Histopathological and Immunohistochemical Studies on the Liver of Chronic Hepatitis C Virus Infected Patients and Hepatocellular Carcinoma in Sharkia Governorate, Egypt

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Abstract: In the present study, seventy five cases which has been previously proved to have HCV by PCR and preserved in the Early Cancer Detection Unit (ECDU) archive belonging to the Faculty of Medicine, Zagazig University, Egypt were submitted for this work. Routine Hematoxylin and Eosin (H&E) stain for histopathological and cytopathological studies, histochemical (Collagen and Reticulin) stains, immunohistochemical stains (AFP & CEA) were applied to predict and evaluate the role of HCV in liver cirrhosis and development to HCC. Normal architecture was still preserved in most of the chronic hepatitis cases while it was partially lost in few cases. Hepatocellular carcinomas (HCCs) and all of the cirrhotic cases completely lost their normal architecture. Liver fibrosis was absent in some of chronic HCV cases and it was observed in severely infected cases. On the other hand, the malignant criteria of HCC appeared in three types, well differentiated HCC, moderately differentiated HCC and poorly differentiated HCC. Immunohistochemical investigations played a good role in evaluating malignant changes of the studied cases, particularly AFP, although absence of AFP sometimes does not exclude the diagnosis of HCC, meaning, that HCC may not produce AFP. We noticed also that, CEA staining is somewhat similar to AFP in that, there was a high significant correlation between the different histopathological changes and the intensity of reaction, while no correlation could be proved between the tumor grades of HCC and the intensity of CEA staining.
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Key words: Histopathology, Immunohistochemistry, Liver, Chronic Hepatitis C Virus, Hepatocellular Carcinoma, Egyptian patients, Sharkia Governorate.

1. Introduction:

The hepatitis C virus (HCV) is one of the most significant health problems affecting the human liver. More than 4 million Americans (1.3% of the U.S. population) and 170 million individuals in the world (3% worldwide) are infected with HCV. The prevalence of HCV infections varies in different parts of the world. For example, the prevalence of HCV in Scandinavia is less than 0.5% of the population, whereas the prevalence in Egypt is over 20%. In the U.S. and Western Europe, the complications of HCV chronic hepatitis and cirrhosis are the most common reasons for liver transplantation HCV is one of several viruses that can cause hepatitis, which is inflammation of the liver. It is unrelated to the other common hepatitis viruses (A, B, D, and E). HCV is a member of the *Flaviviridae* family of viruses. Other members of this family of viruses include those that cause yellow fever and dengue (Fontaine *et al.*, 2004).

One of the major problems with HCV infections is that, 85% of individuals initially infected with this virus will become chronically infected and the other 15% of HCV infected individuals have an acute infection (Saadeh *et al*., 2001).

The clinical picture in patients infected with HCV is highly variable and ranges from a symptomatic virus carriers with normal serum transaminases and liver histology to acute or fulminant hepatitis and liver diseases. HCV related mortality and morbidity are the result of fibrosis development leading to cirrhosis in 10 to 25% of cases (Roudat *et al.*, 1997). HCV infection may be discovered by abnormal serum transaminases but this is not required for the diagnosis. Positive HCV serologies (antibodies or HCV RNA) confirm the diagnosis (Younossi and Mc-Hutchison, 1997). Now, it has become apparent that, HCV infection is a major risk factor for the development of hepatocellular carcinoma (HCC) worldwide. The precise mechanism by which HCV causes HCC is not known. Unlike the HBV, HCV is not a DNA virus and does not become integrated within the genome of hepatocytes. It is more likely that, HCC occurs against a background of inflammation and regeneration, associated with liver injury due to chronic hepatitis. Most, but not all, cases of HCV-related HCC occur in the presence of cirrhosis, suggesting that, it is the underlying liver disease per se that is the risk factor for HCC rather than HCV infection (Adrian and DiBiscegglie, 2002).

On the other hand, Alfa-fetoprotein (AFP) has an important clinical value in patients with chronic hepatitis and cirrhosis, because it is associated with high risk of liver related death or development of HCC (Oka *et al*., 1994). Also carcinoembryonic antigen (CEA) is an important marker for evaluating and predicting malignancy in many organs besides HCC. In addition, DNA image analysis is an advanced and important prognostic tool used in predicting malignancy according to changes of the cell cycle, particularly S-phase fraction, which has a highly significant correlation with variation of DNA ploidy.

The present study was performed to record and follow up the different histopathological and histochemical changes in the liver tissue of hepatitis C virus infected patients and to evaluate any possible deleterious and carcinogenic effects of this virus in Egyptians, particularly in Sharkia Governorate.

2. Patients and Methods

This study has been carried out in the Early Cancer Detection Unit (ECDU) belonging to the Faculty of Medicine, Zagazig University, Egypt.

In the present investigation, seventy-five cases [60 paraffin blocks and 15 fine needle aspiration (FNA) smears] from patients previously proved to have HCV by PCR were studied. All the cases were chosen from the paraffin blocks and unstained slides of FNA biopsies that were preserved in the ECDU archive. The cases in this study were 54 males and 21 females represented (72% and 28%) respectively of all the cases as illustrated in table (1).

Preparation of smears:

Specimens obtained by the fine needle were expressed on glass slides and directly smeared and left to dry in air for about 5 minutes. Some of the smears were subsequently fixed in 95% ethanol for about 30 minutes. The fixed smears were stained with Hematoxylin and Eosin (H&E) for cytological examination.

Preparation of paraffin sections:

Serial 5µm sections were cut from all the paraffin blocks and stained with H&E. Also serial 4μm sections were cut and mounted on charged slides and prepared for immunohistochemistry.

Staining:

Haematoxylin and Eosin Stain was used according to Culling (1974) for routine study of both cytoplasmic and nuclear changes to detect the histopathological and cytopathological state of the liver tissue and the different types of HCCs . Also, for the detection of grades of neoplastic lesions depending on the nuclear changes.

Masson-trichrome stain (Luna, 1972) was used for collagen fibers and Gordon and Sweet's (1936) used for reticulin fibers.

For immunohistochemical studies, Alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) were detected by using streptavidin-biotin immunoperoxidase staining method according to Falini and Taylor (1983). BioGenex streptavidin-biotin kit manufactured by BioGenex, 4600 Norris Canyon Road, San Ramon, CA94583 and monoclonal mouse primary antibodies: anti-AFP and anti-CEA were used.

Interpretation of the results:

Positive test slides showed a brown color precipitate. Alpha-fetoprotein (AFP) showed cytoplasmic staining while carcinoembryonic antigen (CEA) was observed mainly decorating cytoplasmic membrane.

3. Results

In the present investigation, seventy-five cases (60 paraffin blocks and 15 FNA smears) from patients previously proved to have HCV by PCR were studied. All the cases were chosen from the paraffin blocks and unstained slides of FNA smears that were preserved in the archive of the Early Cancer Detection Unit (ECDU) belonging to Zagazig University, Egypt. The males in this study were 54 cases and the females were 21 cases representing (72% and 28% respectively of all the studied cases as illustrated in table (1) and Figs. (1 & 2).

I- Histopathological and Cytopathological results:

Examination of the Haematoxylin and Eosin stained paraffin sections revealed the presence of 40 cases of chronic hepatitis C, 12 cases of liver cirrhosis and 8 hepatocellular carcinomas (HCCs). Examination of the FNA smears showed only 12 cases representing chronic hepatitis and 3 cases representing HCC as shown in tables (2, 3 & 4).

Cytopathological examination of smears of the 12 cases of chronic hepatitis revealed that, 9 cases showed decreased cohesion of hepatocytes and presence of many single cells, swelling of hepatocytes and the cytoplasm was stained less uniformly and appeared paler at the periphery of the cell, (Plate I, Fig. A). Cell membranes, which are indistinct in normal hepatocytes appeared more clearly visible. Fatty changes as well as liver cell necrosis were also observed in the highly diseased cases. Large number of inflammatory cells and Kupffer cells were obviously observed in all cases (Plate I, Fig. B).

