Clinical Utility of Serum Chemerin as a Novel Marker of Metabolic Syndrome and Type 2 Diabetes Mellitus

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Abstract: Background: Chemerin is a novel adipokine which has dual roles in adipose tissue metabolism and regulation of immune response. Its concentrations are elevated in obese, insulin-resistant, and inflammatory states in vivo and suggested to be involved in insulin resistance in obesity and type 2 diabetes. **Objective:** was to evaluate the clinical utility of serum chemerin as a marker of metabolic syndrome and type 2 diabetes and to investigate its correlation with clinical and laboratory parameters of these conditions. **Subjects and Methods**: This study was conducted on 20 patients with metabolic syndrome (group I) and 20 patients with type2 DM (group II), in addition to 15 healthy control subjects. All individuals included in the study were subjected to full history taking, thorough clinical examination including waist circumference and blood pressure measurement, laboratory investigations including: fasting and post-prandial blood glucose, fasting serum insulin, lipid profile, serum chemerin level assessment by ELISA technique. Homeostasis Model Assessment-Insulin Resistance Index (HOMA-IR) and the cardio-vascular risk value were calculated. **Results:** Serum chemerin levels were significantly higher in group I (metabolic syndrome group) when compared to group II (type 2 diabetes mellitus) and control group ($p<0.001$, respectively). In addition, it was significantly higher in group II compared to control group ($p<0.001$). Serum chemerin levels negatively correlated with HDL-C and CAD-risk and positively correlated with systolic blood pressure, diastolic blood pressure, waist circumference, fasting blood insulin, HOMA-IR, total cholesterol, LDL-C and triglycerides in both groups ($p<0.05$, respectively). Multivariate analysis showed that serum chemerin, waist circumference and total triglycerides were the most significant predictors for metabolic syndrome with F value=7.68 ($p <0.05$). ROC curve analysis revealed that the best diagnostic cutoff point for serum chemerin in type 2 DM was 95 ng/mL. This had a diagnostic sensitivity 75%, specificity 80%, positive predictive value 83%, negative predictive value 71% and efficiency 77%. The best diagnostic cutoff point for serum chemerin as a predictor of metabolic syndrome was 140 ng/mL. This had a diagnostic sensitivity, specificity, positive predictive value, negative predictive value and efficiency 100%, respectively. **Conclusion:** In conclusion, the association of high serum chemerin levels with components of metabolic syndrome and type 2 DM indicates that this adipokine represents a novel marker of these derangements and could be considered one of the metabolic risk factors leading to insulin resistance in type 2 DM as well as metabolic syndrome. Moreover, its assessment could be beneficial in early detection of these pathological states and prevention of their unfavorable consequences especially the cardiovascular complications and atherosclerosis.

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Key words: Chemerin; metabolic syndrome; type 2 diabetes mellitus.

1. Introduction

Type 2 diabetes mellitus is a group of disorders characterized by hyperglycemia and associated with microvascular and macrovascular complications. Hyperglycemia results from lack of endogenous insulin or resistance to the action of insulin in muscle, fat and liver in addition to an inadequate response by the pancreatic beta cells ***(Wolfs et al., 2009).***

The metabolic syndrome is a cluster of coronary heart disease (CHD) risk factors including high blood pressure, dyslipidemia, hyperglycemia and central obesity that are associated with decreased ability of insulin to stimulate glucose disposal on peripheral target tissues, referred to as insulin resistance ***(Olufadi and Byrne, 2008).***

Insulin resistance is determined by impaired sensitivity of insulin to its main target organs i.e. adipose tissue, liver and muscle. Obesity, particularly central obesity, is the prominent risk factor for insulin resistance and results in type 2 diabetes and metabolic syndrome ***(Zeyda and Stulnig, 2009).***

Adipose tissue represents an active endocrine organ that releases a large number of bioactive mediators (adipokines) that signal to organs of metabolic importance including brain, liver, skeletal muscle, and the immune system thereby modulating hemostasis, blood pressure, lipid and glucose metabolism, inflammation and atherosclerosis. These adipokines include adiponectin, leptin, omentin, resistin, retinol binding protein-4, tumor necrosis factor-α, interleukin-6, vaspin, visfatin and chemerin ***(Yan et al., 2012).***

Chemerin is an adipokine that has been reported to modulate immune system function through its binding to the chemerin receptor. It is secreted as an

18kDa inactive pro-protein and undergoes extracellular serine protease cleavage of the C-terminal portion of the protein to generate the 16kDa active chemerin which is present in plasma and serum ***(Stejskal et al., 2008).*** It is secreted by the mature adipocytes and can regulate adipogenesis and adipocyte differentiation. Chemerin serves as a chemoattractant for pro-inflammatory cells of the immune system such as macrophages and immature dendritic cells that express the cognate receptor chemokine-like receptor-1 (CMKLR1) ***(Bozaoglu et al., 2010*** and ***Iannone and Lapadula, 2011)*** Furthermore, chemerin and its receptor/ChemR23 are expressed abundantly in adipose tissue, suggesting its function in autocrine/ paracrine fashion ***(Ernst et al., 2012).***

