub>1c</sub>) level was assessed through HPLC technique using Bio - Rad, Hercules, USA kit.
4. Serum visfatin level was assessed through enzyme-linked immunosorbent assay (ELISA) supplied by Adipogen, Soul, Korea. Monoclonal antibody specific for human visfatin had been precoated onto microtiter plate. Standards and samples were pipetted into the wells and any visfatin present is bound by immobilized antibody. The wells are washed and biotinylated anti-human visfatin was added. After washing away unbound biotinylated antibody, hoarse radish peroxidase conjugated anti-rabbit IgG was added. After washing, a substrate solution was added. The color developed in proportion to the amount of visfatin bound. The color development was stopped and the intensity of color was measured at 450 nm.

Calculation of homeostasis model assessment (HOMAIR): [fasting blood glucose (mmol/L) × fasting blood insulin (μU/ml)/22.5].

### Statistical Methods:

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 16. For quantitative data, mean and standard deviation were calculated. Student "t" test: used to test the significance of the difference between two groups.

The association of serum visfatin level with continuous variables was tested with Pearson's correlation. P value was considered significant if  $P < 0.05$  & not significant if  $P > 0.05$ .

### 3. Results:

Parameters including fasting blood glucose, fasting insulin level, HOMA-IR, HbA<sub>1c</sub> and visfatin levels were significantly higher in diabetic than control group while parameters including age, weight, height, BMI, total cholesterol, triglycerides, HDL-cholesterol

& LDL-cholesterol levels showed non significant difference between both groups.

A positive significant correlation was found between serum visfatin & HbA<sub>1c</sub> levels in the diabetic group and no significant correlations were found between serum visfatin and all other parameters in the same group.

HbA<sub>1c</sub>, fasting serum glucose and serum visfatin were significantly higher in uncontrolled than controlled diabetic subgroups while no significant differences were found between age, weight, height, BMI, fasting insulin, HOMA-IR, total cholesterol, triglycerides, HDL-cholesterol & LDL-cholesterol in the same subgroups.

Weight, BMI and serum visfatin were significantly higher in overweight diabetic subgroup than normal BMI diabetic subgroup. No significant differences were found between age, height, HbA<sub>1c</sub>, fasting blood glucose, fasting insulin, HOMA-IR, total cholesterol, triglycerides, HDL-cholesterol & LDL-cholesterol in the same subgroups.

Table (1): The clinical and laboratory parameters of studied groups:

Parameter	T2DM (N=60) Mean ± SD	Controls (N=20) Mean ± SD	p value	Sig.
Age (years)	47.4 ± 6.4	46.6 ± 5.8	0.607	NS
Weight (kg)	85.0 ± 12.5	83.7 ± 10.8	0.688	NS
Height (meters)	1.71 ± 6.9	1.73 ± 6.6	0.525	NS
BMI (kg/m <sup>2</sup> )	28.5 ± 4.2	27.4 ± 0.7	0.460	NS
Fasting glucose (mg/dl)	159 ± 54.0	95 ± 9.0	0.001	S
Fasting Insulin (μU/mL)	17.0 ± 8.6	9.2 ± 4.9	0.001	S
HOMA-IR	6.2 ± 2.9	2.2 ± 1.2	0.001	S
HbA <sub>1c</sub> (%)	8.1 ± 2.3	4.9 ± 0.7	0.001	S
Total cholesterol (mg/dl)	206 ± 41	180 ± 30	0.0819	NS
Triglycerides (mg/dl)	154 ± 77	145 ± 66	0.641	NS
HDL cholesterol (mg/dl)	48 ± 14	47 ± 14	0.783	NS
LDL cholesterol (mg/dl)	127 ± 33	117 ± 25	0.068	NS
Visfatin (ng/ml)	22.9 ± 17.5	14.4 ± 4.2	0.036	S

p value is significant (S) if  $< 0.05$  and non significant (NS) if  $> 0.05$ .

Table (2): Visfatin level correlations in the diabetic group:

Parameter	p value	Significance
Age (years)	0.062	NS
Weight (kg)	0.027	S
Height (meters)	0.165	NS
BMI (kg/m <sup>2</sup> )	0.042	S
HbA <sub>1c</sub> (%)	0.001	S
Fasting Blood Glucose (mg/dl)	0.175	NS
Insulin (μU/mL)	0.762	NS
HOMA-IR	0.625	NS
Total cholesterol (mg/dl)	0.385	NS
Triglycerides (mg/dl)	0.621	NS
HDL-cholesterol (mg/dl)	0.062	NS
LDL-cholesterol (mg/dl)	0.173	NS

p value is considered significant (S) if  $< 0.05$  and non significant (NS) if  $> 0.05$ .

Table (3): The clinical and laboratory parameters of controlled &amp; uncontrolled diabetic subgroups:

Parameter	Uncontrolled subgroup (N=27) Mean $\pm$ SD	Controlled subgroup (N= 33) Mean $\pm$ SD	P value	Sig.
Age (years)	48.9 $\pm$ 6.7	46.2 $\pm$ 5.9	0.0988	NS
Weight (kg)	85.9 $\pm$ 12.0	84.2 $\pm$ 12.8	0.5823	NS
Height (meters)	1.71 $\pm$ 0.07	1.71 $\pm$ 0.06	0.7190	NS
BMI(kg/m <sup>2</sup> )	29.2 $\pm$ 4.6	28.7 $\pm$ 3.8	0.6355	NS
HbA1C(%)	10.0 $\pm$ 1.9	6.6 $\pm$ 0.9	<0.001	S
Fasting Blood Glucose (mg/dl)	182 $\pm$ 65	139 $\pm$ 32	0.0015	S
Fasting Insulin ( $\mu$ U/mL)	16.3 $\pm$ 8.8	17.6 $\pm$ 8.5	0.5641	NS
HOMA-IR	6.6 $\pm$ 2.8	5.9 $\pm$ 2.9	0.264	NS
Total cholesterol (mg/dl)	210 $\pm$ 44	202 $\pm$ 39	0.4586	NS
Triglycerides (mg/dl)	166 $\pm$ 86	145 $\pm$ 69	0.2982	NS
HDL-cholesterol (mg/dl)	48 $\pm$ 17	47 $\pm$ 11	0.7843	NS
LDL-cholesterol (mg/dl)	129 $\pm$ 34	126 $\pm$ 32	0.7267	NS
Serum Visfatin (ng/ml)	32.0 $\pm$ 18.8	15.4 $\pm$ 12.1	0.01	S

p value is considered significant (S) if <0.05 and non significant (NS) if > 0.05.

Table (4): The clinical and laboratory parameters of normal &amp; overweight diabetic subgroups:

Parameter	Normal BMI group (N=10) Mean $\pm$ SD	Overweight group (N= 50) Mean $\pm$ SD	p value	Sig.
Age (years)	47.7 $\pm$ 4.8	47.3 $\pm$ 6.7	0.8603	NS
Weight (kg)	69.6 $\pm$ 4.5	88.0 $\pm$ 11.3	<0.001	S
Height (meters)	1.73 $\pm$ 0.04	1.71 $\pm$ 0.07	0.3870	NS
BMI(kg/m <sup>2</sup> )	23.2 $\pm$ 1.4	30.1 $\pm$ 3.5	<0.001	S
HbA1C(%)	8.5 $\pm$ 2.7	8.0 $\pm$ 2.2	0.53	NS
Fasting Blood Glucose (mg/dl)	152 $\pm$ 40	160 $\pm$ 57	0.6745	NS
Fasting Insulin ( $\mu$ U/mL)	15.1 $\pm$ 9.9	17.4 $\pm$ 8.4	0.4458	NS
HOMA-IR	5.3 $\pm$ 3.1	6.4 $\pm$ 2.8	0.2696	NS
Total cholesterol (mg/dl)	185 $\pm$ 46	210 $\pm$ 39	0.0766	NS
Triglycerides (mg/dl)	135 $\pm$ 42	158 $\pm$ 82	0.3931	NS
HDL-cholesterol (mg/dl)	40 $\pm$ 13	49 $\pm$ 13	0.0504	NS
LDL-cholesterol (mg/dl)	117 $\pm$ 36	129 $\pm$ 32	0.2931	NS
Serum Visfatin (ng/ml)	11.7 $\pm$ 12.3	25.1 $\pm$ 17.6	0.0256	S

p value is considered significant (S) if <0.05 and non significant (NS) if > 0.05.

#### 4. Discussion:

Not all adipokines are diabetogenic. Some of them may be protective against insulin resistance and T2DM. The best example is adiponectin, which has insulin-like effects in liver and muscle and also acts as an insulin sensitizer<sup>(13)</sup>. The adipocyte production of adiponectin is decreased in insulin resistant states, and a low circulating adiponectin level is an independent risk factor for T2DM<sup>(13)</sup>.

An adipose-tissue-derived protein termed visfatin was described with putative antidiabetogenic properties<sup>(13)</sup>. Visfatin was reported to be expressed almost exclusively in visceral adipose tissue and has insulin-like metabolic effects<sup>(12)</sup>. Visfatin molecule was previously identified as a growth factor for early B-lymphocytes termed pre-B cell colony enhancing factor (PBEF)<sup>(11)</sup>. However, the visfatin gene is expressed in

adipocytes, where it is subjected to regulation<sup>(14)</sup>. Furthermore, in humans the gene is expressed predominantly in visceral as compared with subcutaneous human adipose tissue<sup>(15)</sup>. These findings are exciting news and could provide a novel mechanism by which visceral fat accumulation can promote the development of T2DM<sup>(16)</sup>. In particular, effects of visfatin on the liver could be of importance for T2DM and other insulin-resistant disorders because of the portal delivery.

According to the results of this study, age, sex, weight, height and BMI total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol were not significantly different between the patient and control group while HbA<sub>1c</sub>, fasting blood glucose, fasting insulin level & HOMA-IR were significantly higher in the patient group.

According to the results of this study, a significantly higher visfatin/PBEF/Nampt levels were found in the diabetic group when compared with controls. This coincides with *Dogru et al.*,<sup>(17)</sup> who studied visfatin levels in 40 subjects with newly diagnosed diabetes or glucose intolerance and found that visfatin levels were higher in the diabetic patients when compared to controls, but not when compared to glucose intolerant patients (pre-diabetes). Results obtained in this study also coincides with *Chen et al.*,<sup>(18)</sup> who reported that in patients with T2DM (61 patients with T2DM and 59 sex- and age-matched controls), visfatin/PBEF/Nampt plasma levels were significantly increased in T2DM compared with controls, and a significant correlation between plasma visfatin/PBEF/Nampt and T2DM persisted even after adjustment for known biomarkers influencing glucose metabolism, such as age, gender, BMI, WHR, SBP (systolic BP), DBP (diastolic BP), lipid profile and smoking status. The results also coincide with those obtained by *Lopez-Bermejo et al.*,<sup>(19)</sup> who reported that circulating visfatin/PBEF/Nampt was increased in subjects with known T2DM compared with non-diabetic subjects, but not in newly diagnosed subjects with T2DM compared with non-diabetic subjects. Interestingly, visfatin/PBEF/Nampt levels were significantly increased in patients with long-standing T1DM (Type 1 diabetes mellitus) compared with subjects with T2DM or non-diabetic subjects.

*Zhang et al.*,<sup>(20)</sup> studied a group of 814 diabetic patients from the USA and a group of non-diabetic controls (n = 320). They found a significant association between -948C> A and T2DM ( $p = 0.021$ ). In a non-diabetic population (n = 630), the same -948C allele that conferred increased risk of T2DM was significantly associated with higher plasma levels of fibrinogen and C-reactive protein ( $p = 0.0022$  and  $0.0038$ , respectively). However, no significant associations were observed with BMI, waist circumference, serum glucose levels, or fasting insulin levels. They suggested that the visfatin gene may play a role in determining T2DM susceptibility, possibly by modulating chronic, low-grade inflammatory responses.

Elevated visfatin level in patients with T2DM may be explained by the impairment of visfatin signaling in target tissues or the dysregulation in biosynthesis in response to hyperglycemia, hyperinsulinemia, or adipocytokines in state of diabetes<sup>(18)</sup>.

On the other hand, *Erick Ingeleson et al.*,<sup>(21)</sup> reported the absence of statistically significant associations between plasma visfatin and diabetes, obesity (generalized or abdominal and subcutaneous or visceral fat), or dyslipidemia. Potential explanations of *Erick Ingeleson et al.*,<sup>(21)</sup> negative findings include that the study group was a community-based cohort

study, whereas prior investigations were smaller case-control studies of patients with diabetes or obesity or based on patients referred to a hospital for abdominal surgery. In addition, *Bottcher et al.*,<sup>(22)</sup> did not find any association with either T2DM, in a cohort of 503 diabetic subjects and 476 healthy controls, or with T2DM-related traits in 626 non-diabetic subjects from Germany. However, they found an association between the -948 G > T single-nucleotide polymorphism (SNP) and fasting insulin levels in non-diabetic subjects ( $p < 0.05$ ). The ratio of visceral/subcutaneous visfatin mRNA expression was associated with all three genetic polymorphisms studied (rs9770242, -948G > T, rs4730153).

Regarding other types of diabetes, some investigators have reported higher plasma visfatin in individuals with gestational<sup>(23)</sup> and type 1<sup>(19,24)</sup> diabetes. However, other studies have noted opposite findings, e.g., lower plasma visfatin in gestational diabetes<sup>(18)</sup> while another study, *Toruner et al.*,<sup>(25)</sup> demonstrated decreased visfatin in patients with T1DM and inverse relationship between HbA<sub>1c</sub> and visfatin levels.

Current data suggests that visfatin is important to normal insulin secretion, but its relationship with diabetes risk and progression is still a matter of debate. Thus, visfatin may be a compensatory mechanism or part of the pathophysiology of diabetes.