The H & E paraffin sections examined from 40 cases of chronic hepatitis revealed that, the hepatocytes of the 31 cases (77.5%) of the chronic HCV cases were suffering from hydropic degeneration and 26 cases (62.5%) showed fatty changes, while 7 cases (17.5%) showed twinning (Plate III, Figs A, C & D and Plate IV, Figs. B & C). Hepatic necrosis was also observed in variable degrees in the examined sections. Ten cases representing, (25%) of the studied cases appeared to have no necrotic changes, while, necrosis was clearly noticed in (75%) of the cases, (65%) of them showed piecemeal necrosis (interface hepatitis) and minimal focal necrosis (Plate III, Figs. A, B & D). Seven cases (17.5%) showed bridging necrosis, massive necrosis was clearly observed only in all the cirrhotic cases.

Examination of the portal tracts revealed epithelial damage of small bile ducts, lymphoid aggregates and lymphoid follicles in the portal tracts as well as microvesicular steatosis in most of the chronic HCV cases. Ductal proliferation accompanied by epithelial damage was obviously noticed in 24 (60%) of all the chronic hepatitis cases (Plate III, Fig. D). Also, the cirrhotic cases 12 (100%) suffered from bile duct hyperplasia, which was accompanied by epithelial damage as shown in (Plate V, Fig. A).

Infiltration of mononuclear inflammatory cells was observed in the portal tracts of all the studied cases; 35% of them showed diffuse pattern, while, lymphoid aggregates were noticed in 23 cases (57.5%) (Plate III, Figs. A & C). Lymphoid follicles were observed only in 3 cases (7.5%) of chronic hepatitis and all (100%) of the cirrhotic cases.

Regarding the lobular architecture of the hepatic tissue, it was found that, 22 cases (55%) of chronic hepatitis cases showed partial loss of their normal architecture (Plate IV, Fig. B), but it was completely preserved in the other 8 cases representing 45%. All of the cirrhotic cases showed disruption of the architecture of the entire liver (complete loss of their architecture), (Plate IV, Fig. D).

Examination of the liver sections revealed that, 3.5% of the studied chronic HCV cases were negatively stained for collagen and reticulum fibers stain, 22.5% showed reticulum fiber formation, while collagen was mildly to moderately seen with remaining reticulin fibers in 45%. Complete collagenization and regenerated nodule formation were markedly noticed in 100% of the cirrhotic cases, as seen in (Plates VI & VII).

According to the Ishak Modified HAI scoring system of (grading and Staging) (Ishak *et al*., 1995) of chronic hepatitis, the studied cases in the present study can be classified as illustrated in table (5). This score depends on 4 separate categories of well-defined lesions that are added together for the activity grade and 6 architectural changes, fibrosis and cirrhosis for staging. We divided the necroinflammatory activity changes to 3 grades as:

- Grade I: (Mild chronic active hepatitis) and its suggesting score from (0-6)
- Grade II: (Moderate chronic active hepatitis) and its suggesting score from (7-12)
- Grade III: (Severe active hepatitis) and its score was (> 12)

Also stages according to this system were divided as follows:

A- Early stage (The lower end of the fibrosis scale) (0-2)
B- Middle stage (3 or 4)
C- Late stage (5 or 6).

As illustrated in table (5), most of the scored 25 cases representing (48.08%) were observed in the moderate grade of chronic active hepatitis. 13 cases of them representing the middle stage of fibrosis, while 12 cases representing (23.1%) were in the late stage (cirrhosis). By correlating the relationship between the stages and grades of our cases according to Ishak Modified HAI scoring system using Kapa coefficient test, there was a high significant correlation between the middle stage and the moderate grade (P=0.001).

According to the new concept of scoring by (Mangoud *et al*., 2004), the studied chronic active HCV cases can be classified as illustrated in table (6).

As observed in table (6), all of the grades and stages were represented in the table except G1S3 and G3S1. The large number of cases (29, 55.77%) was noticed in G2, while most of them (20 cases, 38.46%) was observed in S2. Stage 3 comprised 12 (23,1%) of the scored cases representing cirrhosis, each of them was present in G3. By correlating the relationship between the stages and grades of our cases according to the new concept of scoring**,** using Kapa coefficient test, there was a significant correlation between the grades and stages and the high significance appeared between S2 and G2, (P=0.001).

On the other hand, the primarily cytologic diagnosis of the HCC cases was rested upon the cytomorphologic criteria of malignancy, which has been illustrated in three cases of all the studied cytological smears. These criteria were monomorphous cell populations composed of variable pleomorphic, polygonal contoured neoplastic cells and abundant or moderate amount of granular and eosinophilic cytoplasm (Plate II, Fig. B). Also, centrally placed oval malignant nuclei with thick nuclear membranes and intranuclear cytoplasmic inclusions have been noticed. Prominent single nucleoli were occasionally seen. These neoplastic

cells may be arranged in variable sized trabeculae,bordered by flat endothelial cells forming the characteristic cell balls (Plate II, Fig. C) ,or were arranged in the form of broad sheets and tubules forming pseudoacinar pattern (Plate II, Fig. A).

The present investigation revealed that, the most common architectural pattern of HCCs was that of the trabecular pattern forming about 2 cases representing (66.7%) of the cytological HCCs. The pseudoglandular (acinar) type represented (33.3%) of HCCs.

Regarding the cytologic variants of the HCCs by routine cytopathologic examination, there was one case (33.3%) revealing hydropic degeneration, fatty changes and ground glass nuclei. The large pleomorphic cell type was detected in 66.7% (2 cases) out of the studied cases.

Depending on the amount and the shape of trabeculae and the nuclear and cytoplasmic changes, HCC was divided into 3 main grades (I, II and III). As illustrated in table (7), grade I (well-differentiated) cell type was found in (2/3) cases (66.7%) of the cytologic smears, while it has been observed in (3/8) cases representing (37.5%) of the histologic cases. Grade II (moderately differentiated HCC) was absent in HCCs smears, while it was observed in 37.5% of the histologic cases. Grade III (poorly differentiated HCC) was noticed in one case representing (33.3%) of the cytological smears and two cases (25%) of the histological sections (table 7).

Well-differentiated HCCs showed minimal atypical changes and were mostly of normotrabecular arrangement (one to two cells thickness).

The cytologic diagnosis of well differentiated HCC was very difficult, but the presence of two or more of the following three criteria in the hepatic aspirates helped in the diagnosis of well differentiated HCC. These criteria are nuclear crowding, increased cytoplasmic basophilia (a sign of enhanced cellular activity) and presence of microacinar formation. The existence of the distinctive trabecular growth pattern or nuclear atypism made the diagnosis of moderately or poorly differentiated HCC more easier than that of the well-differentiated HCC.

The histopathological observations of the paraffin sections showed that the well differentiated (early stage HCC) was more characterized than the moderately and poorly differentiated HCC by increased cell density with an increased nuclear/ cytoplasmic ratios, an irregular thin trabecular pattern and frequent fatty changes (Plate V, Fig. B). Nuclei were large and oval with peripheral condensation of chromatin and usually a large regular nucleolus (Plate V, Figs. B & D).

II- Immunohistochemical results:

a- Alpha-fetoprotein (AFP):

Positively stained AFP particles, appeared as diffused brown color involving the entire cytoplasm or in a part of the tumor cells. Sometimes, it appears as fine granules in the cytoplasm of the tumor cells. Generally, the overall AFP staining was positive, when at least 20% of the immune reactive cells, within a given neoplasm were positive for AFP staining.

The intensity of AFP staining was scored from (-) to (+++). Minus (-) represents negative reaction (absence of AFP), (+) faint staining, (++) moderate staining, (+++) marked staining with dark brown reaction product. The intensity of AFP staining of the positive cases, was greatly varied within most cases and sometimes within the same case, particularly in the different stages of HCCs.