Chemerin serum concentrations are elevated in obese, insulin-resistant, and inflammatory states *in vivo* and suggested to be an obvious cause of insulin resistance ***(Hart and Greaves, 2010)***: Obesity induces inflammation in adipose tissue and since chemerin is a pro-inflammatory cytokine that recruits and activates immune cells and contributes to inflammation by promoting macrophage adhesion to vascular cell adhesion molecule-1 (VCAM-1) and fibronectin, it may link obesity and inflammation ***(Ouchi et al., 2011).*** Muscle insulin resistance is a major risk factor for the pathogenesis of type 2 diabetes. Therefore, a possible relation of chemerin to inflammatory proteins and insulin resistance in obesity and type2 diabetes is suggested. Chemerin is also proved to be associated with components of metabolic syndrome and it may play a role in the pathophysiology of this condition ***(Lambernd et al., 2012)***

Aim of the work: The aim of the present study was to evaluate the clinical utility of serum chemerin as a marker of metabolic syndrome and type 2 diabetes and to investigate its correlation with clinical and laboratory parameters of these conditions.

2. Subjects and Methods:

A- Subjects:

1- Patients' Group (n = 40):

This group included forty patients attending to Ain Shams University Hospitals outpatients Cardiology and Internal Medicine Clinics for medical investigations. They were 17 males and 23 females. They were further divided into two groups:

(a) Group I; Patients with metabolic syndrome (n=20):

This group included twenty non-diabetic patients. They were 10 males and 10 females whose ages ranged from 32 to 69 years (Mean 51.3 ± 11.4). Clinical identification of the metabolic syndrome was done according to the **National Cholesterol Education Program Adult Treatment Panel III (2002) (NCEP ATP III**) guidelines, as described by ***(Lin et al., 2009)*:**

(i) Hypertension was defined as a systolic blood pressure of ≥130 mmHg and/or diastolic blood pressure of ≥ 85 mmHg on repeated measurements, or the patient receiving antihypertensive treatment.

(ii) Abdominal obesity was defined as waist circumference exceeding 102 cm and 88 cm in men and women, respectively.

(iii) Fasting serum triglycerides more than 150 mg/dL (1.7mmol/L) or patient receiving treatment for this lipid abnormality.

(iv) Fasting serum HDL-C less than 40 mg/dL (1.0 mmol/L) and 50mg/dL (1.3 mmol/L) in men and women, respectively or patient receiving treatment for this lipid abnormality.

(v) Fasting plasma glucose ≥ 110 mg/dL (6.1 mmol/L)

Metabolic syndrome was diagnosed by the presence of three or more of the previous criteria ***(American Heart Association, 2004)***

(b) Group II; Patients with type 2 diabetes mellitus (n=20):

This group included twenty patients, 7 males and 13 females, whose ages ranged from 35 to 74 years (Mean 55.3 ± 9.6). Patients were diagnosed according to the Report of the **Expert Committee on the Diagnosis and Classification of Diabetes Mellitus in 1997** and the **follow-up reports in 2003**. Type 2 diabetes mellitus was identified by one of the following criteria:

i) Symptoms of diabetes mellitus plus random plasma glucose concentration ≥ 200 mg/dL.

ii) Fasting plasma glucose (FPG) ≥ 126 mg/dL on more than one occasion.

iii) 2 hours post load plasma glucose concentration ≥ 200 mg/dL during oral glucose tolerance test.

2- Control Group (n = 15):

This group included 15 healthy subjects. They were 9 males and 6 females whose ages ranged from 27 to 69 years (49.1 ± 10.2).

Exclusion criteria: A number of clinical conditions, which were known to be associated with increased serum chemerin levels, were excluded e.g. chronic liver disease, hepatitis B or hepatitis C virus infection, liver cell failure ***(Marra and Bertolani, 2009)***, chronic kidney disease and renal failure ***(Hu and Feng, 2011).***

All individuals included in this study were subjected to:

-Full history taking: focusing on family history of type 2 diabetes mellitus, hypertension, smoking and physical activity.

-Thorough clinical examination including waist circumference and blood pressure measurement.

-Mean arterial blood pressure (MAP) was also calculated. MAP = [(2 x diastolic) +systolic] / 3 -

Laboratory investigations that included: fasting blood

glucose, post-prandial blood glucose, fasting insulin, lipid profile and serum chemerin level.

The Homeostasis Model Assessment-Insulin Resistance Index (HOMA-IR) and the cardio-vascular risk value were calculated.