Regarding the diabetic group & according to the results of this study, there was no correlation between plasma visfatin/PBEF/Nampt concentrations on one hand, and age, gender, fasting blood glucose and fasting insulin level on the other hand. This coincides with *Berndt et al.*,<sup>(26)</sup> *Haider et al.*,<sup>(24)</sup> *Pagano et al.*,<sup>(27)</sup> *Dogru et al.*,<sup>(17)</sup> and *Varma et al.*,<sup>(28)</sup>

There was no correlation between plasma visfatin/PBEF/Nampt concentrations on one hand, and total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, levels on the other hand. *Chen et al.*,<sup>(18)</sup> reported that plasma visfatin level was significantly associated with total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol in simple regression analysis but not in multiple regression analysis.

Our results confirmed that plasma visfatin /PBEF/ Nampt correlates positively with BMI in the diabetic group. This finding coincides with a separate study by *Berndt et al.*,<sup>(26)</sup> in a population of 189 subjects who showed that plasma visfatin/PBEF/Nampt concentrations correlated positively and significantly with BMI and percentage body fat, as well as visfatin/PBEF/Nampt mRNA expression in VAT (visceral adipose tissue). *Berndt et al.*,<sup>(26)</sup> reported a negative correlation between circulating visfatin/PBEF/Nampt levels and mRNA expression in subcutaneous fat. Plasma visfatin/PBEF/Nampt concentrations were not associated with visceral fat mass, which had been calculated by CT (computed tomography) scans in a subgroup of 73 subjects.



**Brendt et al.**,<sup>(26)</sup> explained these findings through a reciprocal regulatory mechanism of subcutaneous visfatin gene expression by increased plasma visfatin concentration. In addition, **Hammarstedt et al.**,<sup>(29)</sup> in a small study composing six healthy subjects and seven untreated patients with T2DM, reported that plasma visfatin/PBEF/Nampt levels and BMI had a weak positive correlation in both groups, whereas there was no correlation between visfatin/PBEF/Nampt concentrations and WHR or waist circumference.

On the other hand **Lopez-Bermejo et al.**,<sup>(19)</sup>, **Haider et al.**,<sup>(24)</sup>, **Chen et al.**,<sup>(18)</sup>, **Dogru et al.**,<sup>(17)</sup> and **Varma et al.**,<sup>(28)</sup> reported absence of correlation between plasma visfatin and BMI and explained this finding by the differential regulation of VF expression in the different adipose depots. Hence, the increase in VAT VF with obesity may be balanced by the decrease in SAT VF, such that plasma VF is not affected by increasing BMI. **Pagano et al.**,<sup>(27)</sup> reported a negative correlation between circulating visfatin/PBEF/Nampt levels and BMI. In contrast, no association between plasma visfatin/PBEF/Nampt and waist circumference, fat mass, was found in lean, as well as obese, subjects.

Regarding glycemic control and according to the results of this study, visfatin level correlates positively with HbA<sub>1c</sub> level in the diabetic group. In addition, in the diabetic group, both visfatin & HbA<sub>1c</sub> were significantly higher in uncontrolled subgroup than the controlled one. This finding coincides with **Lopez-Bermejo et al.**,<sup>(19)</sup> and **Zhu et al.**,<sup>(30)</sup> who reported a significant correlation between visfatin levels and HbA<sub>1c</sub> levels in diabetic patients and reported a reduction in visfatin concentrations from 25.0±6.5 ng/ml at baseline to 20.3±4.7 ng/ml ( $p < 0.01$ ) after 3 months of intensive glycemic control, while HbA<sub>1c</sub> levels decreased from 9.0±1.8% to 6.2±0.7% ( $p < 0.01$ ). **Toruner et al.**,<sup>(25)</sup> reported the presence of a significant correlation between visfatin and hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) even after the adjustment for age, sex, body mass index and duration of diabetes ( $r = -0.48$ ,  $p = 0.005$ ) in the patient group and multivariate analysis showed that significant determinants of visfatin concentrations were HbA<sub>1c</sub> and duration of diabetes ( $r^2 = 0.27$ ).

The relationship between visfatin levels and insulin resistance surrogates was also investigated in this study. However, it was not possible to demonstrate correlation of visfatin with HOMA-IR. This coincides with **Lopez-Bermejo et al.**,<sup>(19)</sup>, **Berndt et al.**,<sup>(26)</sup>, **Dogru et al.**,<sup>(17)</sup> and **Chang et al.**,<sup>(31)</sup> who reported that HOMA-IR did not show correlation with visfatin expression on visceral adipose tissue but was positively associated with visfatin expression in subcutaneous adipocytes.

In conclusion: Diabetic subjects had higher plasma visfatin levels than those of control subjects. Plasma visfatin positively correlated with HbA<sub>1c</sub> and

BMI and not correlated with gender, age, fasting plasma insulin, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, fasting blood glucose and HOMA-IR in the diabetic subjects.

These results indicate that visfatin may play a role in the pathogenesis of T2DM through the unresponsiveness to visfatin actions and the contribution of visfatin to  $\beta$ -cell deterioration in diabetic patients or the increased serum visfatin concentration may be a compensatory mechanism aimed at ameliorating the functional consequences of insulin deficiency. The increased visfatin concentration may also promote insulin sensitivity by its stimulatory effects on insulin receptors.

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## Depression and Anxiety among Males Attending Primary Health Care Centers, Eastern Saudi Arabia: Prevalence and Predictors

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**Abstract: Background:** Psychiatric disorders in Saudi Arabia, mainly depression and anxiety are estimated to have high prevalence. The aim of this study was to assess the magnitude of depression, and anxiety among Primary Health Care Centers (PHCCs) attendees in Dammam and Al-Qatif areas, Eastern Saudi Arabia and to identify possible predictors. **Methods:** This was a cross-sectional study conducted in ten randomly selected PHCCs. The study sample consisted of 822 adult male attendees. Data was collected using an interviewer-administered validated Arabic version of Patient Health Questionnaire (PHQ). The questionnaire consisted of socio-demographic characteristics and questions to assess depression and anxiety. The PHQ scoring system was used to derive severity scores for both conditions. Chi-square test and logistic regression were used for data analysis. **Results:** The overall prevalence of depression was 32.8% with mild depression accounting for 22.9%. The overall prevalence of anxiety was 22.3% with 17.0% of the attendees having mild degree of anxiety. Single marital status, younger age group, lower monthly income, and positive history of psychological and chronic disorders were statistically significantly associated with depression. Single marital status was statistically significantly associated with anxiety. Logistic regression analysis showed that, single marital status and positive history of psychological illness were significant predictors of depression; being single was the only predicting factor for the occurrence of anxiety. **Conclusion:** The prevalence rate of depression and anxiety among PHCCs male attendees was relatively high. Therefore, screening of mental illnesses, especially depression and anxiety should be implemented by PHC physicians during their routine activities.

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**Key word:** Depression, Anxiety, Predictors, Primary Health Care Centers, Saudi Arabia.

### 1. Introduction

Mental disorders are a growing problem worldwide affecting hundred millions of people.<sup>(1)</sup> Westernization of life creates a large burden on health. Mental health takes a big part of this burden. Globally, the burden of mental disorders is estimated to account for 14% of the diseases burden.<sup>(1)</sup> The Epidemiologic Catchment Area (ECA) study of the early 1980s and the National Co-morbidity Survey (NCS) of the early 1990s in United States of America (USA) showed that the prevalence of mental health in USA is 20% of the total population.<sup>(2)</sup>

No country or socioeconomic status is immune from the burden of mental disorders, worldwide.<sup>(3)</sup> Mental disorders, their manifestations and their co-morbidity are considered to have high prevalence at primary health care (PHC) level.<sup>(5)</sup> One out of every three primary care patients presents with clinical problems related to mental illness.<sup>(4)</sup> The first point of contact between individuals and the health care system is the PHC which increases the responsibility of service care providers for initial diagnosis. Even

though studies show that PHC physicians fail to detect depression which remains under-diagnosed in one-third to one-half of the cases.<sup>(5,6)</sup> In Saudi Arabia psychiatric morbidity in PHC is estimated at 30-46%<sup>(7)</sup> with a 20% prevalence of depression.<sup>(8)</sup>

Identification of mental disorders needs valid and reliable instrument of screening.<sup>(4,9)</sup> There are many screening tests for mental disorders in PHC; no clear evidence which shows that one of these tests is better than others.<sup>(10)</sup>

Depression is considered as a major public health issue and as the fourth leading cause of the world diseases burden. One in eight individuals with depression requires treatment during their life time.<sup>(5)</sup> In 2020 the World Health Organization (WHO) in their global prediction considers depression as the second most cause of disability after ischemic heart disease. Depression accounts for 12% of patients attending PHC.<sup>(11)</sup>

Integrating mental health services into PHC is the most viable way of ensuring that people receive the mental health care they need.<sup>(12)</sup> Most people

suffering mental disorders will visit their general practitioner, complaining either of psychological or somatic symptoms.<sup>(13,14)</sup>

The aim of the present study was to assess the magnitude of the most common types of mental disorders namely: depression, and anxiety among Primary Health Care Centers (PHCCs) attendees in Eastern Province, Saudi Arabia and to identify the possible predictors.

## 2. Subjects and Methods

### Statistical Design:

This was a cross-sectional study conducted during November 2010 in ten randomly selected PHCCs in Dammam and Al-Qatif areas, Eastern Province, Saudi Arabia, (8 PHCCs out of the 22 in Dammam city and 2 PHCCs out of the 26 in Al-Qatif area). The study sample consisted of 822 adult males, Saudi and non Saudi citizens above 18 years attending the PHCCs for any reason during the period of the study.

### Technical Design:

Data was collected using an interviewer-administered validated Arabic version of Patient Health Questionnaire (PHQ). PHQ is a psychiatric screening instrument for detection of mental disorders in PHC.<sup>(15)</sup>

Collection of data was done by well-trained fourth year medical students after receiving detailed training about mental disorders in PHC, as well as data collection, and interviewing.

The questionnaire consisted of the following main parts:

1-Socio-demographic characteristics of the patients attending the PHCCs regarding their nationality, age, marital status, occupation, educational level, family income, type of family, crowding index, and family history of psychiatric disease.

2-Questions to assess mental conditions including two main disorders: depression (9 questions), and anxiety (7 questions).

The PHQ scoring system was used to derive severity scores for depressive, and anxiety symptoms. Depression and anxiety severity was calculated by assigning scores of 0, 1, 2, and 3, to the response categories of "not at all", "several days", "more than half the days", and "nearly every day", respectively.

The depression total score for the nine items ranges from 0 to 27. Scores of 5, 10, 15, and 20 represent cut-off points for mild, moderate, moderately severe and severe depression, respectively.

The anxiety total score for the seven items ranges from 0 to 21. Scores of 5, 10, and 15 represent cut-off points for mild, moderate, and severe anxiety, respectively.

For the ease of statistical analysis, associations and interpretation of the results, depression and anxiety

were then grouped into 2 categories only (depression, no depression) and (anxiety, no anxiety).

Necessary permissions to conduct the study were obtained from concerned authorities and confidentiality of the information was strictly adhered to by assuring the attendees that no details about their status will be released and data will be only used for research purpose.

### Statistical Analysis:

The collected data were reviewed, coded, verified and statistically analyzed using the Statistical Package for Social Sciences (SPSS) software version 16.<sup>(16)</sup> Descriptive statistics for all studied variables and Chi-square test were used. Logistic regression analysis was used to find the association between the characteristics of the PHCCs attendees (Independent variables) and the study mental health problems (depression and anxiety) (Dependant variable) and a *P*-value level of <0.05 was considered significant throughout the study.

## 3. Results

The majority of the studied male attendees in the present study were Saudis (90.1%), 52.2% were in the age group of 20-<35 years and married (71.3%). Less than 20% of them were not working, 44.8% had a secondary or diploma level of education, 80.5% were living in nuclear families and 29.7% had less than 5000 Saudi Riyals (S.R.) monthly income. Only 7.5 % of the attendees had positive history of psychological disorders. About 70% of the attendees didn't mention any history of chronic diseases, while 11.7%, 4.9% and 4% of them had history of Diabetes mellitus, bronchial asthma and coronary heart diseases, respectively (**Table 1**).

**Table 2** shows the distribution of primary care male attendees according to their depression and anxiety state. The overall prevalence of various types of depression was 32.8% with a prevalence of mild depression accounting for 22.9%. Also, the overall prevalence of anxiety was 22.3% with 17.0% of the attendees having mild degree of anxiety.

According to **table 3**, there was a statistically significant association between depression state among attendees and their marital status, where 44.4% of singles had depression in comparison to 28.2% of the married attendees (*P*<0.001). Depression was found to be more prevalent (39.5%) among attendees with low monthly income (<5000 S.R.) compared to others with higher monthly income (*P*<0.01). Moreover, age was statistically significantly associated with depression, 37.5% of attendees in the age group 20-<35 years were depressed compared to 29.1% in the age group 35-<50 years. Attendees with a positive history of psychological disorders (51.6%) were significantly having depression more than others (*P*<0.01).

Attendees with history of chronic diseases such as diabetes mellitus (30.2%), coronary heart diseases (30.3%), bronchial asthma (55.0%) were found to have depression in comparison to others ( $p<0.05$ ).

