

Correlative Study between Caspases, Histopathological and Biochemical Changes in Chronic HCV Infection

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ABSTRACT:

Background/Aim: Chronic hepatitis C virus infection is characterized by inflammatory liver damage and is associated with a high risk of development of cirrhosis and hepatocellular carcinoma. Although histological examination of liver biopsies is the current gold standard for the detection of early liver damage, there is a strong need for better non invasive methods. The importance of apoptosis in HCV infection has been originally proposed in view of pathomorphological features, including cell shrinkage and formation of acidophilic bodies and fragmentation of the nucleus particularly in areas of piecemeal necrosis. The key morphological features of apoptosis are mediated by caspases. In the present study, we aiming to detect the caspase-3 in liver and sera of chronic HCV patients and correlate them with liver disease severity, serum transaminases and levels of viremia.

Patients & Methods: In the present study, liver biopsies and sera were taken from chronic HCV patients, as well as controls, for detection of caspase-3 activity. In liver tissue, caspase-3 antigen was detected by immunohistochemical staining with human monoclonal antibodies. Sera from patients and controls were examined for activated caspase-3 products using human caspase-3 ELISA kit.

Results: Caspase-3 was markedly increased in tissues and sera of chronic HCV patients relative to controls.

Conclusion: Caspase-3 activity was significantly correlated with grade of inflammation and stage of fibrosis, so it may be a reliable non invasive marker for disease severity.

Key words: Caspases, Histopathological, Biochemical Changes, HCV Infection.

INTRODUCTION

Hepatitis C (HCV) virus is recognized as a major threat to global health infecting over 3 % of the world population. It is a parentally transmitted highly mutable RNA virus that causes acute and chronic hepatitis ⁰. Egypt has possibly the highest HCV prevalence in the world. The high prevalence of HCV in Egypt was suggested to be the result of the use of unsterile injection or equipment during mass treatment of the general

population with parenteral antischistosomal therapy (PAT) ⁰.

While expression and retention of viral proteins in hepatocytes may influence the severity and progression of liver disease, the mechanisms of liver injury are defined not to be due to the direct cytopathic effects of the virus, but to the host

immune response to viral proteins expressed by infected hepatocytes⁽³⁾

Mechanisms of liver cell injury, inflammation and fibrosis in chronic HCV are both immune mediated and direct cytopathic effects of HCV gene products, the immune mediated process in which apoptosis of liver cells plays a significant role⁽⁴⁾.

Apoptosis is a genetically defined as a major form of programmed cell death enabling the organism to remove unwanted cells (during embryonal development and after immune responses) to select educated immune cells and to eliminate virally infected and transformed cells⁽⁵⁾. The importance of apoptosis in HCV infection has originally been proposed in view of patho-morphological features, including cell shrinkage and fragmentation of the nucleus particularly in areas of piecemeal necrosis, the presence of acidophilic bodies and focal cells of drop out in the liver lobule, which are characteristic features of individually infected hepatocytes⁽⁶⁾. Interestingly, caspase activation appears to correlate closely with the inflammatory response. Data about the role of single HCV proteins either in cultured cells or transgenic animal's models are contradictory, as both pro- and anti- apoptotic effects have been observed. Nevertheless, apoptosis induction upon HCV infection may critically contribute to liver damage, while inhibition of apoptosis may result in HCV persistence and development of hepatocellular carcinoma⁽⁷⁾. The therapeutic benefits of these concepts are that; inhibition of apoptosis may prevent tissue injury and or promote tissue regeneration and restitution, induction of apoptosis of dysplastic cells and transformed cells may be useful in preventing and treating malignant diseases, and lastly, conversion of necrotic inflammatory injury to an apoptotic non-inflammatory process may ameliorate diseases⁽⁸⁾. A variety of proteases have been strongly implicated in apoptosis, including members of the caspase family (previously known as the interleukin 1 β -converting enzyme family of proteases), calpains, cathepsin, and the proteasome⁽⁹⁾. Caspases are present in the cytoplasm of most cells in an inactive form (also called a zymogen).

In this inactive state, a caspase exists as a single polypeptide chain with a prodomain and a catalytic domain. Caspase-3 is one of the major activated cysteine proteases that constitute the caspase family and is a pivotal cog in the apoptotic machine. It cleaves a number of substrates and activates endonucleases, leading to DNA fragmentation which is the hallmark of apoptosis⁽¹⁰⁾. In diseases in which the apoptotic machinery is inappropriately triggered but the cell is not otherwise damaged, caspase inhibition may prove very effective in reversing the pathophysiology⁽¹¹⁾. Caspases are key mediators in liver inflammation and apoptosis. FAS regulated apoptosis plays a major role in the pathogenesis of immune mediated liver disease including viral and autoimmune hepatitis. Down regulation of FAS-FAS L system might have therapeutic value in the treatment of this disease⁽¹¹⁾.

AIM OF THE WORK

The aim of the present work is to investigate the presence of caspases in tissues and sera from patients with chronic HCV, to throw more light on apoptosis process and their correlation with biochemical and histo-pathological changes.