B- Sampling:

Five milliliters of venous blood were collected after 12-14 hours fasting under complete aseptic precautions in plain test tubes without anticoagulant. After coagulation, samples were centrifuged (at 1500 xg for 15 minutes). The separated serum was divided into three aliquots. One was designated for the immediate assay of fasting glucose and lipid profile. The other two aliquots were stored at -20ºC for subsequent assay of insulin and chemerin. Hemolysed samples were discarded. Repeated freezing and thawing was avoided.

C- Methods:

1- Analytical Methods:

(a) Serum glucose level:

The analysis was done using Synchron CX-9 (Instruments Inc.; Scienfitic Instruments Division, Fillerton, CA 92634, 3100, USA.) system auto-analyzer applying enzymatic colorimetric method ***(James et al., 1970)***.

(b) Total cholesterol (TC):

Total cholesterol was assayed on the Synchron CX-9 system auto-analyzer applying enzymatic colorimetric method ***(Dietschy et al., 1976)***.

(c) Triglycerides (TG):

The analysis of TG was done using the Synchron CX-9 system auto-analyzer applying enzymatic colorimetric method ***(McGowan et al., 1983)***.

(d) High density lipoprotein cholesterol (HDL-C):

The HDL-C was assayed on the Synchron CX-9 system auto-analyzer after precipitation of LDL and VLDL by dextran sulfate and magnesium in the separating reagent. The LDL and VLDL portions were then removed by centrifugation. The cholesterol in the HDL fraction which remains in the supernatant was assayed with an enzymatic timed endpoint method ***(Assman et al., 1983).***

(e) Low density lipoprotein cholesterol (LDL-C):

LDL-C value was calculated according to "Friedewald equation":

LDL-C = Total cholesterol-(HDL-C+TG/5)

This equation was applied provided that serum TG level is <400 mg/dL ***(Friedwald et al., 1972*** and ***Warnick et al., 1990).***

(f) CAD-risk percentage:

For the assessment of cardiovascular risk, HDL-C/TC % was calculated ***(Carl et al., 2006)***.

(g) Insulin:

Insulin was assayed by Micro-particle Enzyme Immunoassay (MEIA) on the AxSYM (Abbott Ireland, Diagnostic Division-Lisnamuck, Longford Co. Longford, Ireland +353-43-31000) for the quantitative determination of insulin in human serum or plasma. The used method based on the Micro-particle Enzyme Immunoassay (MEIA) technology. In this assay, sample and reagents required for one test, were pipetted by the sampling probe into various wells of a reaction vessel (RV). The RV was immediately transferred into the processing center. Sample, anti-insulin (mouse, monoclonal) coated micro-particles and assay buffer were pipetted into one well of the reaction vessel. During the incubation of this reaction mixture, the insulin in the specimen binds to the anti-insulin coated micro-particles forming an antibody-antigen complex. An aliquot of the reaction mixture was transferred to the matrix cells. The micro-particles bind irreversibly to the glass fiber matrix. The matrix cell was washed to remove unbound materials. The anti-insulin (mouse, monoclonal) alkaline phosphatase conjugate was dispended onto the matrix cell and binds to the antibody-antigen complex. The matrix cell was washed again to remove unbound materials. The substrate, 4-Methylumbellifryl Phosphate was added to the matrix cell and the rate of fluorescent product formation was measured by the MEIA optical assembly ***(Jennifer et al., 1999)***:

(h) The homeostasis model assessment-insulin resistance index (HOMA-IR): It was calculated using the equation:

HOMA-IR =

$$\frac{\text{fasting glucose}(\text{mg/dL}) \times \text{fasting insulin}(\mu\text{U/mL})}{405}$$

The cutoff point to define insulin resistance corresponds to HOMA-IR ≥ 3.8 ***(Shirai, 2004)***:

(i) Chemerin:

Chemerin concentrations were measured using a commercially available enzyme-linked immuno-sorbent assay (ELISA) kit supplied by BioVendor Laboratory Medicine, Inc (Biovender GmbH, Im Neuenheimer Feld 583, D-69120 Heidelberg, Germany). Surface of the wells in microtitration plate was coated with polyclonal anti-human chemerin specific antibody. Standards, quality controls (QC) and diluted samples were pipetted into the wells. After a 60 minutes incubation followed by washing, biotin labeled polyclonal anti-human chemerin antibody was added and incubated with the captured chemerin for 60 minutes. After another washing step, streptavidin-HRP conjugate was added. After 30 minutes incubation and last washing step, the remaining conjugate was allowed to react with the substrate solution. The reaction was stopped by addition of acidic solution (0.2M H_2SO_4). Absorbance of the resulting product was measured spectrophotometrically at 450nm. The absorbance is proportional to the concentration of chemerin. A standard curve was constructed by plotting absorbance

values versus chemerin concentrations of standards. Concentrations of the unknown diluted samples were determined using this standard curve and then multiplied by the dilution factor to get the actual amount of chemerin in the original serum ***(Bozaoglu et al., 2007)***.