**Table 1: Socio-demographic characteristics of primary care male attendees**

Socio-demographic characteristics	Total (n= 822)	
	No.	%
<b>1-City:</b>		
Dammam	610	74.2
Qatif	212	25.8
<b>2-Nationality</b>		
Saudi	741	90.1
Non-Saudi	81	9.9
<b>3-Age in years:</b>		
20-<35	429	52.2
35-<50	268	32.6
50-<65	96	11.7
≥65	29	3.5
<b>4-Marital Status:</b>		
Single	222	28.2
Married	586	71.3
Divorced	4	0.5
<b>5-Occupation:</b>		
Work	690	83.9
Without work	132	16.1
<b>6-Educational level:</b>		
Illiterate or read and write	39	4.7
Primary or intermediate	186	22.6
Secondary or diploma	368	44.8
University or higher	229	27.9
<b>7-Monthly income in Saudi Riyal(S.R.) :</b>		
< 5000 S.R.	243	29.7
5000-15000 S.R.	478	58.4
>15000 S.R.	97	11.9
Refused to answer	4	0.5
<b>8-Type of family:</b>		
Nuclear	662	80.5
Extended	160	19.5
<b>9-History of psychological disorders</b>		
No	760	92.5
Yes	62	7.5
<b>5-History of chronic diseases:</b>		
No	575	69.9
Diabetes Mellitus	96	11.7
Bronchial asthma	40	4.9
Coronary Heart Diseases	33	4.0
Others	78	9.5

By studying the association between the depression state and other attendees characteristics (nationality, educational level, occupation, and family type), it was found that there was no statistically significant association. Marital status was the only socio-demographic characteristic that was found to be statistically significantly associated with anxiety. About 31.9% of singles were found to have anxiety in comparison to 18.3% of the married ( $P<0.001$ ).

**Table 4** represents the results of the logistic regression analysis of significant factors predicting occurrence of depression and anxiety among primary care male attendees. The following factors were found to be independently and significantly associated with depression: single marital status (OR=0.557, 95% CI=0.384-0.808) and positive history of psychological disorders (OR=1.607, 95% CI=1.140-2.266), ( $P<0.001$ ). Moreover, single marital status was the only predicting factor for the occurrence of anxiety among the attendees (OR=0.516, 95% CI=0.358-0.744), ( $P<0.01$ ).

**Table 2: Distribution of primary care male attendees according to their depression and anxiety state**

Diagnosis	Total (n=822)	
	No.	%
<b>I-Depression</b>		
A-No depression	552	67.2
B-Overall types of depression	270	32.8
-Mild depression	188	22.9
-Moderate depression	61	7.4
-Moderately severe depression	17	2.1
-Severe depression	4	0.5
<b>II-Anxiety</b>		
A-No anxiety	639	77.7
B-Overall types of anxiety	183	22.3
-Mild anxiety	140	17.0
-Moderate anxiety	35	4.3
-Severe Anxiety	8	1.0

**Table 3: Association between socio-demographic characteristics of primary care male attendees and presence of depression and anxiety state**

<b>I-Depression</b>							
Socio-demographic characteristics	No Depression		Depression		Total		Test of significance (P-value)
	No.	%	No.	%	No.	%	
<b>1-Marital Status:</b>							
Single	129	55.6	103	44.4	232	100.0	20.4
Married	421	71.8	165	28.2	586	100.0	(<0.001)
Divorced	2	50.0	2	50.0	4	100.0	
<b>2-Monthly income in Saudi Riyals (S.R.):</b>							
< 5000 S.R.	147	60.5	96	39.5	243	100.0	
5000-15000 S.R.	340	71.1	138	28.9	478	100.0	11.6
>15000 S.R.	64	66.0	33	34.0	97	100.0	(<0.01)
Refused to answer	1	25.0	3	75.0	4	100.0	
<b>3-Age in years:</b>							
20-<35	268	62.5	161	37.5	429	100.0	
35-<50	190	70.9	78	29.1	268	100.0	10.3
50-<65	74	77.1	22	22.9	96	100.0	(<0.05)
≥65	20	69.0	9	31.0	29	100.0	
<b>4- History of psychological disorders:</b>							
No	522	68.7	238	31.3	760	100.0	10.7
Yes	30	48.4	32	51.6	62	100.0	(<0.01)
<b>5-History of chronic diseases:</b>							
No	398	69.2	177	30.8	575	100.0	
Diabetes Mellitus	67	69.8	29	30.2	96	100.0	12.8
Bronchial asthma	18	45.0	22	55.0	40	100.0	(<0.05)
Coronary Heart Diseases	23	69.7	10	30.3	33	100.0	
Others	46	59.0	32	41.0	78	100.0	
<b>II- Anxiety</b>							
Socio-demographic characteristics	No Anxiety		Anxiety		Total		Test of significance (P-value)
	No.	%	No.	%	No.	%	
<b>1-Marital Status:</b>							
Single	158	68.1	74	31.9	232	100.0	19.6
Married	479	81.7	107	18.3	586	100.0	(<0.001)
Divorced	2	50.0	2	50.0	4	100.0	

**Table 4: Logistic regression analysis of significant factors predicting depression and anxiety among primary care male attendees**

Variables	B coefficient	S.E. of B	P-Value	O.R.	95 % Confidence interval of O.R.	
					Lower	Upper
<b>I-Depression</b>						
Marital status	-0.585	0.190	0.002	0.557	0.384	0.808
History of psychological problems	0.475	0.175	0.007	1.607	1.140	2.266
<b>Constant</b>	0.482	0.448	-	-	-	-
<b>Model <math>X^2_{(5)} = 30.6, P &lt; 0.001</math></b>						
<b>II-Anxiety</b>						
Marital status	-0.662	0.187	0.000	0.516	0.358	0.744
<b>Constant</b>	-0.163	0.403	-	-	-	-
<b>Model <math>X^2_{(2)} = 14.5, P &lt; 0.01</math></b>						

#### 4. Discussion

The public health significance of mental disorders, as a chronic community health problem, includes quality of life that is determined by a person's mental state. Many physical disorders have an important mental component, and a large proportion of people who need medical care have mental or brain disorders.<sup>(17)</sup> Accordingly, if environmental factors play a large role, preventive strategies for causes of these disorders can be an urgently needed.<sup>(17)</sup>

The overall prevalence of various types of depression (32.8%) and anxiety (22.3%) in the present study (Table 2) were in agreement with other study carried-out in primary care in Saudi Arabia where psychiatric disorders ranged from 30-46% with 20% prevalence for depression<sup>(8)</sup> and in Qatar (27.8%).<sup>(18)</sup> However, WHO prediction in 2020, considers depression as a second cause of disability that accounts for 12% of patients attending primary health care.<sup>(11)</sup> Also other studies showed that the prevalence of generalized anxiety disorders and major depression in primary care in Belgium and Luxemburg ranged from 4.2% to 8.3%.<sup>(19)</sup> In Bahrain, the prevalence rate of generalized anxiety disorders was 17.3%, life time depression was 19.3% and current depression was 5.6%.<sup>(20)</sup> A study conducted in Qatar to determine the prevalence of mental disorders in adult population attending PHCs, showed that the overall prevalence of mental disorders was 36.6%. Depression (13.5%) was the most common mental disorder, followed by anxiety disorders (10.3%).<sup>(3)</sup>

The significant factors associated with depression among primary care attendees in the present study were age, marital status, monthly income, history of psychological disorders and chronic diseases such as diabetes mellitus, coronary heart diseases, and bronchial asthma. However, the only significant factor associated with anxiety was marital status (Table 3). Other socio-demographic characteristics as nationality, occupation, educational level and type of families were not significantly associated either with depression or anxiety. This finding is consistent with other findings of previous studies that indicated high prevalence of depression among young age group (55.3%), unmarried individuals (35.5%) and people with history of chronic illnesses including psychological disorders (46.1%).<sup>(18,21)</sup> Regarding the age in the present study, high prevalence of depression (37.5%) was observed at younger age group (20-<35years) that is comparable to other studies in Qatar (18-34 years) and US general population (15-24 years).<sup>(18,22)</sup> Also depression was more prevalent among unmarried (single or divorced) male primary care male attendees and this was consistent with other studies<sup>(9,23)</sup> Moreover, other studies carried-out among Kuwaiti patients attending

primary health care setting, and among Saudi elderly reported that chronic illness increase the rate of depression.<sup>(9,23)</sup> However, other studies cannot find a significant relation between depression and different chronic illness as diabetes mellitus, coronary heart diseases or bronchial asthma.<sup>(18)</sup>

The profile of significant predictors for both depression and anxiety considerably differs by the type of mental disorders among primary care male attendees in the present study. Marital status was the common significant factor that predicting both depression and anxiety. In addition history of psychological disorders was the other significant predictor for depression only. These findings are similar to those generally reported by other studies.<sup>(24,25)</sup>

#### 5. Conclusions and Recommendations

The results of the present study concluded that prevalence rate of depression and anxiety among primary care male attendees was relatively high. Marital status and history of psychological disorders were the main significant predictors for depression whereas; marital status alone was the only significant factor which predicts anxiety.

Therefore, it is recommended that screening and early detection of mental health problems, in general, and depression and anxiety, in particular, should be implemented by PHC physicians during their routine daily activity. This is best achieved by proper integration of screening within PHC services. Also all PHC providers particularly physicians should have periodic and in-service training programs about psychological disorders as a part of their continuous medical education. Male patients should be encouraged to report their psychological complaints to their family physicians.

Since the present study was a community questionnaire based, therefore in-depth studies are recommended to identify the real underlying causes of such increase in the prevalence of depression and anxiety in the Saudi community.

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## Mechanical Properties and Morphology Studies of Nanocomposites Based on RSF/Nanoclay Modified /HDPE Nanocomposites

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**Abstract:** The use of natural fibers and nanoclay as reinforcement materials in composites has proved the ability of providing several environmental and economical advantages. In this work, two groups of rice straw fibers (RSF) reinforced high density polyethylene (HDPE) nanocomposites were prepared in the presence of 3wt% maleated polyethylene (MAPE) as coupling agent. To the former group, nanomontmorillonite clay (n-MMT) was added after being treated with cetyltrimethyl ammonium bromide (CTAB), in order to enhance dispersibility, compatibility and interfacial bonding with the polymer. Calcium oxide (CaO) was added as a filler to the second group in fine powder form. These groups have been examined with regard to their mechanical properties and thermal stability. The interfacial adhesion has been also investigated by scanning electron microscopy (SEM). Results revealed that, significant improvement was attained for mechanical properties as the modified nanoclay % increases to ~ 2.5%. Maximum loaded amount of ca. 10% CaO has found to be sufficiently required to enhance tensile strength with reasonable flexural strength.

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### 1. Introduction:

Natural fibers have been increasingly used as reinforcement materials in commercial thermoplastics and thermoset matrix composites. The use of these material has proved the ability of providing several advantages such as low densities, low cost nonabrasive nature, high specific properties and renewable nature [1-4]. Some types such as rice straw fibers and saw dust are considered as important potential reinforcing filler for thermoplastic composite because of their lignocellulosic characteristics. [5]. Composite materials based on cellulosic fibers, namely wood-plastic composites (WPC) [6], nanoscience and nanotechnology have opened up a completely new way to develop wood-plastic composites (WPCs). Nanoparticles are obtained from available natural resources and they generally need to be treated because the physical mixture of a polymer and layered silicate may not form a nanocomposites; in this case a separation into discrete phases takes place. The poor physical interaction between the organic and the inorganic components leads to poor mechanical and thermal properties. In contrast, strong interactions between the polymer and the layered silicate nanocomposites lead to the organic and inorganic phases being dispersed at the nanometer level. As a result, nanocomposites exhibit unique higher properties than conventional composites [7-10]. Modification of clay

due to Hydrophilic clays and hydrophobic polymers are not compatible in their virgin states and a modification of either the polymer or clay is necessary for dispersion, intercalation and/or exfoliation of clay tactoids in the polymer matrix. Surface modification of clay has commonly been used to achieve a greater compatibility of the clay and polymer. Ion exchange of Na<sup>+</sup> or Ca<sup>2+</sup> gallery cations in the mineral by alkyl ammonium ions is frequently chosen to modify the clay [11]. Recently, the use of inorganic nanoparticles as additives to enhance the polymer's performance has been studied extensively. The incorporation of organically modified layered silicate (OMLS) into polymers has been of particular interest because of their demonstrated significant enhancement, relative to an unmodified polymer resin of a large number of physical properties, including barrier, flammability resistance, thermal and environmental stability, solvent uptake, and rate of biodegradability of biodegradable polymers [12]. Montmorillonite (MMT) Of the many type of nano-reinforcements available for polymers, layered silicates clay has attracted the greatest interest in recent years, both in industry and in academia, because of the ability of silicate particles to disperse into individual layers and also the ability to tune their surface chemistry through ion exchange reactions with of organic inorganic cations [13]. The layered silicates

commonly used in nanocomposites belong to structural family known as 2:1 phyllosilicates and the most commonly used layered silicates are MMT. It was discovered in 1847 in Montmorillon France. Among the potential nanomaterials used in polymer nanocomposites, nanoclay (layered silicate) which has been widely investigated primarily because of its remarkable improvement in properties. Furthermore, clay materials are easily available, environmentally friendly, and their intercalation chemistry has been investigated since a long time. These make nanoclay one of the most widely accepted and effective nano-reinforcements [14-16]. Surface modified nanoclay are widely used in, technological applications because of their improved strength [17-19].

## 2. Materials

High Density Polyethylene (HDPE) was purchased from Exxon Mobil Chemical with Density  $0.964 \text{ gm/cm}^3$ , melt index  $8.0 \text{ g/10min}$  and melting temperature  $134^\circ\text{C}$ . Rice straw from Egypt agriculture fields. Montmorillonite clays were used as supplied (Cloisite  $\text{Na}^+$  ( $\text{Na}^+$ -MM), Southern Clay Products Inc. Calcium Oxide (CaO): was from Sigma-Aldrich Company; grade, Assay 96-100.5 % Dicumyl Peroxide (DCP): was from Akzo Chemi, Netherlands, density:  $0.98\text{--}0.99 \text{ gm/cm}^3$ , processing temperature was  $120^\circ\text{C}$ , suitable cross linking temperature:  $150\text{--}170^\circ\text{C}$ . Maleic anhydride: was from Fluke. Cetyltrimethylammonium bromide (CTAB) was purchased from Sigma-Aldrich and used as received.