PATIENTS AND METHODS

The present study was carried out on 37 patients with chronic HCV selected from those referred to Tropical medicine department, Tanta University Hospital, and 5 healthy individuals as a control. They were divided into:

Group I; included, 19 chronic HCV and schistosomiasis.

Group II; included 18 chronic HCV without schistosomiasis.

Control group; included 5 healthy individuals.

Inclusion criteria:

Selected chronic HCV patients were reactive to HCV antibodies and polymerase chain reaction (PCR) with no history of specific therapy for HCV before or at the time of the

study. Schistosomiasis infection in group I was confirmed by history taking, stool analysis for ova, abdominal ultrasonography and rectal snips.

Exclusion criteria:

- Mixed hepatitis B (HBV) & hepatitis C infection.
- Autoimmune hepatitis.
- Active variceal bleeding.
- Decompensated cirrhosis.
- Focal hepatic lesions.
- Cardiac, pulmonary and renal diseases.
- Diabetes mellitus.

All Patients and controls were subjected to the following:

- 1) Liver function tests.
- 2) Quantitative PCR for HCV (genome/ml): by real time PCR, according to the method described by a available commercial kits:
- 3)-Abdominal Ultrasonography.
- 4)-Liver biopsy;

Liver biopsy was done to all selected patients after taking their consent. Liver biopsies of controls were obtained intraoperatively from surgery department, during partial hepatectomy for conditions other than chronic liver disorders (accidents).

- The biopsy specimens then were fixed in 10% diluted formalin and processed in paraffin blocks. Thin paraffin sections (4 μ m thick) from each specimen were prepared for Hematoxylin & Eosin stains for histopathological diagnosis and another section was fixed on adhesive slide for immunohistochemical staining.

- Grading of the necroinflammatory activity and staging of fibrosis was assessed according to modified histological activity index of (Ishak 1995) (12)

- Immunohistochemical staining of liver biopsy.

Immunohistochemical Scoring for caspase-3;

The expression of caspase-3 was measured in 10 successive high power fields (X 400). It was expressed as brown coloration in hepatocyte cytoplasm.

The degree of caspase-3 expression within the hepatic lobule was evaluated semi-quantitatively according to the percentage of positively stained cells and classified into four groups.

(-) = no immunostaining.

(+) = (<25% of cells)

(++) = (25%-50% of cells)

(+++)= (50%-75% of cells)

(++++)= >75% of cells

5) Assessment of activity of serum caspase-3:

Principle of the test: using enzyme-linked immunosorbent assay that selectively recognized a proteolysis neoepitope of the caspase substrate cytokeratin-18 in the sera of HCV patient, (CK-18) was cleaved by caspases at two conserved aspartate residue (amino acids 387-396).

For quantitative measurement of active caspase-3 in the serum, a monoclonal antibody, which selectively recognizes a caspase cleavage-generated Ck-18 at aspartate (Asp396), we used ELISA kit (Human Caspas-3 Instant ELISA kit, Bender MedSystem GmbH Campus Vienna Biocenter 2 A-1030 Vienna, Austria, Europe BMS2012INST hcaspase3 29.07.05(01).

Procedures;

- 1-Serum samples (3ml) from patients and healthy controls were pre-cleared by centrifugation at 10,000g for 15 minutes.

- 2-Two rounds of extraction with 2ml protein G-sepharose for 1 hour reduced the immunoglobulin content.

- 3-Immunoprecipitation was performed in a volume of 4ml in a rotator at 4degrees for 4hours using an anti-CK antibody that also served as a catcher antibody in the ELISA kit at a final concentration of 0.5 μ g/ml.

- 4-The immunocomplexes were precipitated for 1hour with 40 μ l of 50% slurry of protein G-sepharose in phosphate buffered saline

- 5-Precipitates were harvested via short centrifugation (2,000 rpm for 10 seconds at 4 degrees) and washed for four times with cold washing buffer, (20mmol/l hydroxyethyl piperazine-N-2 ethane-sulphonic acid, pH 7.4, 150

mmol NaCl, 10% glycerol, 0.1% Triton X-100, 1 µg/ml aprotinin, 1 µg/ml leupeptin).

6- Proteins were eluted by boiling the precipitates in SDS-loading buffer containing β-mercaptoethanol, separated under reducing conditions on a 12% SDS polyacrylamide gel and subsequently transferred to polyvinylidene difluoride membrane.

7- Membranes were blocked for 1 hour with 5% non fat dried milk powder in Tris-buffered saline, 0.05% Tween-20 then immunoblotted with the caspase-cleaved specific antibody directly coupled to horseradish peroxidase.

8- Finally, the membranes were washed extensively in Tris-buffered saline, 0.05% Tween-20, and developed using electrochemiluminescence reagents.

9- Calculation of the results:

10- The average absorbance values for each set of standard and samples were calculated.

11- A standard curve was created by plotting the mean absorbance for each set of standard concentration on the ordinate against the caspase-3 concentration on the abscissa.

12- To determine the concentration of caspase-3 for each sample, first the mean absorbance value was found on the ordinate and a horizontal line was extended to the standard curve.

13- At the point of intersection, a vertical line was extended to the abscissa and the corresponding caspase-3 concentration was read.