2- Statistical Methods:

Statistical analysis was done using SPSS software, version 9.02, 1998, Echosoft Corporation, USA. The goodness of fit test was done to determine the distribution of data. Descriptive statistics in the form of mean±SD were calculated for parametric data. On the other hand, non parametric data were expressed as median and interquartile range. Between-group comparisons were done using the Student's t test in case of normally distributed data, and Wilcoxon's rank sum test in case of skewed data. Regarding the correlation analysis, Spearman's rank correlation (r_s) was done to evaluate the degree of correlation between two variables if one of them or both had skewed data. p values <0.05 were considered significant, whereas p values <0.01 were considered highly significant. Receiver operating characteristic (ROC) curve analysis was applied to evaluate of the diagnostic utility of chemerin.

3. Results:

The results obtained in the present study are presented in tables 1-3 and figures1 and 2. Systolic blood pressure, diastolic blood pressure, and mean arterial blood pressure were significantly higher in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p<0.001, respectively). Moreover, the waist circumference was significantly higher in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.05 and <0.001, respectively) (Table 2).

Fasting blood sugar, 2-hs post prandial blood sugar and HOMA-IR were significantly higher in group II (type 2 diabetes mellitus) when compared to group I (metabolic syndrome) and control group (p <0.001, respectively) (Table 2). In addition, HOMA-IR levels were significantly higher in group I (metabolic syndrome) when compared to control group, p <0.001. Fasting insulin levels were significantly higher in group I (metabolic syndrome) and group II (type 2 diabetes mellitus) when compared to control group (p <0.001, respectively). However, there was no significant difference between group I (metabolic syndrome) and group II (type 2 diabetes mellitus) concerning fasting insulin levels (p >0.05) (Table 2).

Serum HDL-C was significantly lower in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.05 and p <0.001, respectively). In addition, total cholesterol and LDL-C levels were significantly higher in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.05 and p <0.01, respectively). Moreover, Serum triglycerides were significantly higher in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.001, respectively). However, the difference between group II (type 2 diabetes mellitus) and control group regarding serum HDL-C, total cholesterol and triglycerides did not reach statistical significance, p >0.05; respectively (Table 2).

CAD-risk percentage was significantly lower in group I (metabolic syndrome) when compared to group II (type 2 diabetes mellitus) and control group (p <0.001, respectively). CAD-risk percentage was also significantly lower in group II (type 2 diabetes mellitus group) compared to control group, p <0.05 (Table 2).

Serum chemerin levels were significantly higher in group I (metabolic syndrome group) when compared to group II (type 2 diabetes mellitus) and control group (p <0.001, respectively). In addition, it was significantly higher in group II (type 2 diabetes mellitus) compared to control group (p <0.001) (Table 2).

Our correlation study between serum chemerin level and other studied parameters revealed a significant negative correlation between serum chemerin level and both HDL-C and CAD-risk in metabolic syndrome and type 2 DM (p <0.05, respectively). Moreover, a significant positive correlation was found between serum chemerin levels and each of systolic blood pressure, diastolic blood pressure, waist circumference, fasting blood insulin, HOMA-IR, total cholesterol, LDL-C and triglycerides in both groups (p<0.05, respectively). However, a non-significant correlation was observed between serum chemerin and each of fasting blood sugar and post prandial blood sugar, p >0.05 (Table 3).

Multivariate analysis was done for the measured variables to test them as predictors of metabolic syndrome. It showed that serum chemerin, waist circumference and total triglycerides were the most significant predictors with F value=7.68 (p <0.05).

Receiver operating characteristic (ROC) curve analysis revealed that the best diagnostic cutoff point for serum chemerin in type 2 DM was 95 ng/mL. This had a diagnostic sensitivity 75%, specificity 80%, positive predictive value 83%, negative predictive value 71% and efficiency 77%, with an AUC = 0.786 (Figure 2). The best diagnostic cutoff point for serum chemerin as a predictor of metabolic syndrome was 140 ng/mL. This had a diagnostic sensitivity, specificity, positive predictive value, negative predictive value and efficiency 100%, respectively.

Table 1: Descriptive statistics of the studied parameters in the different studied groups