### 2.1. Modification of montmorillonite with CTAB.

The CTAB-modified MMT was prepared as follows; 5.0 g of MMT was mixed with 3.4 g CTAB in 200 ml of water. The mixture was subjected to mechanical stirring for 8 hours at  $70^\circ\text{C}$  and finally, the modified montmorillonite was collected by filtration and washed thoroughly using distilled water and dried at  $80^\circ\text{C}$ .

### 2.2 Melt Intercalation

In this technique, no solvent is required and the layered silicate is mixed within the polymer matrix in the molten state. The thermoplastic polymer is mechanically mixed by conventional methods such as extrusion and injection molding with organophilic clay at an elevated temperature. The polymer chains are then intercalated or exfoliated to form nanocomposites. [20, 21]. This work rice straw was dried at  $105^\circ\text{C}$  for 24 h, then blended with HDPE and modified nanomontmorillonite in Brabender twin screw for 10 minutes at  $180^\circ\text{C}$  with rotational speed of 60 rpm, to. The rice straw /HDPE composites were then removed from the Brabender twin screw and

pressed to sheet-like sample using the hydraulic press at  $180^\circ\text{C}$  for 5 minutes.

### 2.3 Testing Methods

The flexural and notched izod impact strength properties of the prepared composites were measured according to ASTM standard D790 and D256 respectively. The morphology of the prepared composites was observed by scanning electron microscopy (SEM) type a Joel JXA-840 A.

### 2.4 Water absorption:

Water absorption of the composites was measured according to the ASTM D570 specification. The dried specimens were immersed in distilled water to maintain at  $25^\circ\text{C}$ . for different times. All values were calculated as the mean of three samples. Using the following formula:

$$\text{Water uptake \%} = (W_s - W_1) / W_1 \times 100$$

Where  $W_s$  is the weight of water saturated specimen and  $W_1$  is the weight of oven dried specimen.

## 3. Results and Discussion

### 3.1 Preparation of Maleated Polyethylene (MAPE):

Maleated polyethylene was prepared by grafting of maleic anhydride at high density polyethylene in the presence of the initiator; Dicumyl peroxide (DCP) as initiator [22]. This was carried out by melt mixing of HDPE with maleic anhydride in a Brabender twin screw at  $180^\circ\text{C}$  and 60 rpm. Fourier transform infrared spectroscopy (FTIR) was used to measure the relative maleic anhydride (MA) grafting in polyethylene. Fig. (1) and Fig. (2). Shows the FTIR spectra of HDPE and HDPE-g-MA. New peaks appeared at  $1786 \text{ cm}^{-1}$  is due to asymmetric stretching modes of carbonyl ( $\text{C}=\text{O}$ ) of saturated maleic anhydride [23-25]. The band at  $1859 \text{ cm}^{-1}$  and  $1786 \text{ cm}^{-1}$ , which are characteristic of cyclic anhydride. The backbone molecule, polyethylene, had a strong peak of ( $-\text{CH}$ ) at about  $2917 \text{ cm}^{-1}$  and  $2848 \text{ cm}^{-1}$ , and the attributed band to the rocking vibrations of  $-\text{CH}_2$  bonds in HDPE was observed at  $719 \text{ cm}^{-1}$ .

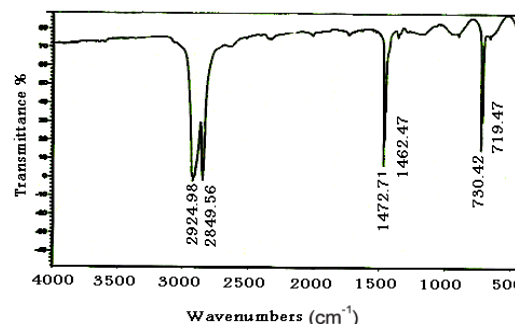


Fig.(1) FTIR for virgin high density polyethylene (HDPE).

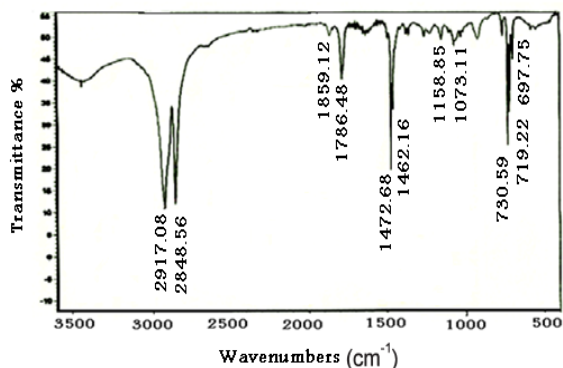
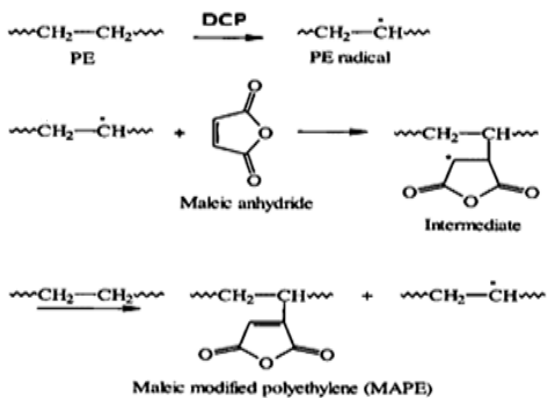


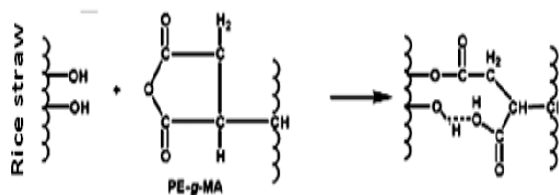
Fig (2): FTIR for Maleated high density polyethylene (MAPE).

### 3.2 Effect of MAPE% as Coupling agent of Composites.

Maleated polyethylene plays a vital role in the improvement of the mechanical properties such as tensile strength, flexural strength and hardness of the prepared composites due to the increase of different ratios of coupling agent compatibility and interfacial bonding between rice straw and HDPE [26]. Different ratios of rice straw /HDPE contents were used in the presence of different ratios of coupling agent (1, 3 and 5%). The obtained results are shown in Fig. (3).



Scheme1: Reaction mechanism of maleic anhydride modification of PE. [27].



Scheme 2: Reaction mechanism interaction between rice straw and PE-g-MA.

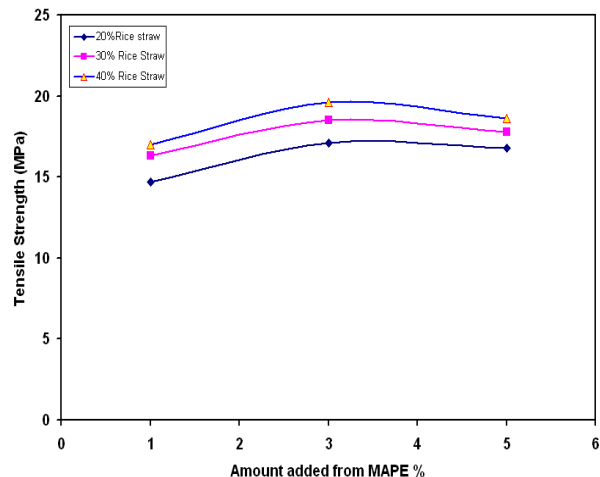


Fig. (3) The change in tensile strength with amounts of coupling agent (MAPE) at different feed ratios

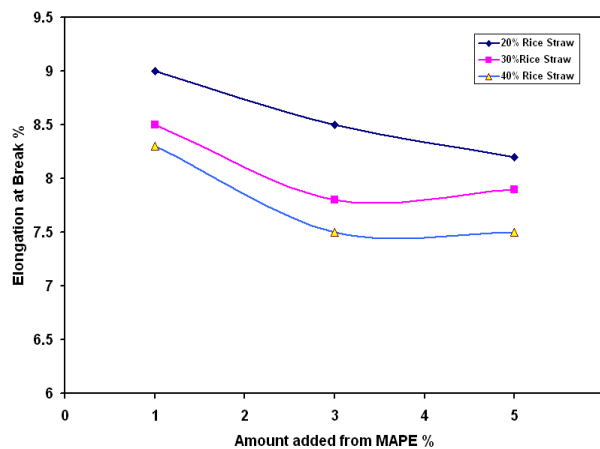


Fig (4): The change in elongation % with the amounts of coupling agent (MAPE) at different feed ratios.

From Fig. (3).one can observe that, the addition of 3% of the coupling agent is more effective than 1% and 5% which gives a slight decrease in tensile strength which increases from 14.7 to 17.1 MPa in case of 20% addition of rice straw and there is a slight decrease in case of 5% of the coupling agent to 16.8 MPa. This indicates that 3% better to improve interfacial bonding in the prepared composites. From Fig(4) indicates that Elongation at break decreases as the amount of rice straw loaded increases due to increasing rigidity of the composites .

**Table(1) :Composition of the studied formulations.**

No.	HDPE (wt %)	Coupling agent (Wt %)	Rice straw flour (wt %)	MMT-CTAB (wt %)	CaO (wt %)
S <sub>1</sub>	37	3	59.5	0.5	-
S <sub>2</sub>	37	3	59	1	-
S <sub>3</sub>	37	3	58.5	1.5	-
S <sub>4</sub>	37	3	57.5	2.5	-
S <sub>5</sub>	37	3	55	-	5
S <sub>6</sub>	37	3	50	-	10
S <sub>7</sub>	37	3	40	-	20

### 3.3Tensile Test:

The effect of nanomontmorillonite loading on the mechanical properties of the composites shows that, proper amount of nanomontmorillonite can improve the tensile, flexural and impact properties of the composites, when nano montmorillonite is organized with cetyltrimethylammonium bromide [28]. From Fig(5) which represents the relation between the tensile strength and the added amount of nanoclay modified with CTAB to RSF/HDPE composites in the presence of MAPE as coupling agent. From the figure. It was found that the tensile strength increases as amount of MMT-CTAB increases. Modification of montmorillonite and presence of maleated polyethylene have important role in improving the mechanical properties of the prepared composites, Koo et al [29], reported that the final morphology and the anisotropic phase formation of MAPE/layered silicate nanocomposites depend on the clay content. With the aid of MAPE. But the elongation % of the prepared composites was found to decrease as the amount loaded with MMT-CTAB increases as seen in Fig.(6).

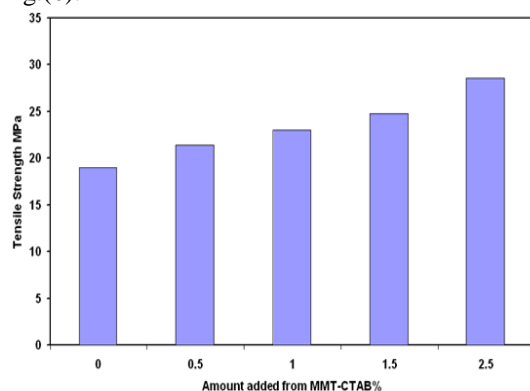


Fig.(5) The relation between Tensile strength (MPa) with different amount of MMT-CTAB.

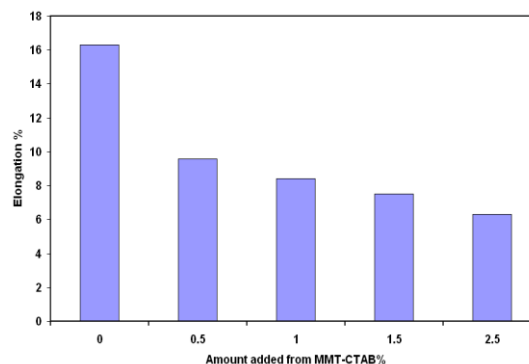


Fig.(6) Represents the relation between the Elongation% (MPa) with different amount of MMT-CTAB.

Fig. (7) Represents the tensile strength of composites in the presence of CaO. Addition of CaO significantly improved the mechanical properties [30]. Good effect was noticed at 10% loaded CaO. It was found that elongation decreases as the amount of CaO increases as clearly shown in Fig.(8).

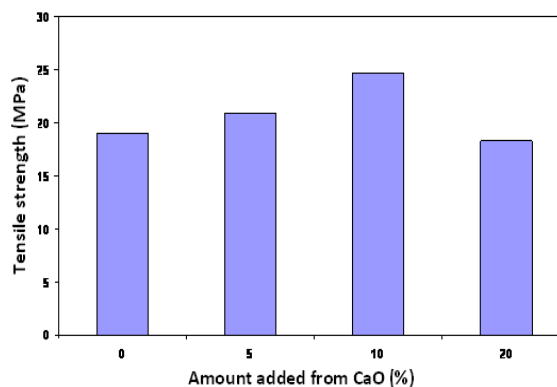


Fig. (7) The relation between Tensile strength (MPa) with different amount of CaO.

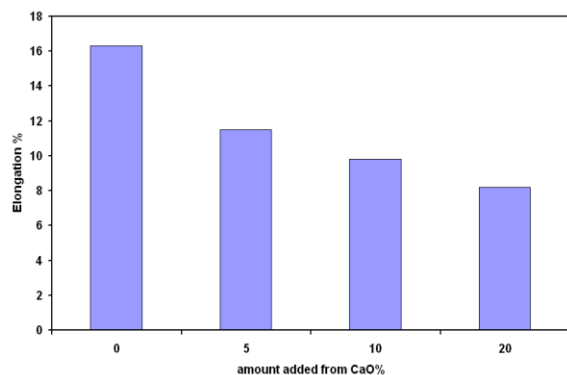


Fig.(8) The relation between Elongation % of the RSF/HDPE composites with different amount of CaO added.

### 3.4 Flexural Test

The flexural strength of the composites measured using 3-point bending tests are shown in Fig. (9) The flexural strength increases with increase of modified nanoclay up to 2.5 wt%. This increase was expected due to the improved adhesion between components in the composites. These results imply that the flexural properties of HDPE/rice straw fiber composites could be enhanced significantly by tailoring the Coupling agent content and the method of nanoclay addition in the composites. [31].