14- Sensitivity;

The limit of detection of caspase-3 was defined as analyte concentration resulting in an absorption significantly higher than that of dilution medium) was determined to be 0.12 ng/ml (mean of 6 independent assay).

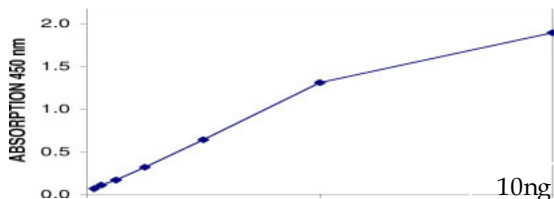


Fig. (1): Caspase-3 concentration (ng/ml), Typical data using the caspase-3 ELISA. Measuring wave length = 450nm, Reference wave length = 620

RESULTS

According to age and sex; the difference between groups of patients in sex & age distribution is statistically insignificant ($p = 0.50$ & $p = 0.83$) respectively.

Biochemical liver test of the studied groups as shown in table (1): As regard to prothrombin activity, the difference between group 1, and 2 as well as group 1 and controls was statistically significant, otherwise the difference was statistically insignificant.

As regard the quantitative PCR (level of viremia) in the studied groups (table 2): The difference between both groups was statistically insignificant.

The serum caspase-3 activity in the studied groups (table 3) revealed that; the difference between group I, group II, and the controls was statistically significant whereas the difference between group I, and group II, was statistically insignificant.

Immunohistochemical assessment of caspase-3 activity in liver biopsy specimens as shown in (table 4): The grading of caspase-3 activity was expressed as percentage of positively stained cells. Minimal activity was found in (31.6%) as seen. Mild activity was found in 26.3%, (picture (2)). Moderate activity was found in 10.5% (picture (3)). Marked activity was found in 21.1% of liver biopsies in group 1, (Pictures (4, 5)).

In group II, minimal activity was present in (50.0%), mild activity in (16.7%), and moderate activity in (27.8%) and marked in (5.6%).

The correlation between, biochemical liver tests level of viremia and caspase-3 activity: The correlation between, biochemical liver tests level of viremia, and caspase-3 activity as shown in (Figure 2); No significant correlation was found between, biochemical liver function tests and level of viremia or serum caspase-3 activity.

Correlations between histopathological picture biochemical liver tests and level of viremia show:

No significant correlation was found between grades of inflammation, stages of fibrosis and biochemical liver tests or level of viremia.

Correlation between histopathological picture and serum caspase-3 levels show: Positive correlation was found between grades inflammation and serum caspase-3, $r = 0.7$, $p = 0.0001$.

Positive correlation was found between stage of fibrosis and serum caspase-3 activity, $r = 0.8$, $p = 0.0001$.

A strong positive correlation was found between grades of inflammation and degree of caspase-3 activity in liver tissue, $r = 0.9$, $p = 0.0001$. A significant positive correlation was found between caspase-3 activity in serum and liver tissue, $r = 0.7$ and $p = 0.002$.

Table (1): Age, sex and some biochemical parameters of the studied groups

	Group I(n=19)	Group II(n=18)	Control (n=5)	F	ISD
Males	12	11	2		
Females	6	7	3		
Age in years (Mean±SD)	32.1±7.9	33.1±5.6	31.2±7.7		
Blood picture:					
Hb (gm/dl)	12.8±1.32	12.2±1.5	13.5±1.1	1.78	0.93 a 0.09 b 0.08 c
RBCs (10 ⁶ /ml)	4.2±0.50	4.3±0.47	4.4±0.74	0.47	0.49 a 0.39 b 0.68 c
WBCs (10 ³ /ml)	4.3±1.1	5.39±0.92	4.4±0.85	1.15	0.84 a 0.15 b 0.19 c
Platelets (10 ³ /ml)	162.0±0.500	165.2±137.3	175.5±56.9	0.06	0.76 a 0.78 b 0.94 c
Liver Function Tests:					
Total bilirubin (mg/dl)	0.66±0.33	0.65±0.19	0.63±0.32	0.52	0.99 a 0.86 b 0.85 c
ALT (u/L)	49.00±27.82	49.22±29.20	26.00±2.45	1.61	0.98 a 0.09 b 0.09 c
AST (u/L)	42.00±20.59	39.11±21.40	25.00±10.25	1.41	0.66 a 0.10 b 0.17 c
Albumin (g/dl)	3.77±0.47	3.80±0.44	4.24±0.18	6.8	0.89 a 0.04* b 0.05*c
Prothrombin conc. (%)	82.05±9.81	89.28±0.32	94.00±5.70	4.3	0.02*a 0.02*b 0.34 c

* = significant

Table (2): Quantitative PCR and the level of viremia in the studied patients

Viremia	Group I (n=19)		Group II (n=18)		Test	p
PCR (u/ml) (Mean±SD)	1089318±1175503.25		401842.83±49166.50		t =0.58	0.56
Level of viremia [n(%)]:						
Low	8	42.1	10	55.6		
Moderate	3	15.8	2	11.1	χ ² =0.302	0.58
High	8	42.1	6	33.3		
Low viremia=<200000 c/ml High viremia=500000 -1million c/ml				Moderate viremia =200000-500000 c/ml		