Group / Parameter	Group I (Metabolic Syndrome) [n=20] $\bar{X}$ ±s/ Median (IR) *	Group II (Type 2 Diabetes Mellitus) [n=20] $\bar{X}$ ±s/ Median (IR) *	Control Group [n=15] $\bar{X}$ ±s/ Median (IR) *
Systolic Blood Pressure (mm Hg)	140±16.9	122±8.9	112±9.7
Diastolic Blood Pressure (mm Hg)	93±10.9	83±6.9	70±7.2
Mean Arterial Blood Pressure (mmHg)	111±9.2	91 ±7.2	85 ±8.2
Waist Circumference (cm) in males	110±12.4	101 ±11.8	80±8.8
Waist Circumference (cm) in females	103±8.7	92 ±9.8	76±7.5
Fasting Blood Sugar (mg/dL)	92 (89 – 99) *	144 (130 – 175)*	75 (67 – 84)*
2 Hours Postprandial Blood Sugar (mg/dL)	111 (100 – 121)*	173 (137 – 197)*	101 (90 – 114)*
Fasting Blood Insulin (µU/mL)	8.7 (7.2 – 12.3)*	9 (6.1 – 13.7)*	4.3 (2.7 – 5.5)*
HOMA-IR	2.1 (1.6 – 3.2)*	3.2 (2.3 – 5.3)*	0.8 (0.4 – 1.2)*
HDL-C (mg/dL)	39±8.7	48±9.4	53±7.4
LDL-C (mg/dL)	133 (110 – 185)*	115 (97 – 137)*	109 (84 – 120)*
Total Cholesterol (mg/dL)	221 (182 – 242)*	192 (157 – 204)*	179 (147 – 198)*
Triglycerides (mg/dL)	170 (123 – 213)*	116 (75 – 130)*	108 (70 – 112)*
CAD-risk (%)	18 (15 – 22)*	26 (25– 30)*	34 (27 – 36)*
Serum Chemerin (ng/mL)	455 (275 – 725)*	112 (80 – 173)*	50 (40 – 90)*

Table (2): Statistical Comparison between Various Groups Regarding Different Studied Parameters

Group / Parameter	Group I (Metabolic syndrome) Vs Controls		Group II (Type 2 Diabetes) Vs Controls		Group I (Metabolic syndrome) Vs Group II (Type 2 Diabetes)	
	t or z*	***p***	**t or z***	***p***	**t or z***	***p***
Systolic Blood Pressure (mm Hg)	6.1	<0.001	2.5	<0.05	4.3	<0.001
Diastolic Blood Pressure (mm Hg)	5.8	<0.001	2.6	<0.05	3.6	<0.001
Mean Arterial Blood Pressure (mm Hg)	5.3	<0.001	2.5	<0.05	3.5	<0.001
Waist Circumference (cm) in male	8.3	<0.001	3.7	<0.001	2.6	<0.05
Waist Circumference (cm) in female	5.3	<0.001	3.5	<0.001	2.5	<0.05
Fasting Blood Sugar (mg/dL)	3.8*	<0.001	4.9*	<0.001	4.9*	<0.001
2 Hours Postprandial Blood Sugar (mg/dL)	1.6*	>0.05	4..0 *	<0.001	3.8 *	<0.001
Fasting Blood Insulin (µU/mL)	3.9*	<0.001	3.6*	<0.001	0.1*	>0.05
HOMA-IR	4.2*	<0.001	4.8*	<0.001	2.7*	<0.001
HDL-C (mg/dL)	5.2	<0.001	1.9	>0.05	2.6	<0.05
LDL-C (mg/dL)	3.1*	<0.001	1.2*	>0.05	2.1*	<0.05
Total Cholesterol (mg/dL)	3.5*	<0.001	1.3*	>0.05	2.5*	<0.05
Triglycerides (mg/dL)	4.6*	<0.001	1.5*	>0.05	3.6*	<0.001
CAD-risk (%)	4.8*	<0.001	2.8*	<0.05	4.3*	<0.001
Serum Chemerin (ng/mL)	5.0*	<0.001	3.1*	<0.001	4.8*	<0.001

t: Student's t test; z*: Wilcoxon's rank sum test (P<0.01): highly significant difference; (P <0.05): significant difference; (P >0.05): non significant difference

Table 3: Correlation analysis between serum chemerin level and other studied parameters in metabolic syndrome and type 2 DM groups using Spearman's rank correlation coefficient

Parameter		Group I(Metabolic syndrome)	Group II(Type 2 Diabetes)
Systolic Blood Pressure	r_s p	0.578 <0.05	0.362 <0.05
Diastolic Blood Pressure	r_s p	0.542 <0.05	0.421 <0.05
Waist Circumference	r_s p	0.642 <0.05	0.532 <0.05
Fasting Blood Sugar (mg/dl)	r_s p	0.087 >0.05	0.196 >0.05
2 Hours Postprandial Blood Sugar (mg/dl)	r_s p	0.127 >0.05	0.207 >0.05
Fasting Blood Insulin (µU/mL)	r_s p	0.367 <0.05	0.284 <0.05
HOMA-IR	r_s p	0.286 <0.05	0.295 <0.05
Total Cholesterol	r_s p	0.556 <0.05	0.461 <0.05
CAD-risk (%)	r_s p	-0.699 <0.05	-0.321 <0.05
HDL-C	r_s p	-0.374 <0.05	-0.311 <0.05
LDL-C	r_s p	0.445 <0.05	0.352 <0.05
Triglycerides	r_s p	0.542 <0.05	0.332 <0.05