Immunohistochemical studies in the current study revealed AFP negative reaction in most of the chronic hepatitis cases (38 case, 95%), while 2 cases (5%) revealed mild to moderate reaction with this monoclone. The histological examination indicated that, the positive-reacted cases showed marked cellular damage as well as marked fibrosis. Eight (66.7%) of the cirrhotic cases showed negative reaction, 3 cases (25%) revealed mild positive reaction, while one case (8.3%) was moderately reacted with AFP as shown in (Plate VIII, Fig. A). Seven cases (87.5%) of the HCCs showed positive reaction with AFP antibodies and one case of HCCs (12.5%) was negatively reacted with AFP as illustrated in table 8 and Fig. 3.

By correlating the AFP staining with the variable histopathological changes observed in this study, AFP positivity was detected in two cases (5%) of chronic HCV, four cases (33.3%) of cirrhotic cases and seven cases (87.5%) of HCCs as illustrated in table (8).

There was a significant association between histopathological changes and AFP staining with $X = 93.36$ and P value <0.0001. By correlating tissue positivity for AFP with the differentiation of HCCs, (+++) degree of staining was noticed in two cases (100%) of grade III and in one case (33.3%) of grade II cases. In two cases (66.7%) of grade II cases (++) degree of staining was detected and in one case (33.3%) of grade I, while (+) degree was noticed in one case (33.3%) of grade II and one case (33.3%) of grade I cases as illustrated in table (9) and Fig. (4), (Plate VIII, Fig. B).

The degree of AFP staining revealed no significant with grading of HCC and significant $X = 1.48$ and P value >0.05

Therefore, from the previous results, it has been concluded that, AFP expression was absent in

approximately all the chronic hepatitis C cases but it was weakly expressed in few cases. Also, AFP expression was low in the well differentiated HCC comparing to moderately and poorly differentiated HCCs. Furthermore, in differentiated HCC, AFP expression was detected in many malignant cells and the distribution of AFP-expressing cells was heterogeneous.

b- Carcinoembryonic antigen (CEA):

Different hepatic lesions, included in the present study showed variable percentage and staining patterns of monoclonal CEA. It has been noticed that, most of the HCCs (6 cases, 75%) were positively stained with CEA monoclone, while (100%) of the chronic hepatitis cases reacted negatively with this monoclone. In addition, few cirrhotic cases (2 cases representing 16.7 %) revealed mild reactivity with CEA.

By correlating the CEA staining with variable histopathological changes noticed in this study, CEA positivity was detected in two cases (5%) of chronic HCV, four cases (33.3%) of cirrhotic cases and seven cases (87.5%) of HCCs, (table 10 and Fig.5). There was a high significant association between histopathological changes and CEA staining with $X^2 = 115.33$ and P value <0.0001.

On the other hand, the sections which were positively stained with CEA revealed two staining patterns. The first pattern was localized to the surfaces of the hepatocytes lining biliary canaliculi and associated with the bile itself. It was depicted as a network of delicate tubules outlining the hepatocytes plasma membranes, or it appeared as a dot like stained canaliculi. Immunohistochemical staining of bile canaliculi with CEA, has demonstrated identifying both benign and malignant hepatocytes, in the sections examined. Bile canalicular staining pattern of CEA was identified in 5 cases (62.5%) of HCCs, which were CEA positive, (Plate VIII, Figs. C&D).

The second CEA staining pattern, which was displayed in the present study, was the intracytoplasmic brown pigmentation of the hepatocytes. This pattern of staining was found in (0%) of positive CEA reacted cases of the noncancerous ones, while it was observed in (1 case, 12.5%) of HCCs (poorly differentiated HCC).

The absence of canalicular CEA staining with or without cytoplasmic staining did not rule the diagnosis of HCC. It has been observed that, CEA staining differs from AFP staining in that, there was no correlation could be proved between the tumor grades of HCC and the intensity of CEA staining (table 11, Fig. 6).

Table (1): Age and sex distribution in 75 studied cases.

Test of sig. *(P)*	HCC (N: 11)	Cirrhosis (N: 12)	Chronic HCV (N: 52)	
				Age (years)
F: 9.6 (0.001)	56.0 ± 12.4	48.9 ± 10.6	39.1± 13.1	X±SD
sig.	34 - 77	31 - 69	21- 67	Range
X^2: 6.58	No %	No %	No %	Gender
(0.018) sig.	9 81.8	8 66.7	37 71.2	Male
	2 18.1	4 33.3	15 28.8	Female

- 54 males and 21 females were included in this study (P < 0.018)
- The minimum age was 21 years and the maximum age was 77
- The mean of age among different groups was significant (P= 0.001)

Table (2): Different pathological lesions of 75 studied cases.

%	No.	Pathological lesion
69.3	**52**	**Chronic HCV**
16.0	**12**	**Cirrhosis**
14.7	**11**	**HCC**
100%	**75**	**Total**

* Chronic hepatitis was the highest percentage in the studied cases (69.3%).

Table (3): Different histopathological lesions of 60 cases of paraffin sections.

%	No.	Histopathological lesion
66.7	40	Chronic HCV
20.0	12	Cirrhosis
13.3	8	HCC
100%	60	Total

* Chronic hepatitis was the highet percentage in the studied cases (66.7%).

Table (4): Different Cytopathological lesions of 15 cases of FNA smears.

%	No.	Cytopathological lesion
80.0	12	Chronic HCV
0.0	0	Cirrhosis
20.0	3	HCC
100%	15	Total

- Chronic hepatitis was the highet percentage in the studied cases (69.3%).

Table (5): Number and Percentage of studied hitopathological changes according to *Ishak et al, (1995)*

Kapa Coeff. *(P)*	%	Total No.	Architectural Changes (Stages)			Necroinflam-matory Activity (Grades)
			Late Stage S. = 5 or 6	Midd. Stage S. = 3 or 4	Early Stage Score = 0-2	
0.01 S.	21.15	11	0	5	6	Mild CAH (Score 0-6)
0.001 S.	48.08	2 5	1	13	11	Moderate CAH (Score 7-12)
0.001 S.	30.77	16	11	4	1	Severe CAH (Score > 12)

Table (6): Number and Percentage of studied hitopathological changes according to a new concept *(Mangoud et al., 2004)*

Kapa Coeff. test *(P)*	%	Total No.	(Stages)			Necroinflammatory Activity (Grades)
			S3 S. = 5 - 6	S2 S. = 3 - 4	S1 Score = 0-2	
0.001 S.	19.23	10	0	2	8	G1 (S. 1-4)
0.001 S.	55.77	29	0	20	9	G2 (S. 5-8)
0.001 S.	25.0	13	12	1	0	G3 (S. 9-12)

Table (7): *Different Grades of all the studied HCCs.*

FNA smears		Paraffin Bloks		Grade of HCC
%	No.	%	No.	
66.7	2	37..5	3	GI (well diff. HCC)
0	0	37.5	3	GII (Mod. dff. HCC)
33.3	1	25	2	GIII (Poorly diff. HCC)
100%	3	100%	8	Total

* GI is the most frequent grade of all the studied HCCs (5 cases, 45.6%).

Table (8): AFP staining in the different hepatic lesions of the studied cases.

AFP Staining Intensity								No	Histopath. Changes
+++		++		+		—			
%	No	%	No	%	No	%	No		
-	-	2.5	1	2.5	1	95	38	40	Ch.Hepatitis
-	-	8.3	1	25	3	66.7	8	12	Cirrhosis
37.5	3	25	2	25	2	12.5	1	8	HCC
5	3	6.7	4	10	6	78.3	47	58	Total

* Test of significant is *Chi- Square* (P value <0.0001)

Table (9): AFP staining in the different HCC grades of the studied cases.

AFP Staining Intensity								No	HCC grades
+++		++		+		-			
%	No	%	No	%	No	%	No		
33.3	1	-	-	33.3	1	33.3	1	3	GI
-	-	66.7	2	33.3	1	-	-	3	GII
100	2	-	-	-	-	-	-	2	GIII
37.5	3	25	2	25	2	12.5	1	8	Total

* Test of significant is *Chi- Square* (P value <0.001)

Table (10): CEA staining in the different hepatic lesions of the studied cases.