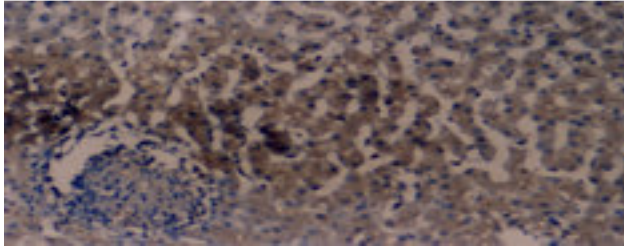
Table (3): Comparison of serum Caspase-3 levels between studied groups

Serum Caspase-3	Group I (n=19)	Group II (n=18)	Control (n=5)
Mean	147.4	145.4	57.2
SD	32.3	25.9	24.2
F	21.0		
p	0.001*		

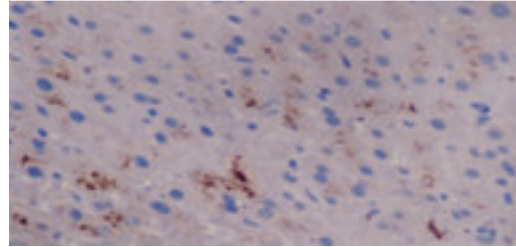
Control group significantly different from groups I and II

Table (4): Caspase-3 activity by immunohistochemistry in liver biopsies of the studied patients and controls.

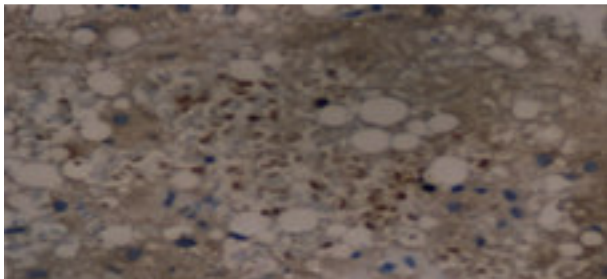
	Group1 (n=19) n (%)	Group2 (n=18) n (%)	Controls (n=5) n (%)
Immunohisto capase3 (no immunostaining)	0 (0.0 %)	0 (0.0 %)	5 (100%)
(minimal)	6(31.6 %)	9(50.0%)	0.00
(mild)	5(26.3 %)	3(16.7%)	0.00
(moderate)	2(10.5 %)	5(27.8%)	0.00
(marked)	4(21.1 %)	1(5.6%)	0.00
χ ²	4.2		
P	0.24		



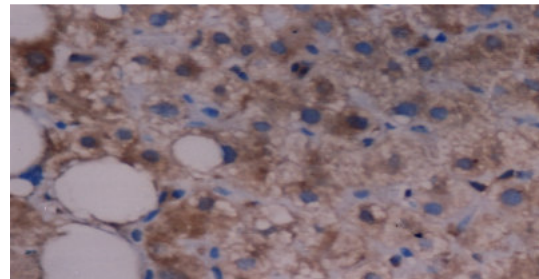
Picture(1) Liver section of chronic HCV patient stained with caspase-3 immunohistochemistry stains showing Dilated sinusoids(X100).



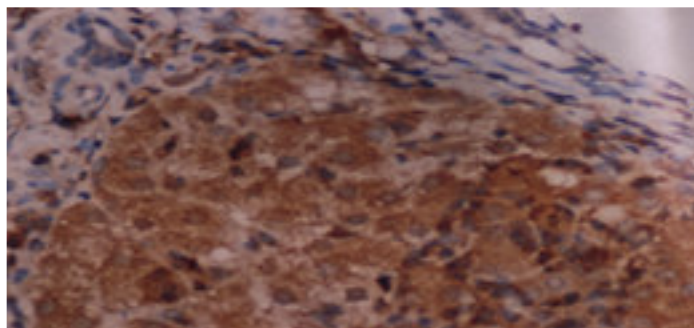
Picture (2) Liver section of chronic HCV patient stained with caspase-3 monoclonal antibody showing positively stained apoptotic bodies with brownish discoloration in focal necrotic areas (mild activity). (Strept avidin Biotin X400).



Picture(3) Liver section of chronic HCV patient stained with caspase-3 monoclonal antibody showing positively stained apoptotic bodies with brownish discoloration in focal necrotic areas (Moderate activity) .(Strept avidin Biotin X 400).