(P <0.05): significant correlation; (P >0.05): non significant correlation

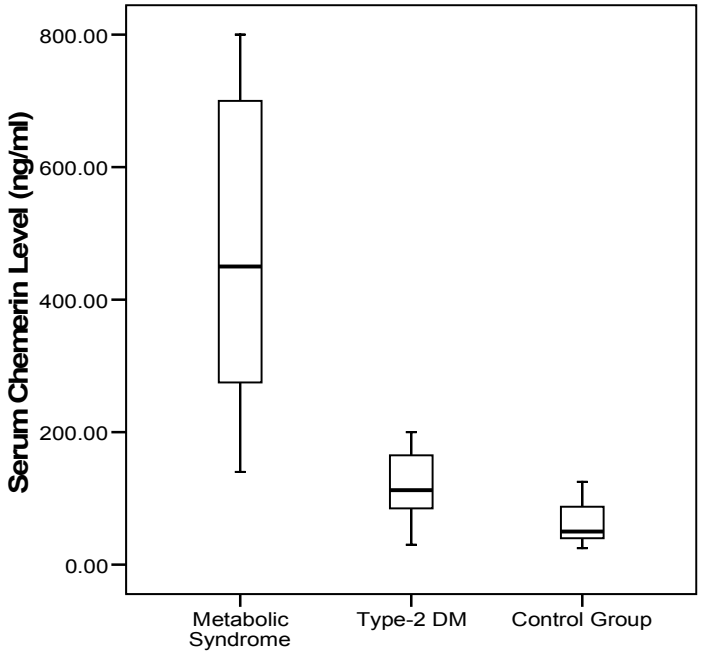

Figure 1: Box-Plot Chart showing difference between study groups concerning serum chemerin level

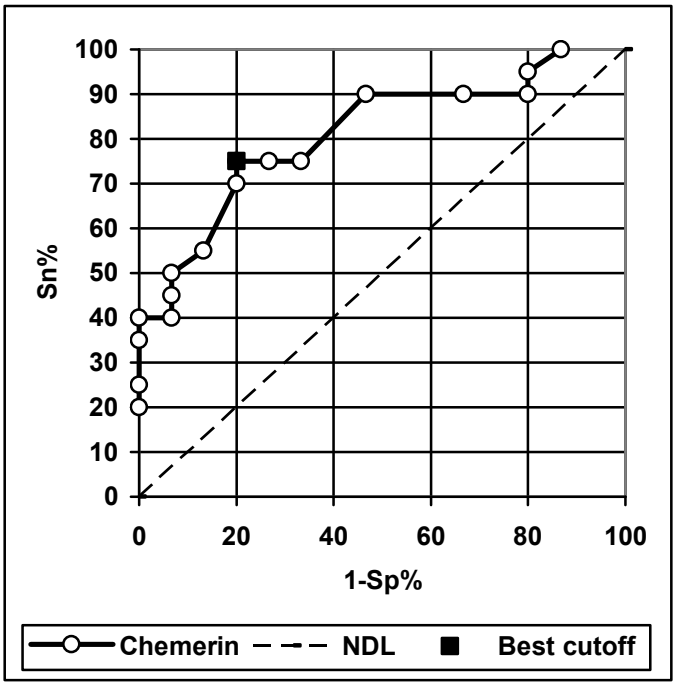

Figure 2: ROC curve analysis showing the diagnostic performance of chemerin in patients with type 2 DM

4. Discussion:

Increased adipose tissue mass, especially in the visceral compartment, is characterized by altered metabolic and endocrine function leading to an increased secretion of pro-inflammatory adipokines ***(Suzuki et al., 2010)***. Chemerin is a recently described adipokine which has dual roles in adipose tissue metabolism and regulation of immune response. Chemerin serum concentrations are elevated in obese, insulin-resistant, and inflammatory states in vivo and suggested to be an obvious cause of insulin resistance in obesity ***(Parlee et al., 2010*** and ***Ferraccioli and Gremese, 2011)***. Chemerin is also suggested to be linked to obesity-induced insulin resistance in type 2 diabetes ***(Hu and Feng, 2011).***

In the view of the previous studies, the aim of our work was to evaluate the clinical utility of serum chemerin as a marker of metabolic syndrome and type 2 diabetes and to investigate its correlation with clinical and laboratory parameters of these conditions.

The results of the present study revealed that the systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, waist circumference, fasting serum insulin, fasting serum glucose, HOMA-IR, total cholesterol, LDL-C, triglycerides and serum chemerin levels were significantly higher in metabolic syndrome group when compared to control group. In addition, serum chemerin correlated positively with each of systolic blood pressure, diastolic blood pressure, waist circumference, fasting serum insulin, HOMA-IR, total cholesterol, LDL-C and triglycerides in metabolic syndrome patients.

These findings were in agreement with those of ***Stejskal et al. (2008), Bozaoglu et al. (2009)*** and ***Dong et al. (2011)***. They proved that subjects with metabolic syndrome had significantly higher serum chemerin levels compared with healthy controls. In addition, they reported that higher serum chemerin concentrations have a strong and independent association with aspects of the metabolic syndrome including serum glucose, waist circumference, fasting serum insulin and lipid profile independent of age and sex in non-diabetic subjects.