CEA Staining Intensity									
+++		++		+		-		No	Histopath. Changes
%	No	%	No	%	No	%	No		
-	-	-	-	-	-	100	40	40	Ch. Hepat.
-	-	-	-	16.7	2	83.3	10	12	Cirrhosis
12.5	1	25	2	37.5	3	25	2	8	HCC
1.7	1	3.3	2	8.3	5	86.7	52	60	Total

* Test of significant is *Chi- Square* (P value <0.0001)

Fourty three (11 HCC, 12 cirrhosis and 20 chronic hepatitis) cases from all the studied cases, were chosen for evaluating DNA ploidy and S-phase fraction (SPF) by DNA image analysis

Table (11): CEA staining in the different HCC grades of the studied cases.

CEA Staining Intensity									
+++		++		+				No	HCC grades
%	No	%	No	%	No	%	No		
-	-	-	-	66.7	2	33.3	1	3	GI
-	-	33.3	1	33.3	1	33.3	1	3	GII
50	1	50	1	-	-	-	-	2	GIII
12.5	1	25	2	37.5	3	25	2	8	Total

* Test of significant is *Chi- Square* (P value =0.001)

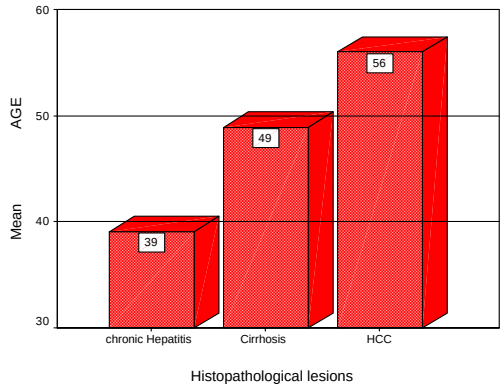

Fig. 1: Age distribution in the studied cases

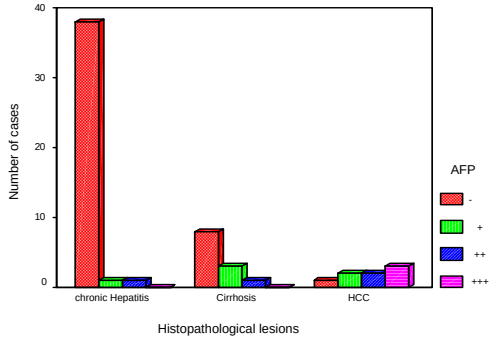

Fig. 3: Comparison of AFP score pattern and different hepatic lesions

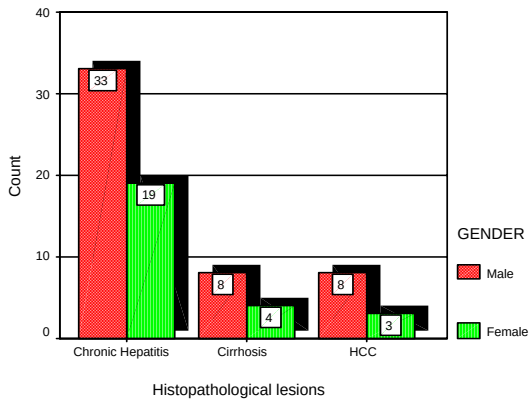

Fig. 2: Sex distribution in the studied cases.

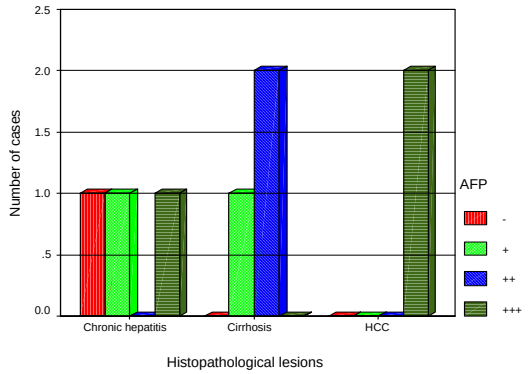

Fig. 4: Association between AFP staining and HCC grades

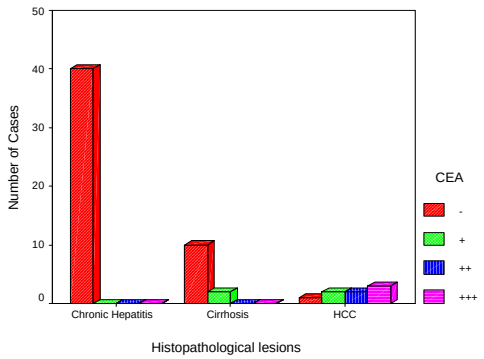

Fig. 5: Comparison of CEA score pattern and different hepatic lesions.

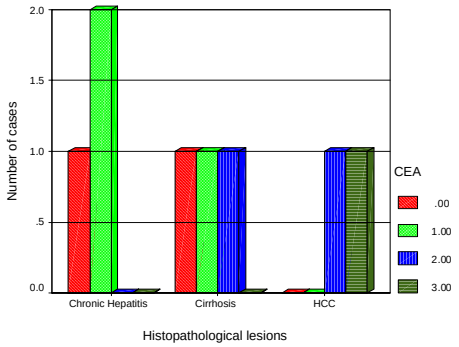

Fig. 6: Association between CEA staining and HCC grades

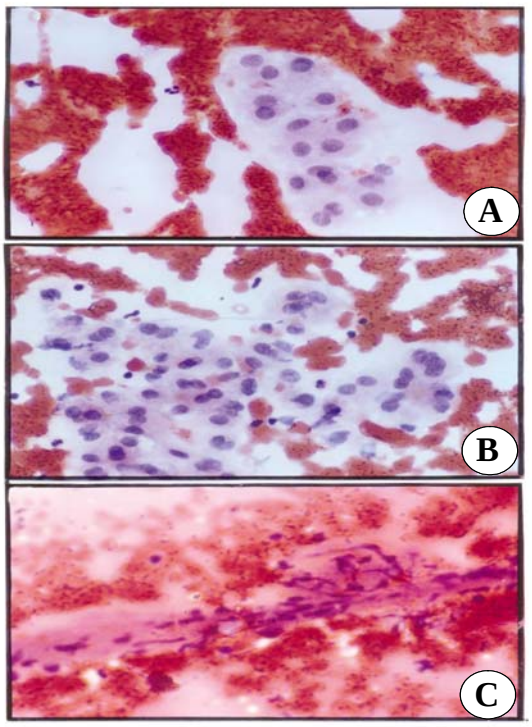

Plate I: Fine needle aspiration (FNA) smears of liver of different cases infected with HCV and stained with H & E.

Fig. (A): A Case of a male patient aged 27 years showing normal hepatocytes with rosette formation and normal nucleo- cytoplsmic ratios. (X 400)

Fig. (B): A case of a female patient aged 33 years showing infiltration of mononuclear inflammatory cells and increased Kupffer cells. The hepatocytes are arranged in more than one cell layer. (X 400)

Fig. (C): A case of a male patient aged 41 years with hepatic cirrhosis showing fibrous threads. (X 200)

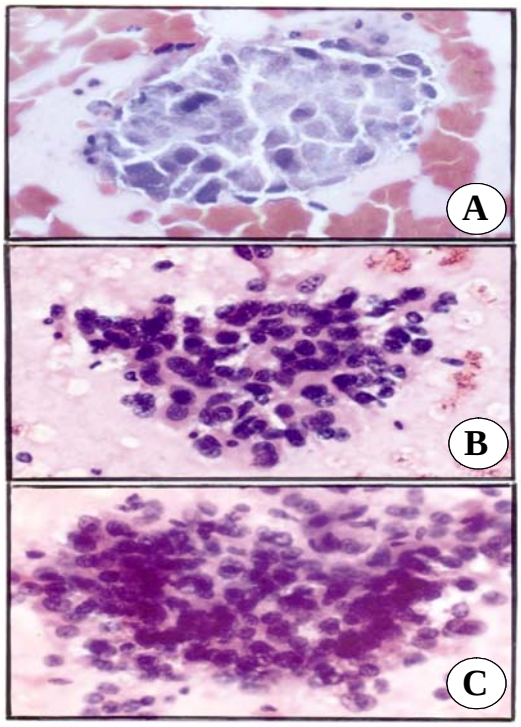

Plate II: Fine needle aspiration (FNA) smears of liver of different cases infected with HCV and stained with H & E.