Picture(4) Liver section of chronic HCV patient stained with caspase-3 monoclonal antibody showing positively stained apoptotic bodies with brownish discoloration in focal necrotic areas (Marked activity, A).(Strept avidin Biotin X400)



Picture(5) Liver section of chronic HCV patient stained with caspase-3 monoclonal antibody showing positively stained apoptotic bodies with brownish discoloration in focal necrotic areas (Marked activity, B). (Strept avidin Biotin X400)

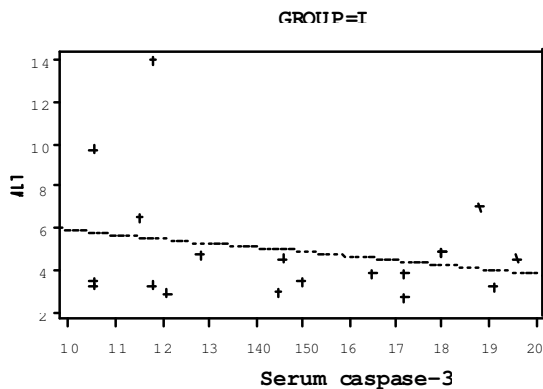


Fig (2): Linear curve between serum caspase-3 and ALT in group I $r=0.05$

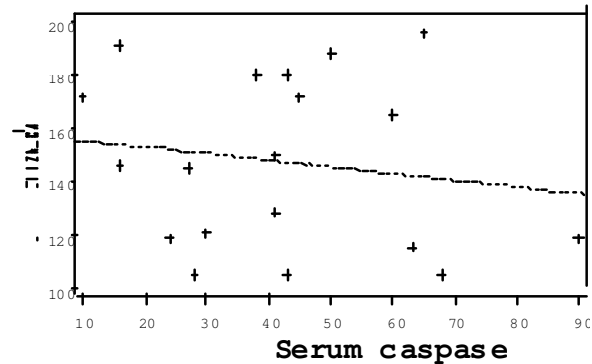


Fig. (3): Linear curve between serum caspase-3 and ALT in group 2, $r=0.05$,

DISCUSSION

Hepatitis C virus infection is a major cause of liver disease, characterized by inflammation, cell damage and fibrotic reaction of hepatocytes (4).

There is increasing evidence suggesting that liver cell damage in chronic HCV infection is mediated by induction of apoptosis (4). The key morphological alterations of apoptosis are mediated by a family of intracellular proteases called caspases (3). Caspase-3 is the central executioner of many if not all apoptosis pathways (4).

Hepatic fibrosis is the main determinant of clinical outcomes of chronic hepatitis C, and liver histology is frequently considered the gold standard for assessing hepatic fibrosis. However, it associated with sampling error, interobserver variability, and potential complications. Thus, there is need for simple, reliable, and noninvasive means to assess disease severity in chronic HCV patients (5).

Non invasive approaches to assessment of severity of hepatitis C include clinical symptoms and signs, routine biochemical and hematologic blood tests, serum markers of fibrosis and inflammation, combinations of clinical and blood test results, and radiologic imaging studies (15).

Symptoms in HCV infection do not appear to be linked to disease activity; neither does appear to be correlated with serum aminotransferase levels or liver histological findings (6). Clinical symptoms and signs are often unreliable in assessing disease severity in patient with compensated liver disease. Likewise, physical finding of chronic liver disease, such as jaundice, pedal edema, hepatosplenomegaly, ascites and encephalopathy, are frequently absent until patients develop decompensated cirrhosis (15).

In the current study, the clinical symptoms encountered in studied patients were varied from absence where the disease is accidentally discovered to mild or moderate in the form of; fatigue, itching, upper abdominal pain as well as impaired quality of life. On clinical examination, no jaundice, no pedal edema, no ascites, hepatosplenomegaly was detected in cases with established cirrhosis. Also in most cases no significant ultrasonographic abnormalities.

This finding was in accordance with (Fontana and Lok 2002) (15) but against Pyonard et al (1997) (17) in two different studies who found that; clinical and histological data have been shown to correlate with rate of fibrosis progression.

Long term follow up of chronic HCV patients reveals that hepatitis C-related cirrhosis is a "slowly progressive disease that may be accelerated by other potential causes of liver disease" HCC is the first complication to develop and the dominant cause for increased mortality (18).

The finding of an abnormal level of serum transaminases may lead to discovery of HCV infection, however an estimated 25% of patients with chronic HCV infection have persistently normal ALT levels (19). Liver enzyme levels in people with chronic HCV infection can be normal, periodically elevated, or persistently elevated (20).

In the present study, the mean serum ALT and AST levels were mostly not exceeding the upper limit of normal ranges and no significant difference was found between patient and controls. These findings were in accordance with previous studies of (Strader et al, 2004) (21), who stated that: The efficacy of elevation of serum alanine aminotransferase (ALT) levels in prediction of severity of liver injury in patients with chronic hepatitis C is debated. Up to 20% of patients with chronic infection have persistently normal serum ALT level.

This finding was not in agreement with (Shiffman et al 2006) (22), who stated that; elevated levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are efficient markers of inflammation associated with liver damage.

Absence of ALT and/AST elevation may be explained by different hypothesis, release of transaminases in the blood of chronic HCV patients depends upon the mechanisms of liver cell injury, whether mediated by necrosis or apoptosis. Apoptosis can occur in HCV-infected patients without overt biochemical changes, explaining the progressive nature of liver disease that can be asymptomatic with persistently normal ALT (22). However, release of transaminases in serum after an apoptotic stimulus in the liver can occur in some circumstances and it has been considered the

result of secondary inflammation and necrosis, excited by the apoptotic process itself (23).

Loeza et al (2006) (24) study revealed that, severe necroinflammatory activity and cirrhosis were more frequent in patients with elevated ALT. Kronenberger et al, (2000) (25) has found that patients with chronic hepatitis C and normal amino-transaminases levels have significantly lower hepatocyte proliferation rates and show a trend toward lower apoptosis rates compared with patients with elevated amino-transaminases levels.