Our results also go hand in hand with ***Shin et al. (2011), Yoo et al. (2012)*** and ***Yan et al. (2012)***. They proved that serum chemerin levels were significantly increased in obese individuals compared with lean controls and circulating chemerin levels had a significant positive correlation with the body mass index, waist circumference, abdominal visceral fat area, blood pressure, fasting serum insulin, HOMA-IR, TC, LDL-C and TG levels in obese individuals. In addition, ***Blüher et al. (2012)*** proved that insulin, triglycerides and chemerin are biomarkers whose dynamics tightly correspond to changes in body weight, with the trend to go to the opposite direction during the weight loss phase. Moreover, ***Ernst et al. (2012)*** stated that CMKLR1(-/-) mice had lower food consumption, total body mass, and percent body fat compared with wild-type controls. These findings suggested that chemerin may play a role in the pathophysiology of obesity and metabolic syndrome.

Sell et al. (2009) explained these findings by the fact that human adipocytes express chemerin and

chemokine-like receptor-1 and chemerin release is correlated with adipocyte volume. Furthermore, higher chemerin release is associated with insulin resistance at the level of lipogenesis by its reversible binding to the extracellular domain of insulin receptor-tyrosine kinase in peripheral tissues and decreasing the rate of auto-phosphorylation and subsequent downstream intracellular signaling cascades. Chemerin also inhibits glycogen synthase kinase phosphorylation, an enzyme necessary for glycogen synthesis and storage, and thus inhibits glucose uptake. In addition, chemerin activates extracellular signal-regulated kinase (ERK). Inhibition of ERK prevents chemerin-induced insulin resistance, pointing to participation of this pathway in chemerin action.

Takahashi et al. (2008) disagreed with this suggestion and postulated that, in adipocytes chemerin has the opposite effect, where it increases insulin-stimulated glucose uptake, and so, it stimulates insulin sensitivity. Hence, the increase in the levels of circulating chemerin is a compensatory mechanism in patients with insulin resistance. Thus, chemerin may exert different actions in endocrine and paracrine/autocrine ways. Moreover, ***Takahashi et al. (2011)*** showed that chemerin-deficient mice are glucose intolerant and glucose intolerance was mainly due to increased hepatic glucose production and impaired insulin secretion. They suggested that chemerin and its receptor were expressed in β-cell and chemerin regulates β-cell function and plays an important role in glucose homeostasis in a tissue-dependent manner.

Our results concerning blood pressure were in agreement with studies done by ***Stejskal et al. (2008)*** and ***Bozaoglu et al. (2009)***. They revealed that chemerin serum levels correlated positively with systolic and diastolic blood pressure. Chemerin may also be a novel regulator of blood pressure because of good correlations with both systolic and diastolic pressure. This hypothesis is supported by the fact that chemerin is highly expressed in the kidney, a key site of blood pressure regulation. Chemerin is structurally related to other circulating factors, as kininogens, whose proteolytic product is the vasoactive peptide bradykinin.

Our results revealed that HDL-C and CAD-risk percentage were significantly lower in metabolic syndrome group compared to control group. Moreover, a significant negative correlation between serum chemerin levels and both HDL-C and CAD-risk were proved in metabolic syndrome patients. Our results agreed with ***Wang et al.(2009) , Dong et al.(2011) , Yoo et al. (2012)*** and ***Yan et al.(2012)*** who revealed that serum chemerin levels were elevated in subjects with metabolic syndrome and were associated with several cardiovascular risk factors. Serum chemerin levels were significantly elevated in metabolic syndrome patients with CAD compared to those without CAD and healthy subjects and negatively correlated with HDL-C levels. Serum levels of chemerin could be considered as an independent predictive marker of the presence of atherosclerosis and CAD in patients with metabolic syndrome.

McCarthy et al.(2008) supported our findings by stating that chemerin expression by endothelial cells in inflamed tissue and its ability for chemotactic recruitment for macrophages and dendritic cells expressing CMKLR1, as well as its ability to promote cholesterol uptake and foam cell formation, suggests a role of chemerin in inflammatory states and possibly atherosclerosis. In addition, ***Lehrke et al. (2009)*** proved that chemerin is strongly related to markers of inflammation as tumor necrosis factor-α, interlukin-6 and CRP. Thus, it is conceivable that chemerin may be up-regulated in states of inflammation such as obesity, metabolic syndrome and atherosclerosis to dampen inflammatory processes and to improve metabolic regulation.

Our study also compared between insulin resistant type 2 DM and insulin sensitive control group. We reported that fasting blood sugar, 2 hours postprandial blood sugar, fasting serum insulin, HOMA-IR, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and serum chemerin levels were significantly higher in type 2 DM compared to control group. In addition, CAD-risk values were significantly lower in type 2 DM compared to control group. A significant positive correlation between serum chemerin levels and each of systolic blood pressure, diastolic blood pressure, LDL-C, triglycerides, total cholesterol and waist circumference and a significant negative correlation between serum chemerin levels and both HDL-C and CAD-risk in type 2 DM group were proved.