Fig. (A): A case of a male patient aged 63 years showing acinar arrangement of malignant hepatocytes. (X 400).

Fig. (B): A case of a male patient aged 51 years showing a cluster of malignant hepatocytes with pleomorphic and hyperchromatic nuclei, intranuclear vacuoles, many mitotic figures, prominent nucleoli and granular eosinophilic cytoplsm. (X 400).

Fig. (C): A case of a female patient aged 64 years showing a tight clusters of malignant hepatocytes, large pleomorphic nuclei, prominent nucleoli and scanty eosinophilic cytoplsm. (X 400).

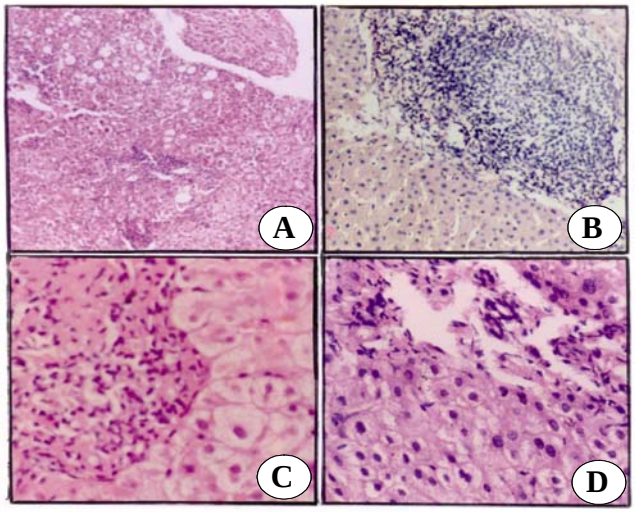

Plate III: Paraffin sections from liver biopsies of patients infected with HCV and stained with H & E.

Fig. (A): A case of a male patient aged 33 years showing ballooning degeneration and fatty changes of hepatocytes. Mild piecemeal necrosis (interface hepatitis) and fibrous septa formation are observed. The portal tract is infiltrated by mononuclear inflammatory cells. (X 100).

Fig. (B): A case of a female patient aged 47 years showing normal architecture of liver cords and the portal tract is invaded by lymphocytic aggregates. (X 200).

Fig. (C): A case of a male patient aged 39 years showing hepatocytic ballooning, which is arranged as a rossette formation with diffuse lymphocytic infiltration of the portal tract. (X 400).

Fig. (D): A case of a male patient aged 51 years showing marked hydropic degeneration and ballooning of hepatocytes. The portal tract shows multiple bile ducts with epithelial damage of some of them, mild inflammatory mononuclear infiltrate and fibrosis spill over parenchyma (mild piecemeal necrosis) (X 400).

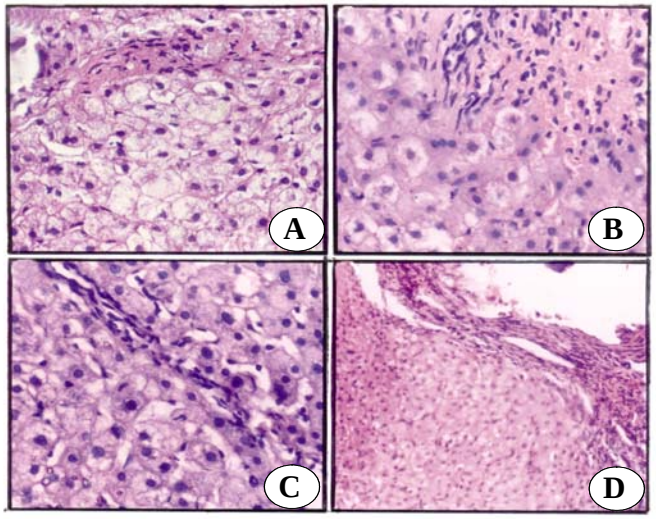

Plate IV :Paraffin sections from liver biopsies of patients infected with HCV and stained with H & E.

Fig. (A): A case of a male patient aged 48 years showing, marked hydropic degencration, piecemeal necrosis (interface hepatitis) and early bridging fibrosis. (X 400)

Fig. (B): A case of a male patient aged 57 years showing, ballooning degeneration of hepatocytes, diffuse infiltration of the portal tract by lymphocytes and fibroblasts and persistance of bile ducts. (X 400)

Fig. (C): A case of a male patient aged 44 years showing bridging fibrosis. (X 400)

Fig. (D): A case of a male patient aged 59 years showing, complete loss of the normal architecture of the hepatic tissue with an active cirrhosis. (X 200)

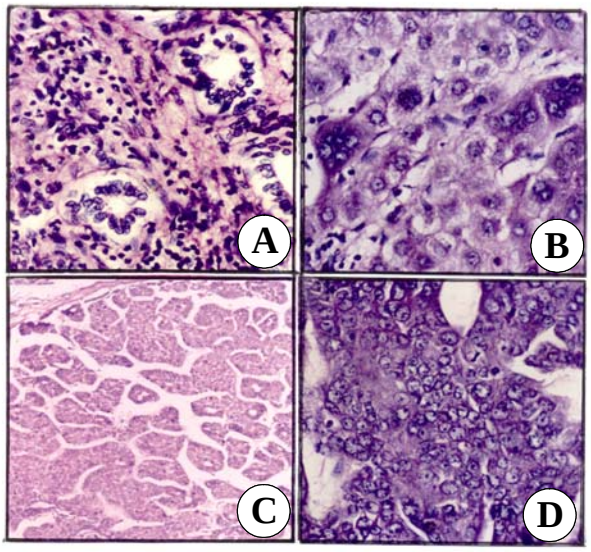

Plate V: Paraffin sections of liver materials of patients previously proved to have HCV by PCR showing different grades of HCC. Sections are stained with H & E.

Fig. (A): A case of a male patient aged 59 years with well differentiated HCC showing infiltration of the portal tract by marked inflammatory cells, and malignant hepatocytes in the form of acini. (X 400)

Fig. (B): A case of a female patient aged 64 years showing trabecular pattern of HCC, the nuclear chromatin shows large prominent nucleoli. Many malignant giant cells are observed. (X 400)

Fig. (C): A case of a male patient aged 71 years showing mixed trabecular and acinar HCC. (X 100)

Fig. (D): A case of a male patient aged 62 years showing a large number of crowded pleomorphic malignant hepatocytes, with large prominent nucleoli and vacuolated nuclei forming trabecular pattern of HCC. (X 400)

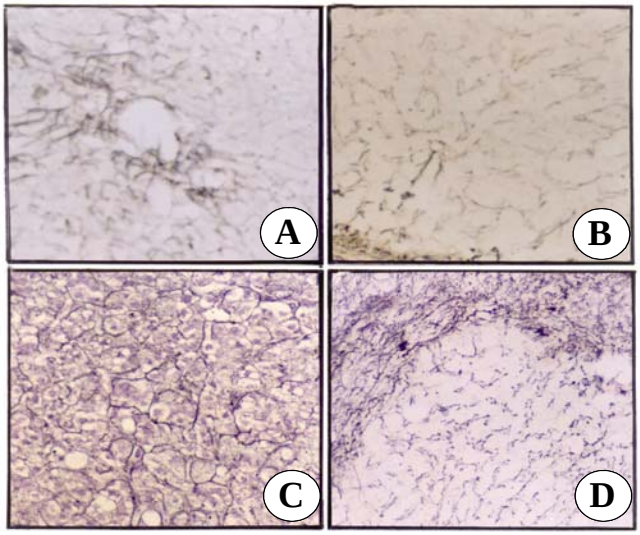

Plate VI: Paraffin sections of liver materials of patients previously proved to have HCV by PCR showing different histopathological lesions. Sections are stained with Masson Trichrome stain to illustrate collagen fibers.