The necroinflammatory activity in our cases was minimal in 63.2% , mild in 31.6%,and moderate in 5.2% of group I patients , where in group II minimal activity was found in 55.5%, mild activity in 33.3% and moderate activity in 11.2% of the studied patients. Fibrosis was absent in 15.8% and 38.9% in group 1 and group 2 respectively but was extensive (S4) in 21.1% and 11.1 % and cirrhosis was found in 16.0% and 56%. No significant correlation was found between histological activity and serum transaminases levels, a finding that in agreement with; Benhamou et al, (2002) (26) who stated that serum Alt levels reflect liver injury, but the correlation between ALT levels and necroinflammatory and fibrosis score is poor.

Prothrombin Time (PT) measures the amount of time needed for blood to clot. Clotting factors are made in the liver. PT elevations may occur during acute hepatitis. In chronic hepatitis, PT usually remains normal, though abnormal PT prolongation usually accompanies progression to cirrhosis. PT is elevated in cirrhotic individuals, because a significantly damaged liver may not be able to produce enough clotting factors with increased risk of bleeding (27).

In the present study, the studied patients in both groups were divided according to level of viremia into 3 groups; low, moderate, and high viremia. No correlations were found between level of viremia and grade of activity or stage of fibrosis; a finding that agrees with previous studies of Sherman 2002 (28).

Liver biopsy examination remains the most reliable method to assess the extent of

necroinflammatory activity and fibrosis⁽²⁹⁾ The role of biopsy examination is more debatable in patient with persistently normal alanine transaminase (ALT) levels and those with genotypes 2 and 3. In those with clinical evidence of cirrhosis or portal hypertension, a liver biopsy examination may add little further information unless an additional diagnosis is suspected, and may be associated with more complications⁽³⁰⁾.

In the present study, histological examination of liver biopsies of the studied patients and controls was done by the usual H & E stains (hematoxyline and eosin) as well as by immunohistochemistry stains for detection of caspase-3 activity. The histopathological features included necroinflammation {portal inflammation, interface hepatitis (piece meal necrosis) and intralobular necrosis}, steatosis, sinusoidal dilatation, bile duct proliferation and ballooning degeneration.

Apoptosis could not be seen by H & E stains. Fibrosis of different degrees 1-6, was diagnosed by the by H and E stains. By immunohistochemistry caspase-3 activity in liver tissue was detected as brown stains in cell cytoplasm of chronic HCV liver tissue but not in controls. The intensity of the stain was marked in higher grades of inflammation and higher stages of fibrosis; the intensity of immunohistochemistry was positively correlated with the grade of inflammation and stage of fibrosis. Apoptotic bodies were also found in immunohistochemistry stained tissues.

These findings were in agreement with previous studies by Ibrahim (2004)⁽³¹⁾ who obtained a direct evidence for variable degree of liver cell apoptosis in the liver of patients with chronic hepatitis C has been obtained and a significant correlation was found between the amount of apoptosis, expressed as apoptotic index and inflammatory activity in the liver. He also found no correlation between extent of liver cell apoptosis and serum transaminases levels or the serum viral RNA levels.

Also the results of the present study are in agreement with Bantel et al, (2001a)⁽³²⁾ who found that liver cell damage in chronic HCV

infection is mediated by induction of apoptosis. Caspases are activated in human biopsy specimens of chronic hepatitis patients, activation of caspase-3 correlated significantly with inflammatory activity but not with transaminases or viral load.

Similar results were also found by; Bantel et al, (2004)⁽²⁹⁾ who found that chronic HCV infection is characterized by massive caspase activation. Hepatocyte exhibiting immunoreactivity for active caspases or caspase-cleaved substrates was in most cases morphologically normal and did not reveal late apoptotic features. So, they suggested that measurement of caspase activation may be a suitable marker for detecting very early signs of liver damage.

In the present study, caspase-3 activity in sera of patients and controls was estimated by ELISA kit, where higher levels were found in patients than controls. Caspase-3 levels were positively correlated with grade of inflammation and stage of fibrosis but not with serum transaminases or viral load. This finding was in agreement with Bantel et al, (2004)⁽²⁹⁾ who found higher caspase-3 activity in sera of chronic HCV patients that wasn't significantly correlated with serum transaminases or viral load.

The exact mechanism leading to the secretion of caspases and CK-18 fragments in blood of HCV patients may be due to disruption of the keratin network and formation of small spheroidal cytoplasmic inclusions containing cleaved CK-18 as well as activated caspases⁽³³⁾.

There are several possibilities that could explain the lack of strict correlation between the serum levels of transaminase and caspase activity. Whereas transaminases are released during necrosis, generation of CK -18 requires apoptotic caspase- activation, and their release might occur during secondary necrosis. In vivo studies demonstrated that hepatocyte apoptosis is associated with increased transaminase values, but the release of transaminase is lower in apoptosis than necrosis (34) It is also conceivable that different ways of cellular sequestration are responsible for the differences in serum content of aminotransferases and CK-18 cleavage products⁽³⁾.