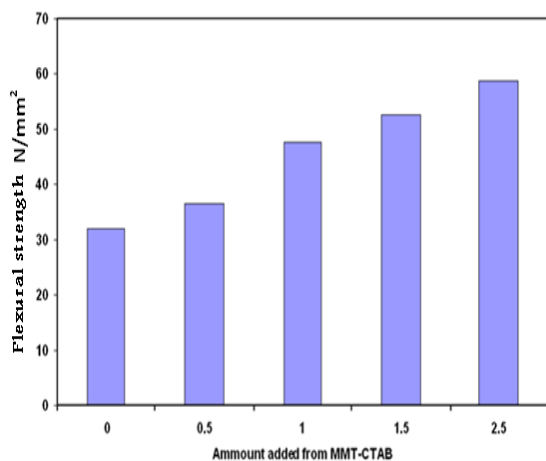


Fig.(9) Effect of added amount of MMT-CTAB on the flexural strength (N/mm<sup>2</sup>) of the prepared nanocomposites.

The effect of adding CaO amount on the flexural strength of the prepared polymer composites was studied. Results are shown in fig (11) which reveals flexural strength increases as calcium oxide increases.

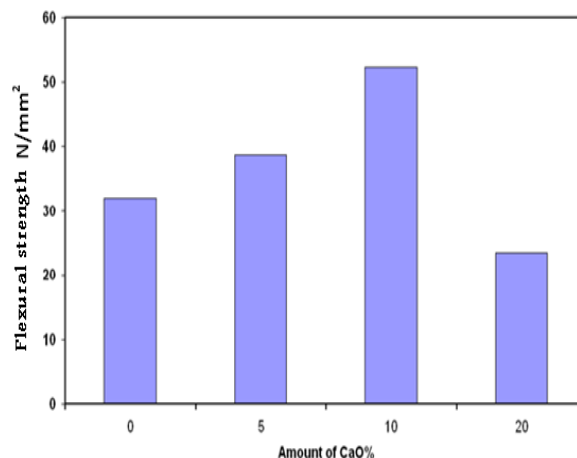


Fig.(10) The relation between the tensile strength (MPa) with different amounts of CaO.

### 3.5 Charpy Notched Izod Impact Test

Effect of (Na-MMT) treated with cetyltrimethylammonium bromide (CTAB) content on rice straw fibers and high density polyethylene composites on the notched izod impact test are shown in Fig.(11). Represents the effect of MMT-CTAB amount loaded on the impact strength of RSF/HDPE composites. The impact strength decreases at small amounts loaded of Nanoparticles MMT-CTAB, followed with increasing the impact strength sharply with increasing the loaded Nanoparticles. Fig (12) it was observed that, the impact strength decreases as the calcium oxide increases,

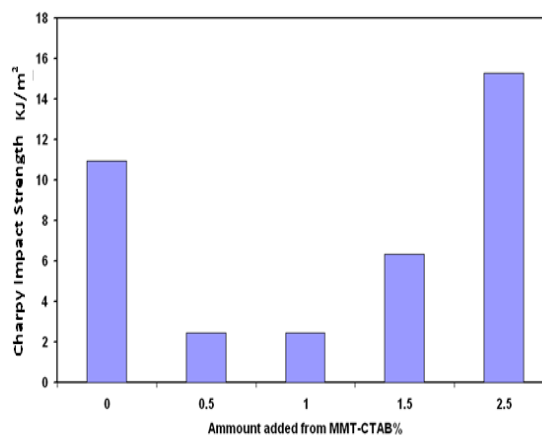


Fig.(11) The change in notched Izod impact strength with the amount of MMT-CTAB increase.

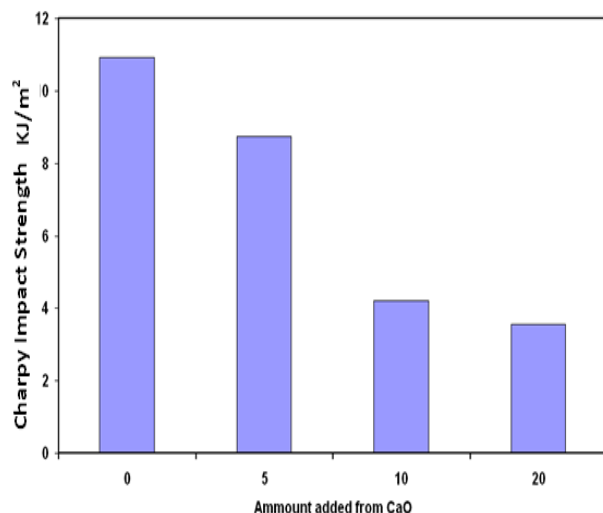


Fig.(12): The change in notched izod impact strength with the amount of CaO loaded with RSF / HDPE composites.

### 3.6 Hardness:

Hardness is an important test for different applications of rice straw /high density polyethylene/MMT-CTAB composites. The change in the Hardness with loaded amounts from MMT-CTAB on the RSF/HDPE composites was studied. The obtained results are shown in Fig (13).hardness of the composites increased with increasing the nano-particles MMT-CTAB in the polymer composites. From fig (14) hardness increases as amount of calcium oxide increase.

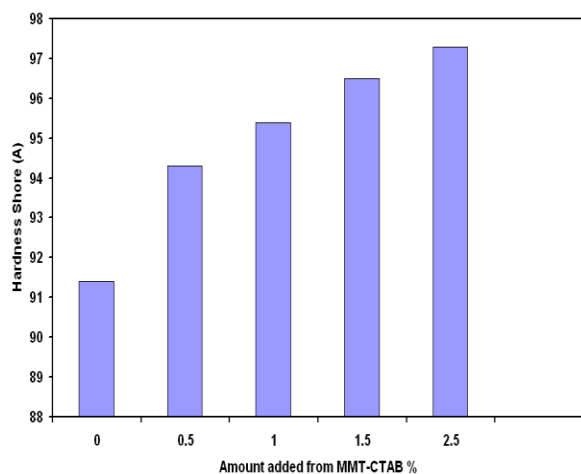


Fig.(13) The change in Hardness (Shore A) with different amount of MMT-CTAB.

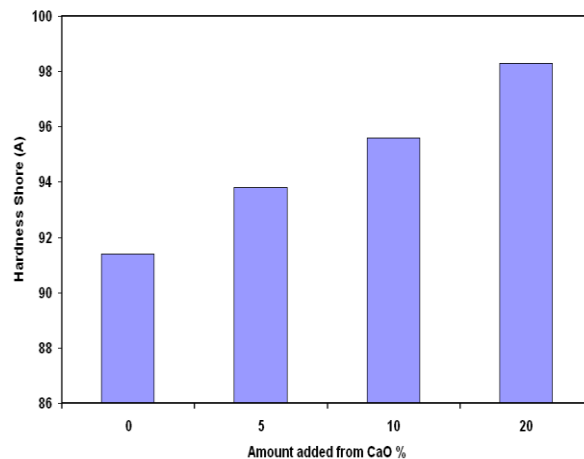


Fig. (14) The change in hardness (shore A) with different amount of CaO.

### 3.7 Water uptake:

The moisture penetration into polymer matrix composites is explained by three different mechanisms [32]. The first type the direct diffusion of water molecules into the matrix and with much lesser extent, into the fibers second is the capillary form of flow of water molecules along the fiber-matrix interface followed by its diffusion into the bulky polymer matrix. It tends to occur preferentially along the polymer-filler interface. However, this is expected to happen only when the debonding between the fibers and the matrix occurred, and the debonding which occurred is often as a result of water attack at the interface. The third mechanism is the transport of water through micro cracks or other forms of micro damage in the matrix, such as pores or small channels already present in the material or expanded by water due to swelling. Thus, each of them becomes active only after the occurrence of specific damage to the composites. The activation of these mechanisms is distinguished in increasing both the rate and the maximum capacity of moisture absorption in an auto accelerative manner. [33].In this study moisture uptake gives the water absorption characteristics of the rice straw/HDPE/MMT-CTAB composites. The obtained results for the water uptake with time are shown in Fig(15).which Indicates that nanoclay modified with CTAB reduces the affinity for water absorption.

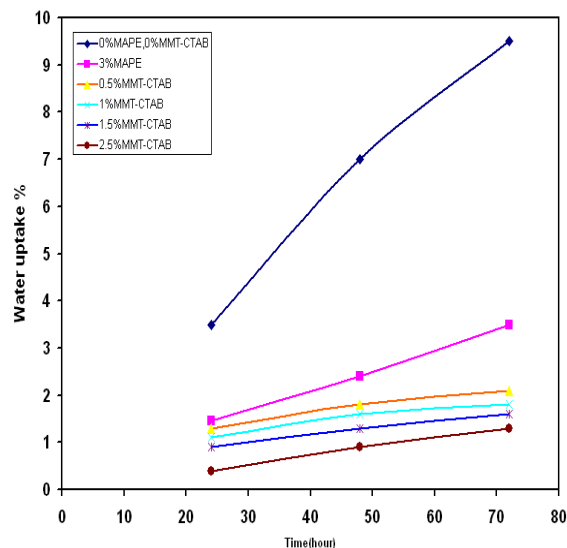


Fig.(15) The change in the water uptake % with time.

### 3.8 Thermal stability of the prepared Rice Straw /HDPE composites.

For studying the thermal stability of the prepared polymer composites, the Thermogravimetric analysis (TGA) was carried. The obtained results for the weight loss% changed with temperature were recorded and the obtained results are shown in Fig(16). From the figure it can be detected that at lower temperature until 200°C there is no large difference between the prepared composites formed from RSF/HDPE in presence of MAPE as compatibilizer and that after adding 2.5% Nanoclay-CTAB, also when adding 10% CaO instead of nanoclay, compared with pure PE.

In the second part of the curves in the range of 220-425 °C large difference between the different types of composites. It was found the most stable for the pure PE, followed by the composites formed in presence of CaO, than that containing nanoclay modified with CTAB and the lowest stable composites, is that formed from RSF /HDPE only when it lost 3% by weight at the beginning of this region and reached to 30% at 425 °C. For PE the loss weight was 0% at 220 °C and reached to 10% at end of this region. For the third region between 425 °C up to 500 °C. Opposite behavior was detected; the loss weight for pure PE increased, but the different type of polymer composites, the residue present was at about 11%. From the Thermogravimetric study it can be concluded that, the thermal stability of pure PE is higher than that of any other type of prepared polymer composites up to high temperature (above 220 °C) but at normal temperature for using of polymer composites instead of rice straw (up to 220 °C) there is no difference between Pure PE and the prepared RSF/HDPE composites. The thermal

decomposition of each sample took place in a programmed temperature range of 25°C to 600°C. According to TGA analysis, results the increase of rice straw fiber level increases the thermal degradation of composites in early stage, but decreased in late stage [34].

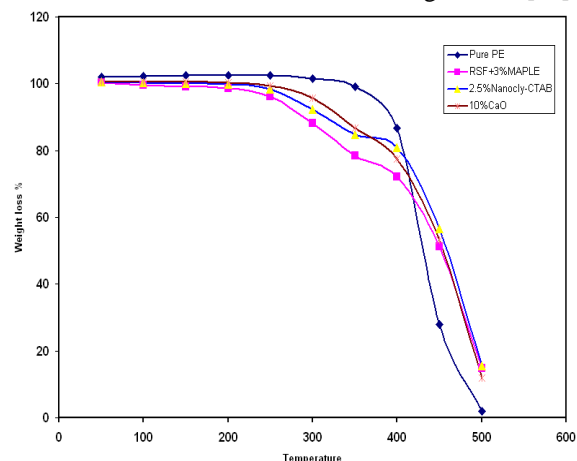


Fig. (16) Relation between Weight loss % at different Temperatures.

### 3.9 Scanning electron microscopy (SEM)

Samples were immersed in liquid nitrogen and fractured to ensure that the microstructure remained intact. Then samples were sputter coated by gold using a sputter-coater to avoid charging under the electron beam. Fig (17) and Fig (18). Represents the cross section of the specimens for RSF /HDPE / 2.5 %MMT-CTAB nanocomposites, RSF/HDPE/CaO composites respectively. It was found that fiber bundles are embedded in the matrices. Modified nanomontmorillonite added to rice straw fibers/HDPE composites improves dispersibility, compatibility and interfacial bonding between rice straw fiber and HDPE as seen from figure(17), bonding between the filler and the matrix polymer is strong, and this reflects good dispersion of Nanoparticles in the prepared composites. This indicates in the presence of compatibilizing agent (MAPE). Improved interfacial bonding leads to improved tensile property, which is reflected in the increased strength. It is seen in fig (17a) fiber pulled out due to fracture of the fiber after immersion in liquid nitrogen. Fig (18) shows the effect of calcium oxide in homogeneity of the composites also good dispersion of the particles of CaO that are more dispersed with rice straw and HDPE networks.