Our findings were in agreement with those of ***Wang et al. (2009)*** and ***Yang et al. (2010)*** who stated that plasma chemerin levels were found to be markedly increased in patients with type 2 diabetes mellitus with hypertension compared with normal controls. Our results are also supported by a study done by ***Ernst et al. (2010)*** who revealed that recombinant chemerin administration exacerbated glucose intolerance in obese and diabetic mice. This study provided evidence that serum chemerin levels are elevated in obesity and diabetes and that chemerin exacerbates glucose intolerance in these models by decreasing serum insulin levels and glucose uptake in liver tissue.

Our results go hand in hand with ***El-Mesallamy et al.(2011)*** and ***Hu and Feng (2011)*** who proved that serum chemerin levels were significantly increased in patients with type 2 diabetes and in patients with type 2 diabetes with ischaemic heart disease compared with healthy control subjects. Interestingly, chemerin levels

were found to be significantly correlated with BMI and SBP. Moreover, ***Chakaroun et al. (2011)*** proved that chemerin mRNA is significantly expressed in adipose tissue of patients with type 2 diabetes mellitus and correlates with circulating chemerin, BMI, percentage body fat and HOMA-IR. Obesity surgery-induced weight loss causes a significant reduction on subcutaneous chemerin expression. Decreased chemerin serum concentrations significantly correlate with improved glucose infusion rate independently of changes in BMI.

In contrast to our findings, ***Bozaoglu et al. (2007)*** describes important findings that circulating chemerin levels in type 2 diabetes human subjects were not significantly higher than those in normal control subjects, given that quite probably a proportion of their type 2 diabetic study subjects may have been taking anti-diabetic drugs. ***Tan et al. (2009)*** reported for the first time that metformin (an oral hypoglycemic drug employed by most physicians in both developing and developed countries) significantly decreases circulating chemerin levels with a concomitant decrease in insulin resistance in diabetic subjects.

The lack of significant correlation between serum chemerin levels and each of fasting blood sugar and 2 hours postprandial blood sugar in our study could be attributed to the anti-diabetic drugs taken by the diabetic patients' group beside that the cases of metabolic syndrome group are selected carefully to be non-diabetic with normal fasting glucose levels. In addition, it is conceivable that other factors such as the level of glycemic control, medication history, duration of diabetes, and the presence of complications such as renal disease may have an impact on the relationship between circulating chemerin levels and blood glucose levels ***(Bozaoglu et al., 2009).***

In the present study, multivariate analysis was done to choose the best predictors of the metabolic syndrome. It revealed that serum chemerin, waist circumference and total triglycerides were the most significant predictors with F value=7.68 (p<0.05) which supports the hypothesis that serum chemerin can be added to the conventional parameters as a new marker for the metabolic syndrome.

These results are in agreement with ***Bozaoglu et al.(2007)*** who proved that after adjusting age and sex, chemerin levels were significantly associated with measures of WC and metabolic syndrome components (fasting glucose, fasting insulin, triglycerides and blood pressure) in non-diabetic subjects. After further adjustment for WC, serum chemerin levels were still independently associated with metabolic syndrome components, including systolic blood pressure and plasma triglycerides. When the data were adjusted for age, sex, WC and triglycerides, chemerin levels were only associated with systolic and diastolic blood pressure. These results clearly demonstrate that circulating chemerin levels are associated with components of metabolic syndrome.

Lastly, the ROC curve analysis revealed that the best diagnostic cutoff point for serum chemerin in type 2 DM was 95 ng/mL. This had a diagnostic sensitivity 75%, specificity 80%, positive predictive value 83%, negative predictive value 71% and efficiency 77% with an AUC = 0.786. The best diagnostic cutoff point for serum chemerin as a predictor of metabolic syndrome was 140 ng/mL. This had a diagnostic sensitivity, specificity, positive predictive value, negative predictive value and efficiency 100%, respectively.

Our results were in accordance with those of ***Stejskal et al.(2008)*** who found in a study done to evaluate serum chemerin as a marker for the metabolic syndrome, that serum chemerin levels had a sensitivity of 75 % and specificity of 67 % with an AUC = 0.75 at a chemerin cutoff concentration = 240 ng/mL. Their study proposed that serum chemerin should be added up to the profile used regularly to diagnose metabolic syndrome.

In conclusion, the association of high serum chemerin levels with components of metabolic syndrome and type 2 DM indicates that this adipokine represents a novel marker of these derangements and could be considered one of the metabolic risk factors leading to insulin resistance in type 2 DM as well as metabolic syndrome. Moreover, its assessment could be beneficial in early detection of these pathological states and prevention of their unfavorable consequences especially the cardiovascular complications and atherosclerosis.

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