Finally, as reported for the characteristic extracellular release of cytochrome c or glutathione during apoptosis, CK-18 cleavage fragment might be extruded by other still-unknown mechanisms and thereby could explain differences in the release of aminotransferases and CK-18 cleavage products⁽³⁵⁾.

Although the pattern of HCV/S.mansoni infection frequent in many geographic areas and previous reports showed more severe liver disease in coinfecting patients, the inter-actions between HCV and S.mansoni are not fully understood⁽³⁶⁾.

In the present study no significant difference was found between co-infected patients in group 1 and mono-infected patients in groups 2 as regard to liver disease severity. This was in agreement with Blanton et al., (2002)⁽³⁷⁾ study, in Egypt who found that there was no interaction between S.mansoni infection or disease and the prevalence or severity of hepatitis C. For both infections, the intensity or prevalence of infection was a poor predictor of morbidity. The prevalence of chronic liver disease in the Egyptian population from different pathogens suggests a generalized susceptibility to inflammatory liver disease.

But this finding was not in agreement with Angelico et al, (1997)⁽³⁸⁾ and Kamal et al, (2000)⁽³⁶⁾ who stated that, concomitant schistosoma and HCV infection is common in Egypt and other developing countries. Patients with concomitant chronic HCV and S.mansoni have higher incidence of cirrhosis and hepatocellular carcinoma than patients with chronic HCV mono-infection matched for age, disease duration, and HCV genotype. Kamal et al., (2001)⁽³⁶⁾ found that, fibrosis was significantly accelerated in coinfecting patients than mono-infected patients and explained the cause of fibrosis progression by the additive effects of 2 injurious agents or the difference in immune response and alteration of the host signaling pathway inflicted by S.mansoni, resulting in fibrogenesis.

The results of the current study may be explained on the basis of the small number of the studied patients. The effect of coinfection of HCV and S.mansoni needs long term follow up, which is not included in the current study, younger age of studied patients and short duration of HCV infection.

Repeated courses of antischistosomal therapy which is a very common attitude in Egyptians may greatly diminish the effect of schistosomal infection.

From the current study we can state that, serum caspase-3 activity might be a more sensitive and noninvasive marker for detecting liver fibrosis in chronic HCV. However, the identification of endogenous caspase inhibitors and some other evidences suggest that within a cell the extent of caspase activation may be restricted and may not necessarily lead to cell death⁽³¹⁾

Inhibition of caspase activity may help to reduce liver damage as was explained by a study of Shiffman et al., (2006)⁽⁴¹⁾, who found that a pancaspase inhibitor PF-03491390 (formerly IDN-6556), is well-tolerated and effectively reduces raised transaminases levels in chronic active Hepatitis C (HCV) Patients. Pancaspase inhibitors are included in future therapies for HCV infection.

CONCLUSIONS

From the present study we can conclude that:

- 1- Serum transaminases are poor indicators of hepatic injury in chronic HCV infection.
- 2- Caspase-3 could be a non invasive measure for detection of extent of liver injury in chronic HCV but needs further studies for specificity and validity.

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الملخص العربي

دراسة العلاقة بين الكسبيس و التغيرات الباثولوجية والكيميائية الحيوية فى مرض الالتهاب الكبدى الفيروسى (سى) المزمن

تعد العدوى بفيروس الالتهاب الكبدى سى من أخطر المشاكل فى العالم، ويعانى الشعب المصرى من امراض الكبد بسبب هذا الفيروس حيث بلغت نسبة وجود الاجسام لمضدة لهذا الفيروس بين المصريين من ١٥ الى ٢٠ بالمائة. ويعد الحقن بالمقار لمضد للبلهارسيا من أهم عوامل الخطورة التى وضعت مصر نون بقية دول العالم فى هذا الوضع، ولايزل فحص عينة من الكبد هو المقياس المفضل فى تحديد شدة لمرض، ولكها وسيلة صعبة وغير مقبولة عند لمرضى وقد تنتج عنها مضاعفات شديدة . وقد استخدمت كثير من الدلالات الكيميائية لتحديد مدى تلف الكبد ولكنها لم تكن ليعتمد عليها، لذلك دعت الحاجة الماسة الى البحث عن دلالات أخرى أمنه و أكثر ثقة فى تحديد مدى التلف فى خلايا الكبد وخصه فى مرضى الالتهاب الكبدى سى ذوى المستوى الطبيعى لإنزيم الالانين الناقل للامحاض الامينية وذوى الاعداد القليلة من الفيروس بالدم.

وقد اثبتت الدراسات ان انزيمات الكسبيس -وهى انزيمت متخللة فى عملية الموت المبرمج للخلايا - يزداد معدل نشاطها فى انسجة الكبد وأصمالم مرضى الالتهاب الكبدى الفيروسى المزمن سى، وأن معدل نشاطها يختلف تبعاً لاختلاف شدة المرض؛ وعلى ذلك فإن من الممكن أن يكون قيسل مدى نشاط هذه الانزيمات عند هؤلاء لمرضى أكثر حساسية من الدلالات التقليدية مثل إنزيم الالانين الناقل للامحاض الامينية، و لذلك كان هدف هذه الدراسة هو قياس مستوى انزيم الكسبيس فى سيج الكبد والمصل عند مرضى الالتهاب الكبدى الفيروسى المزمن سى ودراسة مدى تأثيره بدرجة لتلف بخلايا الكبد وعلاقته بمدى شدة المرض، و مستوى إنزيم الالانين الناقل للامحاض الامينية و مستوى الفيروس بالدم.