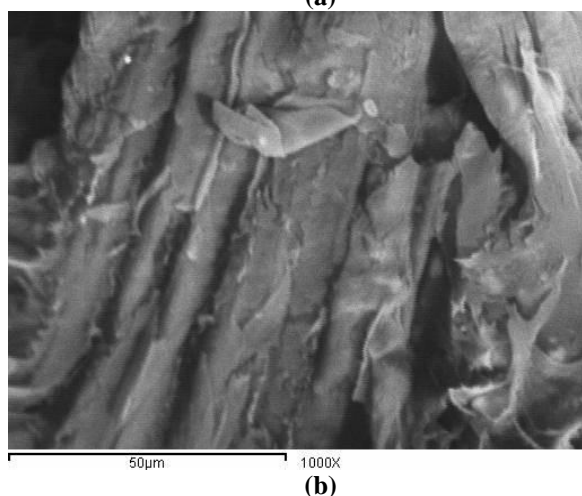
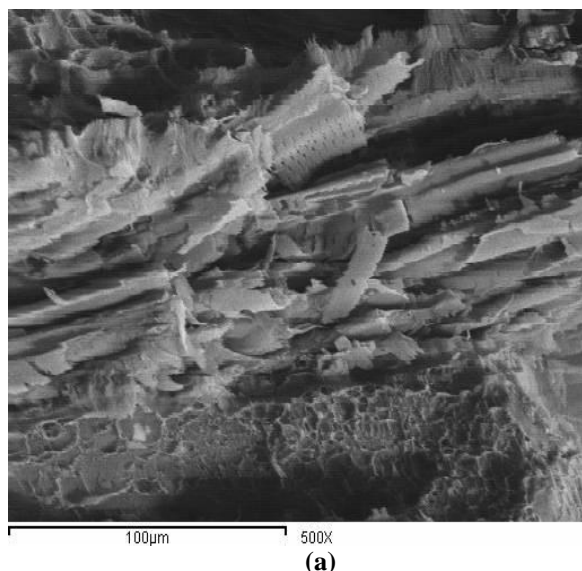


Fig. (17) SEM observations of the composites (a)RSF/HDPE/2.5% MMT-CTAB nanocomposites with magnification 500x (b) RSF/HDPE/2.5% MMT-CTAB nanocomposites with magnification 1000x.

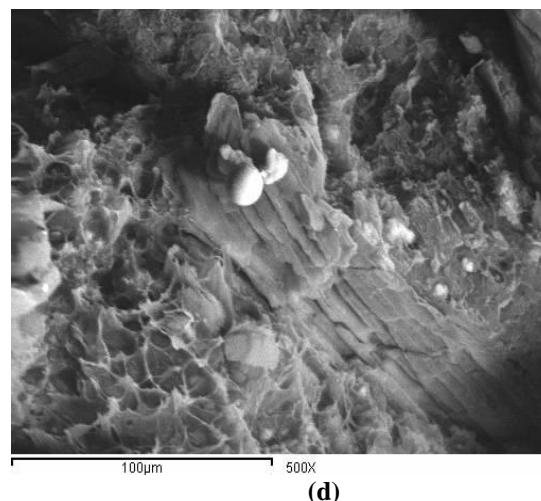
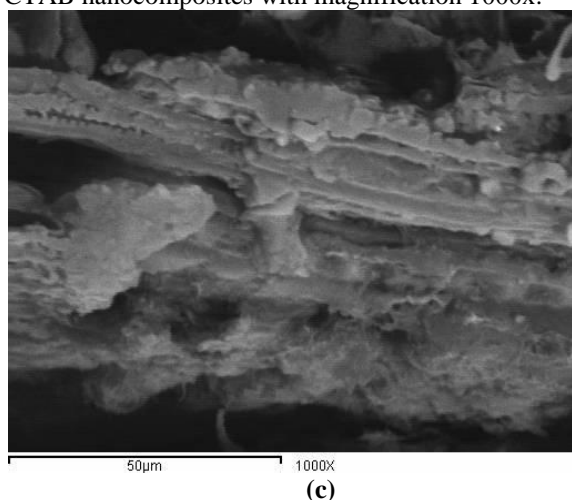


Fig.(18)SEM observations of the composites (c) RSF/HDPE/CaO composites with magnification 1000x (d) RSF/HDPE/CaO composites with magnification 500x.

#### Conclusions:

The aim of this research was to study the rheological behaviors of HDPE/Rice Straw Fiber/MMT-CTAB nanocomposites in presence of MAPE as a coupling agent. The physico-mechanical properties were evaluated. Tensile strength, flexural strength and impact strength of the prepared polymer nanocomposites were found to be improved by increasing the loaded amounts of modified MMT Nanoparticles. Morphology was investigated by SEM which show modified nanomontmorillonite added to rice straw fibers/HDPE composites improves dispersibility, compatibility and interfacial bonding between rice straw fiber and HDPE. Also CaO has a good effect to improve the physico- mechanical properties when added in 10% of ratio.

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## Cloning of HBV S Gene with Artificial Mutation Sites in 'a' Determinant and Analyzing the Protein Antigenicity Expressed

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**[ABSTRACT]** HBV (hepatitis B virus) vaccine is an effective way to control HBV infection, so viruses must be in variance to escape the clearance of host's immunity. The mutations induced by HBV vaccine majored in 'a' determination. In order to study mutations those how to influence the biologic characteristic of S gene, especially in antigenicity and immunogenicity of HBsAg, We constructed series variant clones of HBV S gene 'a' determination according to data of epidemiology. After verified by sequence, we constructed a series variant clone of HBV S gene with "site-mutation method of PCR (gene SOEing PCR, gene splice of overlap extending PCR)". After cellular expression, the antigen expressed by different mutant clones were all recognized by corresponding monoclonal antibody and not done by common HBsAb with immunofluorescence assay. In a conclusion, the mutations in "a" determination can alter the biologic characteristic of S gene, especially in the antigenicity of HBsAg, in a extend. The series mutant clones of "a" determination, which we constructed, may be provide the basic theory for how to develop new HBsAg detected kit, vaccine or HBV immunoglobulin(HBIG).

[Xiaofei Li, Zujiang Yu, Dongliang Yang, Jun Zhang, Youhua Hao, Baoju Wang, Kan Quancheng. **Cloning of HBV S Gene with Artificial Mutation Sites in 'a' Determinant and Analyzing the Protein Antigenicity Expressed**. Life Sci J 2012;9(3):143-147]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 19

**Key words:** HBV; 'a' determination; variance; antigenicity

### 1 Introduction

Hepatitis B virus (HBV) is a major human pathogen causing acute and chronic liver inflammation. It bears the clearance from host, and must avoid it through gene variance or other ways. Hereinto, variance of surface gene is the most common form. Surface antigen mutations of HBV may lead to immune escape and cause failure of immunization. In Europe and North America, HBV with mutations in the portion of the S gene coding the "a" determinant of the hepatitis B surface antigen (HBsAg) have been documented in small numbers of infants born to HBV-infected mothers following post-natal HBV vaccine and hepatitis B immune globulin (HBIG) prophylaxis and in many liver transplant recipients who develop HBV re-infection despite HBIG prophylaxis<sup>[1]</sup>. In order to study mutations in 'a' determination of HBV S gene those how to influence the biologic characteristic of S gene, especially in antigenicity and immunogenicity of HBsAg, we used gene SOEing PCR (gene splice of overlap extending PCR), and constructed expression plasmids of HBV S gene containing mutation sites (T126S, M133L, D144A) in 'a' determinant. According to investigates of epidemiology, these mutations are frequently encountered in patients with HBV infection [2,3]. Data of cellular expression presented below confirmed the importance of the mutation in 'a' determinant.

### 2 Materials and Methods

#### 2.1 Plasmids and cells

pcDNA3 were obtained from Invitrogen company(USA), pCR3.1/SHBV(contain wild HBV S gene) and BHK-21 cells are of our lab.

#### 2.2 Chemicals and enzymes

Pfu enzyme, EcoR I, Sma I and Xho-I endonuclease were obtained from Kejian company, Wuhan; CIAP, 123bp mark, goat anti mice fluorescence antibody from Gibco BRL (USA).

#### 2.3 Methods

2.3.1 Site-directed mutation in 126, 133 and 144 positions of HBV S gene

#### 2.3.2 PCR3.1/SHBV and pcDNA<sub>3</sub>

PCR3.1/SHBV and pcDNA<sub>3</sub> were both digested by EcoR I, 37°C, 5 hours (hereinto, the linear pcDNA<sub>3</sub> after digestion were prepared for next clone. Complex from the former after digestion were dephosphorylated by CIAP, connected with the linear pcDNA<sub>3</sub> and provided the templates of the next gene SOEing PCR.

#### 2.4 Gene SOEing PCR:

##### 2.4.1 Primers

Left primer, ZL: 5'-GCGCTGAACATGGAGAACATCAC-3' (nt157 to nt180);

Right primer, ZR: 5'-CCATCTTTTTGTTTTGTTAQQG-3' (nt860 to nt838);

Primer SP6R: 5'-AGCATTTAQGTGACACTATAQAATAQG-3' (located at SP6 promoter of pcDNA<sub>3</sub>). Sited-directed mutation primers at 126, 133 and 144 positions in HBV S gene: 126 position, 126L (left primer): 5'-CTGCATGACTAQTGCTCAAGGAAC-3' (nt439 to nt415), 126R (right primer):

5'-TTCTTGAGCACTAGTCATGCAGGTCC-3' (nt460 to nt434) ; 133 position, 133L (left primer): 5'-CCAGGAACCTCTGTATCCCTCC-3' (nt 455 to nt 431), 133R (right primer): 5-AGGGATACAGAGAGG TTCCTTGAGCA-3' (nt476 to nt 450); 144 position (left primer): 5'-AACCTTCGGCCGAAATTGC-3' (nt 492 to nt 472), 144R (right primer): 5'GTGCAATTTCGGCCGAAGGTTTG-3' (nt 513 to nt 489).

#### 2.4.2. Gene SOEing PCR

Methods were executed as described by Servant, et al<sup>[4]</sup>. In precise, the first PCR: the upstream -part of S gene with different position mutations were amplified by ZL and 126R or 133R or 144R; the downstream-part of S gene with different mutations were amplified by SP6R and 126L or 133L or 144L. The second PCR were all amplified by ZL and SP6R. We extracted the second PCR productions of HBV S gene with T126S, M133L or D144A mutation.

The fragments for amplified by PCR were rounded to be 92°C 30 s, 50°C 45s and 72°C 60 s for 30 cycles, and the final extension step being 10 min at 72°C. Amplification products were resolved by agarose gel electrophoresis, stained with ethidium bromide, and quantified for the next PCR ligations or cloned.

#### 2.5 Clone and determination of variant HBV S gene

##### 2.5.1 Clone of HBV S gene with sited-mutation

After digesting pcDNA3 by XhoI and EcoR V, we purified the linear vector. All second PCR productions were digested by XhoI, connected with the above vector, and at last transferred into *E.coli*. Ligation products were transferred into DH5  $\alpha$  with routine principle, sifted and maintained at 37°C, poke out single clone of DH5  $\alpha$  and cultured, Both of the newly-constructed vectors were confirmed by restriction endonuclease digestion, PCR with specific

primers and finally by DNA sequencing (Baosheng Co., Dalian, China).

#### 2.5.2 Analysis by sequencing

After sifting and determination by digestion with EcoR I and XhoI, mutation clones were sequenced and analyzed by ALIGN soft.

#### 2.6 Cells and transfection

To study the biological property of mutant clone, BHK cells were divided into 5 groups (wild strain group, negative control, T126S, M133L and D144A group) and seeded in 8-well dishes at the density of  $1\sim 2 \times 10^4$  cells/cm<sup>2</sup> in DMEM containing 10% fetal calf serum. cells were cultured for 3 days continued with DMEM. BHK-21 cells were transfected with the appropriate plasmids constructs (20  $\mu$ g of purified DNA per 10cm dish) by lipofectamine (Boehringer Mannheim Biochemicals Co.USA. At the end of incubation, cells were fixed and detected by immunofluorescence assay 3 days post-transfection with monoclonal antibody of HBsAg or common HBsAb.

### 3 Results

#### 3.1 Results of Gene SOEing PCR (the first and second PCR)

The site-mutation PCR were executed by SOEing PCR, the products of the first PCR and second PCR were showed. The ladder-liked fragments with different length were amplified in the first PCR (Fig 1.1, 1.2) and the same length of products were emerged in the second PCR (Fig 1.3).

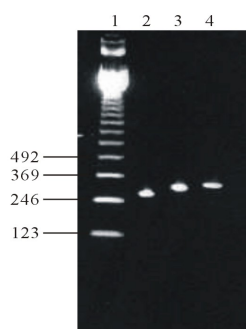


Fig 1.1

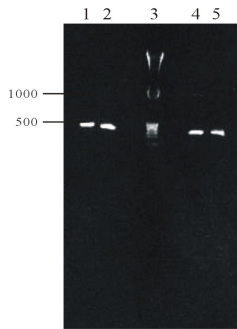


Fig 1.2

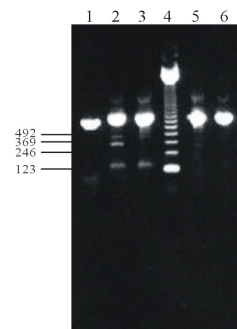


Fig 1.3

The upstream-part of S gene by first PCR (figure.1.1) the downstream-part of S gene by first PCR (figure.1.2) the second PCR (figure. 1.3). Note Fig. 1.1: lane 2,3 and 4 were the upstream-part of S gene by first PCR with mutations at 126,133 and 144 position and the weight were 378 bp, 399bp and 432bp separately; lane 1 is 1kb Mark. Fig.1.2: lane 1,2,4 and 5 were the downstream-part of S gene by first PCR with mutations at 126, 133,144 and 144 position, and weight is 380bp, 359bp and 326bp separately (all containing muticlone sites and full-length SP6 promoter of pcDNA<sub>3</sub>); lane 3 is 1kb Mark. Fig3. 1.3: lane 1 is the product by ZL and ZR, 703bp; lane 2,3,5 and 6 are products through ZL and SP6R for 126,133 and 144 position separately, 758bp (all containing muticlone sites, full-length SP6 promoter and mutation sites); lane 4 is 123bp Mark.