Fig. (A): A case of a female patient aged 32 years infected with chronic HCV showing mild collaginization around the portal tract extending around the hepatacytes. (X 250).

Fig. (B): A case of a male patient aged 44 years infected with chronic HCV showing moderate collaginization extending around liver lobules. (X 200)

Fig. (C): A case of a male patient aged 51 years proved to have HCV and cirrhosis showing marked fibrosis separating regenerated nodules of hepatocytes with bile duct proliferation. (X 100)

Fig. (D): A case of HCC of a male patient aged 71 years showing extensive fibrosis of collagen type. (X 100)

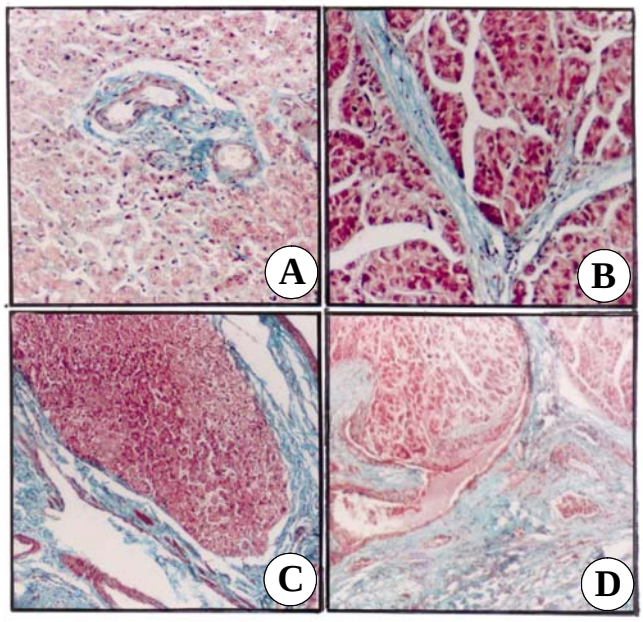

Plate VII: Paraffin sections of liver materials of patients previously proved to have HCV by PCR showing different histo-pathological lesions. Sections are stained with Gordon and Sweet's stain to illustrate reticulin fibers.

Fig. (A): A case of a female patient aged 32 years infected with chronic HCV showing mild reticulin fibers around dilaled central vein. (X 100)

Fig. (B): A case of a male patient aged 29 years infected with chronic HCV showing mild reticulin fibers around hepatocytes in a lobular area. (X 100)

Fig. (C): A case of a male patient aged 48 years and has been proved to have HCV and cirrhosis showing marked appearance of reticulin fibers intra-hepatocytes. (X 200)

Fig. (D): A case of a female patient aged 55 years and has been proved to have HCV and cirrhosis showing active cirrhosis appearing as a marked expression of reticulin fibers. (X 100)

4. Discussion

Many studies have been carried out in Egypt for evaluating the percentage of presence and transmission of HCV in the rural areas and introducing the different solutions and prevention programs for this serious problem (Abdel-Aziz *et al.,* 2000**).** The disease was presented with different clinical manifestations and progressed to liver cirrhosis and even cancer, while in some patients the effect was minimal (Habib *et al.,* 2001**).** The time of infection till the appearance of the manifestations ranges in most previous world studies between 10-30 years. Among Egyptians, because of the endemic conditions, environmental habits and human behaviour, the course of the disease and its complications are short and rapid with the development of cirrhosis before 40-50 years of age (Habib *et al*., 2001**).**

The present study revealed that, the mean of ages (39.1, 48.9 and 56.0) for the chronic hepatitis, cirrhotic and HCC cases respectively, was significantly increased (P value = 0.001) with the degree of histopathological lesion. Also the severity of HCV was proportionally increased among males compared to females. These observations are in agreement with those of Abdel-Aziz *et al* (2000) and Habib *et al* (2001)**.**

The histopathological characteristics of chronic hepatitis C virus in the infected patients have been the subject of many articles, (Badizadegan *et al.,* 1998)**.** Lymphoid aggregates, sinusoidal lymphocytosis, ductal proliferation and ductal epithelial damage were the main histopathological changes noticed in the present study. These observations coincide with those of Kage *et al*. (1997) who noticed approximately the same changes in 109 Japanese patients.

The histopathological and cytopathological picture of the 75 studied cases varied as follows, (52 cases, 69.3%) suffered from chronic hepatitis, (12 case, 16%) were cirrhotic and (11 cases, 14.7%) had HCC. 35% of chronic HCV cases showed diffuse pattern, while portal lymphoid aggregates were present in (57.5%) of these cases. Bile duct damage and epithelial changes were noticed in (60%) of these cases. According to Czaja and Carpenter (1993), the mechanism of the damage of these ducts is still unclear.

A minor hepatocellular damage was also one of the main characteristic changes noticed in most of the

cases of the present study. Badizadegan *et al*. (1998), suggested that, this minimal hepatocellular damage had a diagnostic value helping to distinguish hepatitis C from other types of chronic hepatitis, particularly autoimmune hepatitis, in which hepatocellular damage is more pronounced. According to Dienes and Popper (1982), lobular activity and changes in the liver cell plate with formation of rosettes and ballooning degeneration may be due to direct cytopathic effect of HCV.

A prominent histopathological feature of chronic hepatitis C infection was pericellular fibrosis, which was typically seen around the central veins and occasionally within the lobules away from the central veins. Although the pathogenesis of centrilobular pericellular fibrosis can only be speculated at this point, its association with pericentral necroinflammatory activity raises the possibility of direct hepatocellular damage in this region as a possible fibrogenic mechanism. This lesion could also represent a sequela of a previous episode of acute hepatitis (Badizadegan *et al.,* 1998). The cases of chronic HCV with minimal to moderate necroinflammatory activity had reticulin fibers starting in the centrilobular area progressing towards the portal tracts. Connective tissue stains (Masson trichrome for collagen fibers and Gordon stain for reticulum fibers) have long been considered the "gold standard" for assessing liver histology disease activity and liver fibrosis (Friedman, 2003). It has been noticed that, the 12 cases sufferring from cirrhosis and in which the necroinflammatory processes appeared to be severe had collagen fiber accumulation and bridging fibrosis with thickened portal tracts.

In the present study, an increase in the number of the cirrhotic cases (16%) and HCCs (14.7%) was recorded comparing to all the previous studies. This evidenced the hypothesis "cirrhosis is the end stage of fibrosis of liver parenchyma meaning that cirrhosis is irreversible" (Friedman, 2003). Contrary to that, Kweon *et al.* (2001) and Poynard *et al.* (2002) postulated that, there is unequivocal and mounting evidence that cirrhosis can be reversible. Based originally on anecdotal evidence, this conclusion is now additionally drawn from studies involving large number of patients. The feature common to all cases of cirrhosis improvement is the elimination of the underlying cause of liver disease, whether due to eradication of HCV or HBV, decompression of biliary obstruction in chronic pancreatitis (Hammel *et al.,* 2001) or immunosuppressive treatment of autoimmune liver disease. Moreover, there is an ample evidence of reversibility in animal models, through the experimental studies carried out by Iredale *et al.* (2001). A more recent study has now established that, cirrhosis can regress following HCV eradication with α-interferon /ribavirin (Poynard *et al.,* 2002).

The average histological activity based on the Modified HAI grading scheme reported in the series of Ishak *et al*. (1995), is approximately in accordance with the observations noticed in the present study. There was a significant difference in the stage of fibrosis between the different groups. Most of the scored cases (25, 48.08%) were present in the moderate grade of chronic active hepatitis and the large number of them (13 cases, 25.0%) represented the middle stage of fibrosis. Also as has been observed previously 12 cases (23.1%) were present in the late stage (cirrhosis).