تم لختيار الكسبيس ٣ نظراً لاهميته فى عملية الموت المبرمج للخلايا، وقد اجريت هذه الدراسة على سبعة وثلاثين مريضاً مصاباً بالالتهاب الكبدى الفيروسى سى المزمن وخمسة اصحاء كعينة ضابطة، وقد قسم المرضى الى مجموعتين :

المجموعة الاولى وشملت تسعة عشر مريضاً مصاباً بالبلهارسيا، **لمجموعة الثانية** وشملت ثمانية عشر مريضاً لم يثبت لديهم الإصابة بالبلهارسيا، وقد شملت **المجموعة الثالثة** اصحاء كعينة ضابطة، وقد تم اختيار المرضى على أساس وجود الاجسام المضادة للفيروس و الحمض لنوى بالدم باجراء اختبار تفاعل الحمض النووى المتسلسل (نوعى) للتأكد من وجود الفيروس.

وقد تم فحص جميع المرضى اكلينيكياً و اجريت لهم الابحث الالائى:وظائف الكبد الكاملة، وقد تم تحديد مستوى الفيروس فى الدم بالقياس الكمى (بى سى ار) كما تم فحص المرضى بأشعة الموجات فوق الصوتية.

تم أخذ عينات لبرية لمرضى من الكبد لدرستها هستولوجياً، وقد تم تقييم درجة لتلف بخلايا الكبد باستخدام مقياس ليزك (١٩٩٥)، كما تم تعيين نشاط إنزيم الكسبيس ٣ فى خلايا الكبد باستخدام الكيمياء لنسجية المناعية ومقارنتها بعينات كبدية من مجموعة الاصحاء، كما تم قياس نشاط إنزيم الكسبيس ٣ فى الدم للمرضى والاصحاء باستخدام الاليزا .

وقد لوحظ ارتفاع معدل نشاط إنزيم الكسبيس ٣ فى أصمالم المرضى فى المجموعة الاولى ولثانية مقارنة بالمجموعة الثالثة، حيث بلغ المتوسط الحسابى لمستوى الإنزيم فى الدم (١٧٤ ± ٣٢.٣٣) فى المجموعة الاولى و (٢٥.٩٨ ± ١٤٥.٤٤) فى المجموعة الثانية مقابل (٥٧.٢٠ ± ٢٤.٢٢) فى المجموعة الضابطة .

وقد أظهرت نتائج الفحص الباثولوجى لعينات الكبد وجود التهاب كبدى بسيط فى ٣١.٦٥% من لحالات ومتوسط فى ٥.٢٠% وتليف كبدى بسيط فى ١٥.٨% ومتوسط فى ١١.٢% و شديد فى ١٦% فى المجموعة الاولى، بينما وجد التهاب كبدى بسيط فى ٣٣.٣% من الحالات ومتوسط فى ٣١.٦% وتليف كبدى بسيط فى ٥.٦% ومتوسط فى ٣٨.٩% و شديد فى ١٦.٧% فى المجموعة الثانية.

وقد لوحظ ارتفاع نشاط إنزيم كسبيس ٣ فى المرضى مقارنة بالمجموعة الضابطة كما وجد ان هناك ارتفاعاً ملحوظاً فى مستوى نشاط الإنزيم مع زيادة درجة التلف فى خلايا الكبد؛ و بدراسة العلاقة بين نشاط الإنزيم ودرجة التلف فى خلايا الكبد وجد ان هناك ارتباطاً إيجابياً بين مستوى الإنزيم ودرجة التلف فى خلايا الكبد بينما لم يوجد أى ارتباط بين مستوى الإنزيم و مستوى الالانين الناقل للامحاض الامينية أو مستوى الفيروس بالدم. أما بالنسبة لمستوى نشاط إنزيم كسبيس ٣ بالدم وجد أن هناك ارتباطاً إيجابياً بين مستوى الإنزيم ودرجة التلف فى خلايا الكبد بينما لم يوجد أى ارتباط بين مستوى الإنزيم و مستوى الالانين الناقل للامحاض الامينية أو مستوى الفيروس بالدم، وبمقارنة لمجموعتين الاولى والثانية لم يوجد أى تأثير للإصابة بالبلهارسيا فى درجة شدة تأثير خلايا الكبد بالفيروس.

وقد أوصت الدراسة بالآتى:

إجراء اختبارات مسحية للتشخيص المبكر للالتهاب الكبدى الوبلى وقيام بمعالجته فى مراحل المبكرة قبل حدوث التليف الكبدى. كما يوصى بدراسات مستقبلية واسعة لتحديد جدوى الكسبيس ٣ فى تحديد مدى الاعتلال الكبدى المصاب بالالتهاب الفيروسى سى المزمن.