3.2 Align of postulated translation after sequencing

The data were testified after sequencing. After analyzing by ALIGN soft, the homologies of amino acids was almost in 100% among the strains of Wshbvaa, T126Saa, M133Laa, and M144aa, except for the mutant sites we constructed (Wshbvaa amino acid sequence which was translated in theory is simplify for the wild strain sequence of HBV s antigen in “a” determination; T126Saa for the mutant strain sequence at 126 site of HBV s antigen in a determination; M133Laa for mutant strain at 133 site and D144Aaa for mutant strain at 144 site). (Figure 2)



Figure 2. Align of postulated translation after sequencing

3.4 Detection of immunofluorescence assay

The cells were transfected by the differenced mutant clones of HBV S gene and 3 days later, the cells were recognized by monoclonal antibody of HBsAg or HBsAb. The fluorescence were found in the cells of different group by monoclonal antibody, but there were no sign in common HBsAb reorganization (The data not shown) (10×40).(Figure 3)

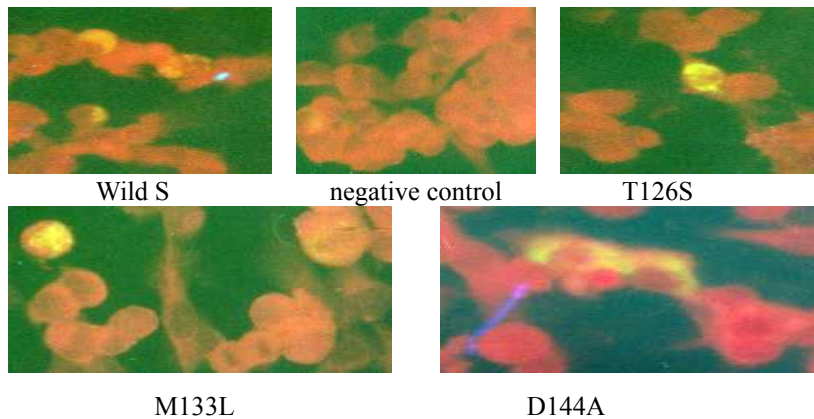


Figure 3. Detection of immunofluorescence assay

## 5. Discussion

HBV vaccine has been proven to be effective in prevention HBV infection. During the natural infection and vaccination, 'a' determinant in HBsAg is thought as the most important motif to elicit the protective antibody. After vaccination, about 90-95% of people can induce the protective antibody against 'a' determinant. But there are still some people can be infected by HBV, though in these patient HBsAb can be detected. Data showed that the variances of HBV S gene 'a' determinant may lead to the above phenomenon. Further more, the variances may escape detection by certain commercial HBsAg kit a, have a selective advantage in carriers treated with passive immunization (HBIG) and become a dominant clone<sup>[4,-6]</sup>.

Carman et al found G145R strain in a carrier of HBV and persisted for 5 years. Synchronously, the G145R variant sera from patients could infect chimpanzee, auto-replicate completely, and have pathogenic effect<sup>[7, 8]</sup>.

Recently, Sayiner AA, et al (2007) found that different commercial kits recognized variant HBsAg depressively, even could not, expressed by G145R, M133A or other mutation positions plasmids, and those variant positions are most common emergence in patients<sup>[9]</sup>. In the chronic, variance of HBV S gene is also very common; positions of mutation are focus on loop I of 'a' determinant. In addition, in patients with unsuccessful vaccination or protection for reinfection after HBIG therapy (such as organ graft), variances are also found in 'a' determinant, but those often mustered on loop II<sup>[10]</sup>.

All sites, single or with others, in HBV 'a' determinant can be elicited to be variance and those variances can cause antigenic alteration of HBsAg more or less in different patients. So, it is crux to definitude which variant strains may alter antigenicity and immunogenicity of HBsAg in vivo (especially high frequent mutation positions), how escape the immune supervision and lead to persistence of HBV infection<sup>[11]</sup>. Therefore, the hinge to solve above problem is to analyze biological characteristics of protein HBV S gene expressed in vivo and in vitro.

According to data of epidemiology, we used gene SOEing PCR and constructed the expressed plasmids of HBV S gene containing mutation sites (T126S, M133L and D144A) in 'a' determinant. Sequence analysis confirmed that the homologies of nucleotides or amino acids between the wild HBV and mutants were 100% except for the target mutation sites. Meanwhile, variant clones with different mutation sites were transfected into BHK cells and showed the variant clones constructed can be expressed in eukaryotic cells after detection by immunofluorescence assay. The

results showed that proteins expressed could precisely fold, maintain natural second construct, and take on good antigenicity. So, the series variant clones we constructed can be used in developing new blended multivalence vaccine, HBIG or HBsAg detected kits, otherwise, also provide the basis for analyzing biological characteristic of S gene variant in vivo, especially in antigenicity and immunogenicity in the future.

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## Implementation of Real-time Handwriting Recognition System Using Touch Panel Based on Neural Network

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**Abstract:** Based on neural network, this study contributes to propose a real-time handwriting recognition system with Arabic numbers and lowercase letters. It includes two parts which are hardware design and software algorithm. In hardware design, after pressing the touch panel surface, analog signals are obtained and transformed into digital ones by A/D converter. In software algorithm, recognition architecture is constructed by three level back-propagation neural network and learning samples of Arabic numbers and lowercase letters are collected from nine schoolmates. Based on the illustration, the proposed handwriting recognition system of this study can achieve about 90% correction rates and satisfy the market standard.

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**Keywords:** Touch panel, Handwriting recognition, Back-propagation neural network

### 1. Introduction

Handwriting is a natural part of the daily lives of human beings. In the past, people have only been able to interact with a computer by typing on a keyboard or using a mouse. When people are unfamiliar with a method by which to input language, they must spend time studying and practicing. As technology has developed, the innovativeness of touch products has gradually improved communication between humans and machines. It would be convenient if we could control machines by only using a touch panel instead of a keyboard or mouse. The advantages of handwriting recognition include the direct inputting of characters and without having to learn an input method. Touch panel can form an important bridge between people whose learning ability is poor and the machines they are trying to operate.

Neural networks have been used in most of research applications such as classifications, automatic control, estimation, signal processing, recognition, etc. (Ramadan et al., 2012; Hanafy, 2011; Arif et al., 2012; Yousefi et al., 2008; Gao et al., 2010). Compared with developing conventional expert systems, the main advantage is that neural networks often can be quickly constructed using available data at a very low cost. In recent years, these artificial intelligence techniques have also been successfully applied in the area of handwriting recognition (Abbas et al., 2010; Zhang and Wu, 2010). Generally speaking, handwriting recognition can be divided into two approaches: on-line and off-line. In on-line technology (Lin and Yen, 1997), information is acquired during the writing process

through the use of a tablet and touch pen. In off-line technology (Chen and Wei, 2010; Dilruba et al., 2006; Tay et al., 2001; Wang et al., 2009), the text data are obtained by a scanner after the writing process is over. Therefore, the recognition technique for on-line handwriting is more complex than is the case with off-line situation. Because everyone has different writing habits and because noise usually exists in the acquisition process, these factors cause the recognition process to occur after acquisition.

In this study, an on-line recognition technique is adopted, and a method by which to develop the recognition system is viewed as a very challenging issue. We use a touch panel to communicate with a machine. After determining the coordinate handwriting location, we utilize in the recognition process to obtain more accurate coordinates and extract feature by obtain stroke characteristic. Finally, the handwriting recognition system uses an artificial neural network to obtain a recognition result. Furthermore, determining how to implement an on-line handwriting recognition system utilizing is the primary goal. The target of the proposed system is to identify handwriting in various font sizes and to create a system that can be utilized by anyone.

### 2. Material and Methods

#### Overview of the system architecture:

There are four main structures in the system architecture, a four-wire resistive touch panel, an A/D converter, a Micro-Control Unit (MCU) development board and a PC. The system architecture diagram is shown in Figure 1. Descriptions of these four main structures are as follows:

- i. Four-wire resistive touch panel: This is the handwriting input device with a two dimensional plane. By touching the surface with the finger or a touch pen, the touch panel controller can calculate the x-axis and y-axis coordinates using the KVL principle.
- ii. A/D converter: Because the data for the touch coordinates is an analog signal, an A/D converter is used to transform the position signal into a digital signal. In this study, an ADS7846 chip is used for the data sampling.
- iii. Micro-Control Unit (MCU): FS7805 GPIO ports are utilized to deliver and read measurement data from an ADS7846 chip. After analyses of the operation, the MCU sends the final data to the PC through the RS232.
- iv. PC software: Visual C++ 2008 software is used to implement the handwriting recognition and to design the graphic user interface (GUI). The firmware of this system is coded by keilC software.

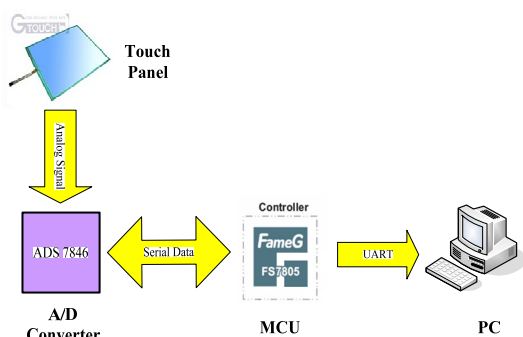


Figure 1. The system architecture diagram.

**Hardware circuit design:**

In the design of the circuit interface, the touch panel's X+, X-, Y+, Y- pins are respectively connected to the ADS7846, and the DCLK, DOUT, DIN, CS, BUSY pins are respectively combined with the FS7805 GPIO port. The schematic diagram of the related control pins is shown in Figure 2. The first transmission between the FS7805 and the ADS7846 is a send control byte on the DIN including the start bit, channel selection, 8/12bit mode, differential/single-ended and power mode. The FS7805 sends the corresponding control byte through the GPIO interface, and the BUSY pin is used to detect the ADS7846 converter status. Then, the FS7805 reads the conversion results from the ADS7846 and obtains coordinates data and the ADS7846 setting configurations. After the above processes, the FS7805 and ADS7846 communication is finished.

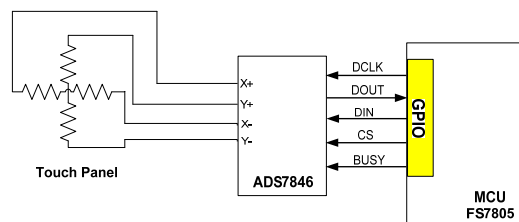


Figure 2. Schematic of connecting interface.

**ADC timing diagram:**

In this study, the timing diagram of the ADS7846 controller is considered (Brown 2005). The CS# is the chip-selected signal. Because this study uses a 12 bit resolution, the sampling data is 0~4095. When the CS# converts from a high voltage level to low voltage level, this means that the controller begins to operate. On the other hand, it also expresses the end of the operation. This system uses 24 clock cycles to represent an operational process. The first eight clock cycles are used to receive commands from the FS7805. At this moment, the DIN (called the "control byte") is set to capture the address coordinates. The control byte and the configuration of differential mode input are shown in Table 1 and 2. At that point, when BUSY signals are moving from high voltage level to low voltage level, the other sixteen clock cycles are started to send commands to the FS7805. Then, the DOUT of the ADS7846 begins to return the coordinate value (called the serial data output) to the FS7805. Finally, the process of the system measurement is completed. The timing diagram is shown in Figure 3.

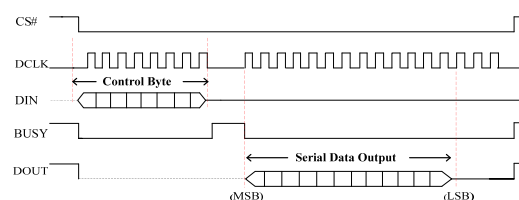


Figure 3. ADS7846 conversion timing diagram.

Table 1. Control byte of ADS7846.

Bit map	Name
Bit7(MSB)	S
Bit6	A2
Bit5	A1
Bit4	A0
Bit3	MODE
Bit2	$\overline{SER/DFR}$
Bit1	PD1
Bit0(LSB)	PD0



Table 2. Configuration of differential mode input.

A2	1	0
A1	0	0
A0	1	1
Y-		
X+		+IN
Y+	+IN	
X-Position	Measure	
Y-osition		Measure
X-Drivers	On	Off
Y-rivers	Off	On

**Firmware Coding on the MCU:**

All types of touch panels must contain pressure detection on their input surfaces, X coordinate measuring, Y coordinate measuring, and repeated detection. At the beginning of the programming design, this system must do the MCU initialization. After touching the touch panel, the ADS7846 pen-interrupt signal starts. The MCU generates a timing sequence to the ADS7846 and sends a control-byte to measure the X and Y coordinates. Therefore, the touch panel firmware design is focused on the control-byte settings. Figure 4 shows the FS7805 firmware flowchart.

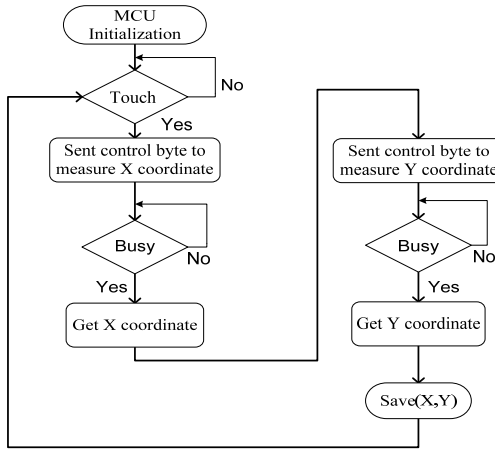


Figure 4. Flowchart of firmware design.

**Algorithm of handwriting recognition:**

Before handwriting recognition occurs, a great deal of data and information about the coordinates obtained from the touch panel must be set up and transmitted to the PC processor through the RS232. The handwriting recognition algorithm can be divided into several steps that include the coordinate acquisition, pre-processing, feature extraction and the neural network module. Figure 5 shows the software schematic design.