The Ishak Modified HAI system ignored many histopathological changes which have been observed in the current study such as: bile duct damage and proliferation and immunohistochemical (AFP and CEA) findings as well as age and gender variations. These notes were also summarized by Brunt review (2000), who recorded that, bile duct inflammation and damage; lymphoid follicles; steatosis, mild, moderate, or marked hepatocellular dysplasia; adenomatous hyperplasia; iron or copper overload; intracellular inclusions (eg. PAS-positive globules, Mallory bodies); and immunohistochemical findings have been noted but not scored. Information on viral antigens, lymphocyte subsets, or other features, when available, should be recorded and may be semi-quantitatively expressed. Westin *et al.* (1999) noted that using of X 10 objective for the evaluation of necroinflammatory loci raises concerns of reproducibility, because the size of the field may vary among microscopes. In addition, definitions of a "focus" of lymphocytic aggregates, apoptotic hepatocytes, or confluent necrosis may vary among pathologists.

Brunt (2000) concluded that, for a system to be effective in every day diagnostic practice it must be simple to understand and to apply and it must communicate effectively to the treating clinician, and it must be clinically relevant. Also as documented by this researcher, it is to the credit of many dedicated pathologists that, liver biopsy continues to have a central role in clinical evaluation and diagnosis; indeed biopsy evaluation remains the "gold standered" for many of the current clinical investigations in chronic hepatitis. Building on the background reviewed in Brunt review and in the spirit of the contributions of the last 50 years, pathologists will continue the efforts to participate in the expanding clinical and scientific knowledge of chronic hepatitis.

By applying the new concept of scoring (Mangoud *et al.,* 2004), this system was somewhat in

aggreement with the observations of the present study. Also there is a significant correlation between the scoring by using Ishak Modified HAI score and using the new concept of scoring by Mangoud *et al.* (2004).

Hepatocellular carcinoma (HCC), was the common hepatic tumor observed in this study, which represented (100%) of the malignant cases. This finding is in accordance with Mathew and Affandi (1989), who showed that, hepatocellular carcinomas account for 90% of all primary cancers of the liver in the chronic hepatitis cases.

Hepatocellular carcinoma is one of the most common cancers worldwide. There is a striking variation in HCC incidence rates between various countries. The high-risk populations are clustered in Sub-Sharan Africa and Eastern Asia, (Chen *et al.,* 1997). It is frequently occurring in individuals in many developing countries including Egypt. Important factors have been demonstrated including chronic carriage of hepatitis B and hepatitis C viruses and other environmental factors such as exposure to aflatoxin, consumption of alcohol, tobacco smoking and long term use of oral contraceptive pills (Chen *et al.,* 1997).

Because the natural history of chronic HCV infection is characterized by a predominantly a symptomatic course and a variable clinical outcome, it has been difficult to define the rate of progression to cirrhosis and HCC. Many prospective studies on the histological progression of posttransfusion C hepatitis have shown that, during the early course of the disease (during the first 10-15 years after initial infection), liver cancer is a rare occurrence (Koretz *et al.,* 1993 and Mattson *et al.,* 1993). Also, Tong *et al.* (1995), indicated that, generally HCV infection precedes the development of HCC by a long time lag, between two and three decades after transfusion, although in some cases HCC has been shown to develop in less than 10 years from the onset of hepatitis.

Hepatitis B virus (HBV) is known to induce carcinogenesis by integrating into the genomic DNA of the host. In contrast, HCV is an RNA virus, and the role of HCV in the development of HCC is still unknown (Shiratori *et al.,* 1995). In our trial to discover this role, it has been clearly observed in the present study that, chronic hepatitis and cirrhotic cases have an obvious hepatocellular damage and replication as well as many symptoms predicting probability of development to HCC. These findings are accepted with Kashari *et al.* (1998), who revealed that, when hepatocytes are continuously damaged and replicated in chronic hepatitis C infected patients, the frequencies of genetic alterations probably increase, leading to the development of HCC. It is more likely that, HCC occurs against a background of inflammation and regeneration, associated with liver injury due to chronic hepatitis. Also, Di Bisceglie *et al.* (1994), noticed that, most but not all cases of HCV-related HCC occur in the presence of cirrhosis, suggesting that, it is the underlying liver disease per se that is the risk factor for HCC rather than HCV infection. At the same manner, the frequency of hepatocellular carcinoma occurring in patients with cirrhosis is high, with reports varying from 5% to 40%. These statistics are skewed to underestimate the relationship between cirrhosis and cancer since, once cirrhosis develops, a patient‘s subsequent life expectancy is shortened to approximately 5 years, thus constraining the allowable tumor latency period.

The present findings also revealed that, the high percentage of HCC was clearly observed in the cases aged over 40 years, (8 cases, 72.7%), while the persons aged below 40 years were3 cases represented (27.3%). Davis (1999) stated that, HCC caused by HCV infection occurred in old patients either due to the need for a long time of infection to cause cirrhosis and subsequent HCC or due to the late acquiring of HCV infection. Nearly similar findings were found in many Egyptian studies (Sabry *et al.,* 1995). Namieno et al, (1995) found the same results in South African blacks. In Italy, Farinati *et al.* (1992) found higher age incidence that was slightly lower in males than in females, while some previous studies by El-Serag and Mason (1999) and Fung *et al.* (2000) demonstrated that, the incidence of HCC is rising in the United States over the past two decades, with age-specific incidence shifted toward younger persons.

Males in this study were highly infected comparing to females (7.3: 1) and this ratio is lower than that previously reported in Egypt (9.2 : 1) (Sabry *et al.,* 1995). The male predominance may be explained by occupational causes, where most of the patients were farmers and presented with hepatic cirrhosis due to viral hepatitis and bilharziasis. Derbala *et al.* (1999) stated that in Egypt male predominance may be explained by a higher prevalence of HBsAg carrier state, greater exposure to environmental carcinogenic factors and chronic liver disease is more frequent in men than in women. The difference in prevalence in the present study compared to previously mentioned studies might be due to the difference in the number of patients involved in these works.

Diagnosis of HCC can not easily be accomplished by cytological examination alone because regenerating hepatocytes for example in hepatitis or active cirrhosis may show changes, which led us to apply immunohistochemical techniques and DNA image cytometry.

Immunohistochemistry by using tumor markers has been considered as an effective technique in the diagnosis of HCC from other malignant hepatic tumors, in which the cytologic criteria alone may be insufficient for their diagnosis. According to Jiang *et al.* (1997), alpha-Fetoprotein (AFP) is a reliable diagnostic tumor marker for hepatocellular differentiation in malignant hepatic tumors**.** In the present investigation, the procedure of immunostaining was carried out on paraffin sections only and not on the available smears. According to Orell *et al.* (1992)**,** the interpretation of immunohistochemical stains is more difficult in smears than in tissue sections, because of the fragility of cellular cytoplasm which torn from the stroma and due to cellular dispersion in the background. The presence of admixture of blood, serum or secretory products and contamination of the all, makes it difficult to ascribe any positive staining to specific identifiable cells. On the other hand, it is not clear weather all AFP-producing cells are malignant (Ohguchi *et al.,* 1998)**.** Though in situ hybridization studies in noncancerous liver failed to reveal the AFP-expressing cells, a weak but consistent AFP expression observed by Northern blot analysis suggests the existence of AFP-expressing cells in noncancerous liver (Ohguchi *et al.,* 1998)**.** Some cancer cells might harbor in the noncancerous cells in the liver and may express AFP. These observations are in accordance with our results which revealed that AFP expression was absent in most of chronic HCV cases (38/40, 95%), while it was weakly expressed in two cases (5%) which were highly affected with HCV and some cirrhotic cases (33.3%). Also in the present study, seven cases (87.5%) from HCCs showed positive staining reaction for AFP. This percentage is higher than that recorded by Tsuji *et al.* (1999)**,** who reported that, only (17%) of the studied HCCs were AFP positive, while it was negative for the typical hyperplasia cases. Also, they suggested that, these findings of AFP immunostaining are not useful in the differential diagnosis between a typical hyperplasia and well-differentiated HCC, while it was well expressed in moderately and poorly differentiated HCCs. Our results are in agreement with this suggestion, where the poorly and moderately differentiated HCCs revealed high AFP expression than that of the well differentiated HCCs. Ng *et al.* (1998), attributed this discrepancy to a heterogeneous distribution of cellular AFP in the tumor, so that sampling errors have to be taken into account. Another reason may be that, AFP production depends on the degree of differentiation of HCC, i.e., that it was low in well-differentiated HCC and high in poorly differentiated one. In addition, separation between false-positive immunohistochemical stains from a true positive reaction depends upon the ability of the individual investigator. Adding to the strength and affinity of anti-AFP, antibodies may affect on the sensitivity of the different immunohistochemical techniques (Nishizaki *et al*., 1997). Moreover, Semenkata *et al*. (1997) reported that, AFP positive cells showed either coarse brown cytoplasmic granules or longer clumps and lobules. It was noticed in the present study diffusing of finely granular brown pigmentation involving the entire cytoplasm in some cases and in a part of the cytoplasm as well as preninuclear arrangement.