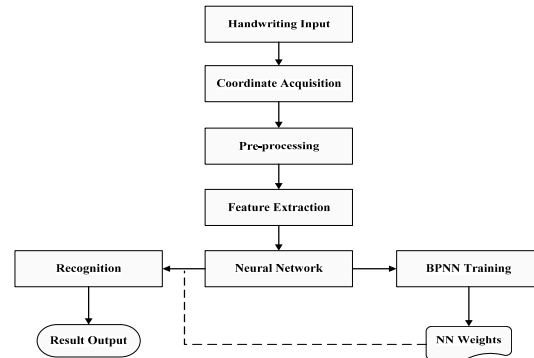


Figure 5. Flowchart of software design.

**Coordinate Acquisition:**

In the previous parts, the MCU has been processed to control the method by which coordinate formation from the touch panel is received. The formula is shown as follows:

$$\begin{aligned}
 X &= 2^{11} \cdot x_{11} + 2^{10} \cdot x_{10} + \dots + 2^0 \cdot x_0 \\
 Y &= 2^{11} \cdot y_{11} + 2^{10} \cdot y_{10} + \dots + 2^0 \cdot y_0
 \end{aligned}
 \tag{1}$$

where  $X, Y$  is the coordinate value after quantification.  $x_0 \sim x_{11}$  and  $y_0 \sim y_{11}$  are defined as the returned coordinate data from touch panel.

**Pre-processing:**

The system uses a 4-wire resistive touch panel with a voltage divider to obtain the voltage value and to convert it to a digital value using an A/D converter. When a finger or stylus touches the panel, this might cause the voltage divider to be incorrect for the location and the calculation may obtain a neighborhood location. Therefore, the noise filter gives a threshold if the coordinate is too close to eliminated. If the difference between the current coordinate information and the next one is greater than 5, the information is recorded, or the filter automatically considers it to be meaningless jitter. Through this approach, the noise jitter can be removed.

**Feature Extraction:**

This study uses two main steps: First, users write a character, and the system begins to normalize. This step can conform the range of the original character to the optimally judged process range. Regardless of whether the sizes of the characters are big and small, the optimum area can be judged effectively. After obtaining the judged range, the maximum X coordinate, maximum Y coordinate, minimum X coordinate and minimum Y coordinate can be picked out. Then, the system can extract the features of the determinant handwriting region. Secondly, because the writing sequences from

everybody are different, this study is aimed at identifying the character correctly while neglecting the writing sequences. By holding the maximum and minimum coordinates, the regions of Arabic numbers and lowercase alphabet letters are separately divided into grids measuring  $8 \times 6$  and  $8 \times 8$ . When the segment of a character is through the grid, the system records "1" from the present grid; otherwise, it records "0". Figure 6 shows that the feature extraction and the features of the Arabic number "3" are recorded as 000000; 011110; 000010; 011110; 000010; 000010; 011110, and 000000. The features of the Lower-case alphabet letter "b" are recorded as 01000000; 01000000; 01100000; 00111110; 00100010; 01100010; 01101110, and 00110000.

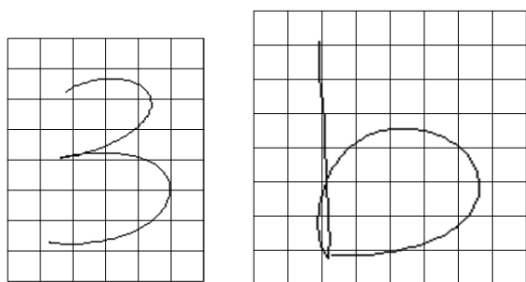


Figure 6. Two examples of the feature extraction.

### BPNN training algorithm

The back-propagation algorithm uses a method of gradient descent that can optimize the weights of the neural network. It is utilized iteratively to adjust weights, which can achieve the minimum error between the input and target values, and the ANN will finally be convergent. The complete flow of learning is shown as follows, and Figure 7 shows the flow chart for the learning model.

- Step 1: The numbers for the ANN, which contains the input, hidden, and output layer numbers of the neuron are chosen first. Because the BPNN belongs to a supervised learning network, it is needed to provide the sample numbers and to target into the ANN.
- Step 2: The initialized weights that adopt the random hypothesis are set up.
- Step 3: The patterns of learning, which include the input and target dimensions are inputted.
- Step 4: The ANN output values, which include the neurons from both the hidden and output layers, are calculated.
- Step 5: The magnitudes of error, which include the neurons from both the hidden and output layers, are calculated. This key point returns the output values from the output layers into the neuron of the hidden layers, which can

adjust the magnitudes of error and obtain new weights for the ANN.

- Step 6: All weights, including the hidden and output layers of the ANN, are adjusted.
- Step 7: If the error achieves the standard of convergence, the learning process is stopped and the weights are saved, or it repeats again from step 3.

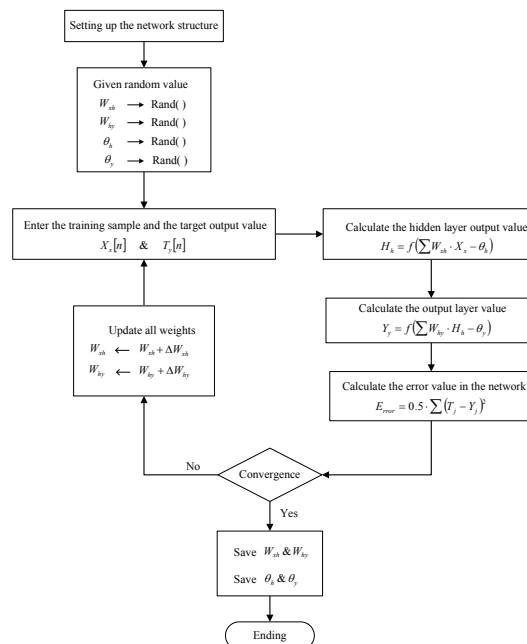


Figure 7. Flowchart of BPNN learning process.

### BPNN structure design:

This study uses a three-level layer back-propagation neural network, which includes input layers, hidden layers, and output layers as the framework for the neural network.

- i. Design of the input and output layers: Due to treating the samples differently, the numbers of input neurons are changed from the size of the input data. In this study, the Arabic numbers mode and lowercase alphabetic mode are set at 48 and 64, respectively. The output layer is used to describe the results of the network output. And then, the numbers of the output layer neurons in the Arabic number mode and the lowercase alphabetic mode are set at 10 and 26, respectively.
- ii. Design of the hidden layer: This layer is used to describe the neuron interaction between the input and output layers. Number of layer numerous is very important. If the numbers of layer numerous are set suitably, the optimal weights can be found easily, and the error function can converge better. The optimized formula is shown as the following equation:

$$h = \sqrt{x \times y} + c \tag{2}$$

where  $x$  is the number of input layer neurons,  $y$  is the number of output layers neurons;  $h$  is the number of hidden layers neurons, and  $c$  is chosen to be a number between 1 to 5. In this study, the numbers of hidden layer neurons in the Arabic number mode and the lowercase alphabetic mode are set at 18 and 230, respectively.

iii. The determination of activation functions:

Sigmoid function  $f(x) = 1/(1 + \exp(-ax))$ ,  $a > 0$  can be used as the activation function, where  $a$  is the slope parameter of the sigmoid function, and by changing the value  $a$ , the activation function with a different slope can be obtained. Sigmoid function is shown in Figure 8.

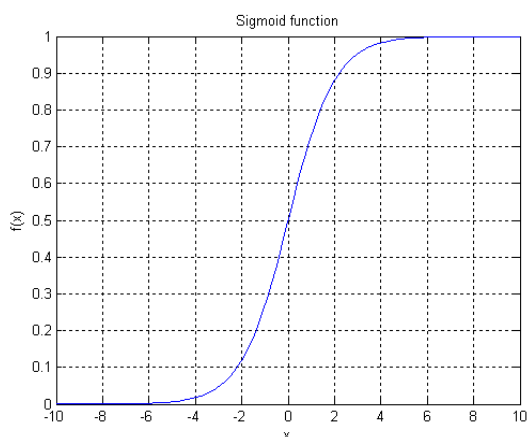


Figure 8. Sigmoid function.

### 3. Results

#### Illustration and Testing:

Before testing the system must collect training samples that include Arabic numbers and lowercase letters. The handwriting samples are written randomly on the touch panel. Based on the training intended to adjust the BPNN weights, the convergence of the mean square error (MSE) can be reached (Choudhary et al. 2010).

#### Illustration in Arabic Numbers:

In part of the Arabic numbers training, complete stroke of the feature extracting contains 48 parameters, which set 48 neurons in the input layer and 9 neurons in the output layer representing Arabic number (0~9). We adjust the neuron numbers in the hidden layer and discuss the numbers in the hidden layer. All parameters for the BPNN are shown in Table 3, which follows. Figure 9 compares the numbers in the hidden layer neuron of each MSE convergence situation. After trial and error, the

BPNN have convergence below 0.01 via the neuron amount 18 from the hidden layer.

Table 3. Neural network structure of Arabic part.

Parameters	values
Input neurons	48
Hidden neurons	18
Output neurons	10
Learning rate	0.5
Momentum Term	0.5
Activation function	Sigmoid function
Initial weights and biased values	Randomly set values between 0 and 1

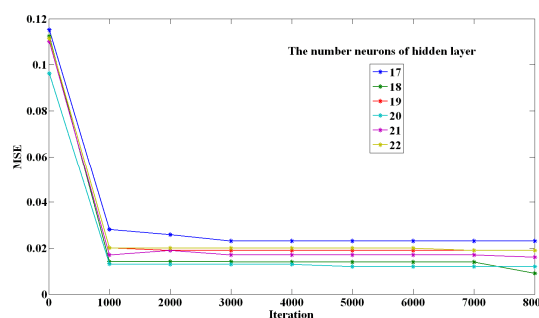


Figure 9. Schematic of MSE.

In this part, the training samples are written with unlimited strokes on the touch panel, which writes the Arabic number (0~9) 1000 times. After training, we ask nine people to write an Arabic number 100 times. Table 4 shows the recognition results with candidate situation of Arabic number can reach 92%. The recognition index was defined as follows:

Recognition Rate =

$$\frac{\text{The number of the correct recognition}}{\text{Amount of the samples}} \times 100\% \tag{3}$$

Table 4. Testing results of Arabic number.

Arabic number	Recognition rate	Arabic number	Recognition rate
0	97.5	6	95
1	97.5	7	92.5
2	92.5	8	85
3	92.5	9	98.75
4	93.75	Average	92.6
5	81.25		

#### Illustration in Lowercase Letters:

In the part consisting of training in the lowercase letters, a complete stroke of the feature extraction contains 64 parameters, which set 64 neurons in the input layer and 26 neurons in the

output layer representing lowercase letters (a~z). In this section, we adjust the neuron numbers in the hidden layer and discuss the numbers in the hidden layer. All parameters of the BPNN are shown in Table 5, which follows. Because training in lowercase letters have a high level of complexity, we use the decrease in the learning rate to substitute for the increase in the hidden layer in order to obtain good convergence. Improper selection of a learning rate may cause a local minimum problem or large training runs, and as a result, can decrease ANN performance (Abbas et al., 2010; Zhang and Wu, 2010). Figure 10 compares the numbers of the hidden layer neurons with each MSE convergence situation.

Table 5. Neural network structure of letters part.

Parameters	Values
Input neurons	64
Hidden neurons	300
Output neurons	26
Learning rate	0.2
Momentum Term	0.2
Activation function	Sigmoid function
Initial weights and biased values	Randomly set values between 0 and 1

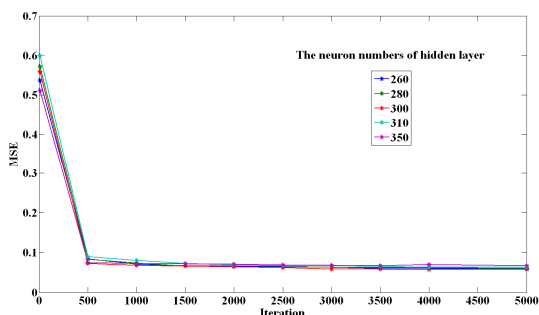


Figure 10. Schematic of MSE.

In this section, the training samples are written with unlimited strokes on the touch panel, which writes the lowercase alphabet (a~z) 2600 times. After training, we ask nine people to write 130 times. Table 6 shows the recognition result with candidate situation can reach 93%.

Table 6. Testing results of lowercase alphabet.

Alphabet	Recognition rate	Alphabet	Recognition rate
a	94.3	o	97.1
b	94.3	p	94.3
c	94.3	q	88.6
d	97.1	r	97.1

e	86.4	s	94.3
f	94.3	t	94.3
g	94.3	u	94.3
h	88.6	v	97.1
i	83	w	97.1
j	97.1	x	97.1
k	97.1	y	83.5
l	94.3	z	94.3
m	85.7	Average	93.2
n	94.3		

#### 4. Discussions

In this experiment, we consider handwriting strokes that are simple or one directional to complete to have a high recognition rate. Poor handwriting trajectory, it may cause incorrect results. Therefore, the system compares the situation with join candidate word condition. The recognition results can be more than 90%. Figure 11 shows the comparison of the experimental results.

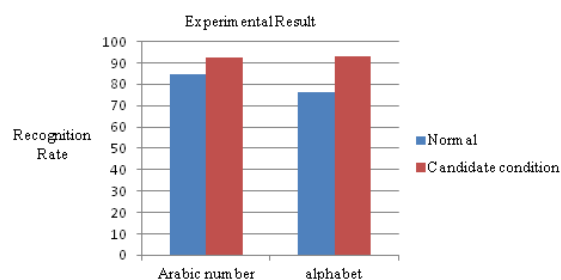


Figure 11. Comparison of illustration.

#### 5. Conclusion

In this study, an on-line handwriting recognition system was implemented by using the artificial neural network. The proposed system includes hardware design and software algorithm. In hardware design, analog signals from the touch panel surface are transformed into digital ones by A/D converter. In software algorithm, recognition architecture is constructed by three level back-propagation neural network. Based on the illustration, the proposed handwriting recognition system of this study can achieve about 90% correction rates and satisfy the market standard.

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