In the present study, only one case was primarily diagnosed as HCC based on cytomorphologic ground with routine stains has revealed negative AFP staining. This negativity did not exclude the hepatocellular origin of these neoplasms, so the authors of the present study agrees with that reported by Yamashita *et al*. (1996) and Aoyagi *et al*. (1996), who stated that, AFP is a reliable diagnostic but insensitive immunocytochemical marker for hepatocellular differentiation in hepatic tumors.

In the present study, it has been noticed that, heterogeneous cellular distribution of transcripts for AFP was the common in positive reactive cases. The extremely heterogeneous AFP expression in HCC nodules was obviously observed with higher magnifications. This discrepancy may be explained by the difference of sensitivity of the method and /or the probes used. The present findings are in agreement with some previous immunohistochemical studies (Kinoyama *et al*., 1996)**,** which have shown heterogeneous distribution of AFP in HCC nodules. The in situ hybridization study by Ohguchi *et al.* (1998)**,** revealed that, clear cellular localization of the transcripts for AFP in individual cells is due to presenting precise information of the gene expression. Malignant tumors might be derived from clonal proliferation, which would suggest a more homogenous expression of these genes, which suggests that, each cancer cell of HCC nodule is in a different cell cycle and may possess different characteristics.

In the present study, we tried to correlate between the different hepatic lesions of the cases truly infected with HCV, which ranged from chronic hepatitis to HCC, and AFP expression in tissue and also, between the expression of AFP by the malignant hepatocytes and their grades. With regard to the grades of HCCs, 2 cases of HCC GI showed only faint staining for AFP, while the rest of this grade showed negative reaction. Three cases of HCC GII showed moderate AFP staining, while the three cases of HCC GIII showed marked AFP reactivity. These findings are in accordance with the work done by

Ogawa *et al.* (1996)**,** who noticed that, AFP positivity was most frequently observed in GII 21% and GIII 36% of studied HCC.

Also, it has been observed in the present study that, some chronic hepatitis cases of high grades and many noncancerous ones were stained positively with AFP, while, many HCCs reacted negatively or were stained weakly with AFP. This finding agreed with Ohguchi *et al*. (1998)**,** who suggested that, AFP is not the ideal option for targeting HCC cells.

The immunohistochemical results of CEA staining in this study revealed two patterns of staining, the first pattern was bile canalicular and the other one was the diffuse cytoplasmic staining. The bile canalicular staining pattern was found in 6 cases (75%) of HCC cases, and in 2 cases of the other lesions. Koelma *et al.* (1986) explained this pattern of bile canalicular staining in HCC cases by the presence of BGP cross reactivity with CEA. They also added that, the persistance of bile canalicular structures in HCC implied that, the formation of these structures was a basic feature of hepatocytes, which could be expressed even in poorly differentiated liver cells. Bile canalicular staining pattern was also observed in the aspirates from HCC cases with prominent dispersed pattern, apparently even the thinly stretched and fragmented HCC. This may be explained that, HCC plasma membranes retained much of its canalicular structures and glycoproteins antigenicity in these cases (Wolber *et al.,* 1991)**,** because of CEA staining pattern of canalicular type was negative in metastatic tumors and cholangiocarcinomas. This finding indicated that, staining with CEA antiserum was a useful adjunct in the differential cytologic diagnosis of malignant hepatocytes lesion. When it was present, it became strongly indicative of HCC differentiation.On the other hand, HCC without bile canalicular staining pattern tends to be very poorly differentiated, it represented a limitation to this method of staining (Goodman *et al.,* 1985)**.**

Christensen *et al.* (1989) stated that, the immunocytochemical staining of bile canaliculi with CEA specifically identified both benign and malignant hepatocytes. The results of the present study revealed that, this pattern of CEA staining was seen in normal hepatocytes of two cirrhotic cases. Wolber *et al.,* (1991) stated that, bile canalicular CEA staining has not been observed in any malignant lesion other than that of hepatocellular origin. So it was considered as a highly specific and relatively sensitive method for determining hepatocellular differentiation in a malignant aspirate.

Wong and Yazdi (1990) noticed that, 90% of the studied HCCs showed marked positive CEA staining. This percentage is approximately accepted with the present findings, where the positive CEA-reacted cases was (87.5%) of all the studied HCCs. They also, suggested that, the highly percentage of staining is due to the type of the fixative they used, (Methanol acetic acid) which was considered as an ideal fixative for immunocytochemical staining. It generally preserves antigenicity better than formalin fixative, which was used in our study, and may be in other series. Regarding to the other pattern of CEA staining (the diffuse cytoplasmic one), which appeared as brown cytoplasmic pigmentation, it has been noticed that, (12.5%) of HCCs were stained positively with this pattern of staining, while (0%) of the nonmalignant cases was positively stained. The CEA cytoplasmic stained cases were poorly differentiated. These findings are in accordance with Goodman *et al.* (1985) and Wolber *et al.* (1991) who mentioned that, the absence of canalicular CEA staining with or without cytoplasmic staining was suggestive of a metastatic carcinomas, but did not rule out a diagnosis of HCC.

Kilpatric *et al.* (1993) found that, CEA staining helped also in diagnosis of mixed tumor of HCC-CC. They noticed that, CEA positivity of bile canalicular pattern was demonstrated in the regions of hepatocellular differentiation, while neoplastic cells in glandular areas showed more diffuse cytoplasmic staining. No similar composite staining pattern was seen in this study, where no mixed HCC-CC cases were included.

In their immunohistochemical study, Tsuji *et al.* (1999) showed that, immunostaining was regarded as positive when more than 5% of cells were stained. Also, they observed that, CEA (polyclonal antibody) did not stain the cytoplasm of all adenomatous hyperplasias (AH) and HCC, but stained two(25%) of eight AH and 10 (23%) of 30 HCC in a bile canalicular staining manner. These results suggest that, immunostaining of CEA, AFP are not useful in the differential diagnosis between AH and well-differentiated HCC. So, absence of AFP did not exclude the diagnosis of HCC, meaning that, HCC may not produces AFP, but, if a tumor that seems to be primary in the liver and produces AFP, this may be taken as an evidence of its hepatocellular origin

In conclusions, The infected patients with chronic hepatic diseases particularly HCV were predominantly affected by HCC. Using cytopathological side by side histopathological examination and application of the advanced systems of chronic hepatitis grading and staging provide a high accurate means of diagnosis, which will be of a great benefit to the patients. The immunohistochemical investigations are considered as early predectors of cellular abnormality and different malignant criteria, which lead to early and

well diagnosis as well as rapid manipulation of the patients.

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