ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com
Journal DOI:10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

The Effect Of TNF-α , IL-8 In Pathogenesis Of Hepatitis C Virus Patients.

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Manuscript Info

Manuscript History:

Received: 15 April 2016
Final Accepted: 22 May 2016
Published Online: June 2016

Key words:
Hepatitis C virus , Asymptomatic,Chronic liver disease ,Acute liver disease ,IL-8 ,TNF-α,ELISA, Cytokines.

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Abstract

The hepatitis C virus (HCV) is amajor cause of liver disease that infect approximately 130 million people worldwide and can lead to chronic liver disease , cirrhosis and hepatocellular carcinoma (HCC), the aim of the study was to find out if hepatitis C infection influenced the levels of interleukine (IL-8) and Tumor necrotic factor (TNF-α) in liver disease patients.Atotal of (1126) patients were involved in this study.The levels of cytokines in serum of HCV positive was measured by commercial ELISA Tests.Results showed positive for anti- HCV Abwere 84(7.46%) out of 1126 cases including22 (26.19%) for clinical isolates , 5 (5.95%) for dialysis patients , 26 (30.95%) for thalassemia patients and 31 (36.90%) for blood donors patients . Our results showed significant differences in serum level of IL-8 ($p<0.05$) and (TNF-α) ($p<0.001$) between investigated groups .Results of cytokines were significantly related with histopathological changes of liver biopsis ,there is scattered inflammatory cell infiltration and marked portal fibrosis for liver tissues.

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Introduction:-

Hepatitis C virus (HCV)is a *Hepacivirus*genusamember of the *Flaviviridae*family, frequentlyconcerned in most hepatic disorders, includingchronic hepatitis that may developinto cirrhosis in approximately10–20% of cases and then to hepatocellular carcinoma(HCC) in 1–5% of cirrhotic (1). HCV is the most common cause of parenterally transmittedhepatitis. The majority of HCV infections are transmitted byblood transfusion and other parenteral ways assharing of needles, occupational exposure to blood andhemodialysis (2). Prenatal transmission and sexual transmission are relatively rare. However, the route of transmission is mysterious in about 50% of individuals with HCV infection (3).

The appearance of HCV-specific T cells can be identified according to some studies of experimentally infected chimpanzees, the following picture of early events in acute HCV infection emerges. After virus entry,hepatocytes are stimulated to produce IFN-α and IFN-β, which induce Kupffer cells to produce macrophage inflammatory protein–1a, that recruits natural killer (NK) cells,so, in turn, secrete IFN-ɤ this interferon then up-regulates chemokines that direct liver-infiltratinglymphocytes into the hepatic parenchyma. These effector cells recognize major histocompatibility complex class I–HCV peptide complexes on the surface of infected hepatocytes and bring about their clearance by apoptosis (4**)**.

Thl cytokines, including interleukin-2 (IL-2) and interferon-gamma (IFN-gamma), promote a cell-mediated immunity (CMI) response whereas Th2 cytokines including IL-4 and IL-10 are involved in antibody mediated immunity. Th1 and Th2 responses have been shown to interact in a HCV infection (5) and the imbalance between Th1 and Th2 responses favors humeral immune responses and down regulates cell mediated immunity, which is important for host defense against viral infections (6). Our study aimed to know role of IL-8 and TNF-α in progress of changes in liver for HCV infected groups.

Materials and methods:-

Patients:-

1126 patients in different ages,werejoining to primary health care centers in Al-Muthana province (Al- Samawa 1 ,Al- Samawa 2 , Al-Rumaitha , Al- Khidhir) with jaundicee or signs and symptoms expressive of acute and chronic viral hepatitis patients.

Blood Samples:-

(5-10) ml was drawn from each clinical patients ,thalassemic patient(latent), blood donors and dialysis patient (asymptomatic).then the blood samples were centrifuged at (4700 RPM) for (5 min.) to obtain blood serum then frozen at (_20 °C) until collected sufficient number for performing ELISA technique to estimate the HCV patients.

Liver biopsies:-

Three liver biopsies were obtained from acute and chronic HCV patient biopsies were fixed with formaline (10%) for histopathological examination.

Serum cytokine:-

Measurements of cytokines inthe serum were performed by ELISA test . Absorbance was measured in duplicateswith a micro plate reader (Beckman Coulter). Thefinal concentration was expressed in pg/ml.

Statistical analysis:-

Statistical analysis wasconducted by using Chi-square (χ^2) test todetermine the statistical differences amongdifferent groups by using a design statisticalpackage for social science (SPSS 19). Theprobability of ($P \leq 0.05$) was considered to bestatistically significant.

Results and discussion:-

The results during ELISA test done for anti-HCV Ab showed that positive test were 84(7.46%) out of 1126 cases including22 (26.19%) for clinical isolates , 5 (5.95%) for dialysis patients , 26 (30.95%) for thalassemia patients and 31 (36.90%) for blood donors patients as shown in table (1) and diagram (1):

Table (1) Positive cases for positive HCV

NO.	Positive cases	Number	Percentage%
1	Clinical signs patients	22	26.19%
2	Dialysis patients	5	5.95%
3	Thalassemic patients	26	30.95%
4	Blood Donors patients	31	36.90%

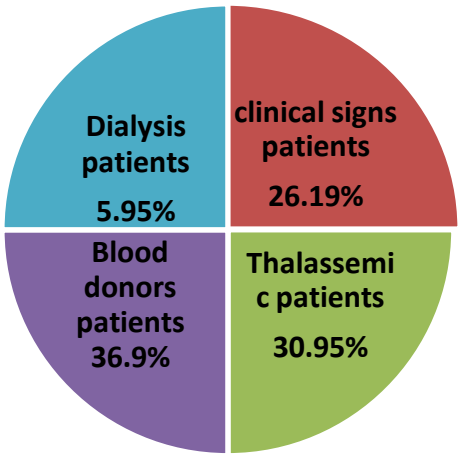


Diagram (1) positive cases for HCV

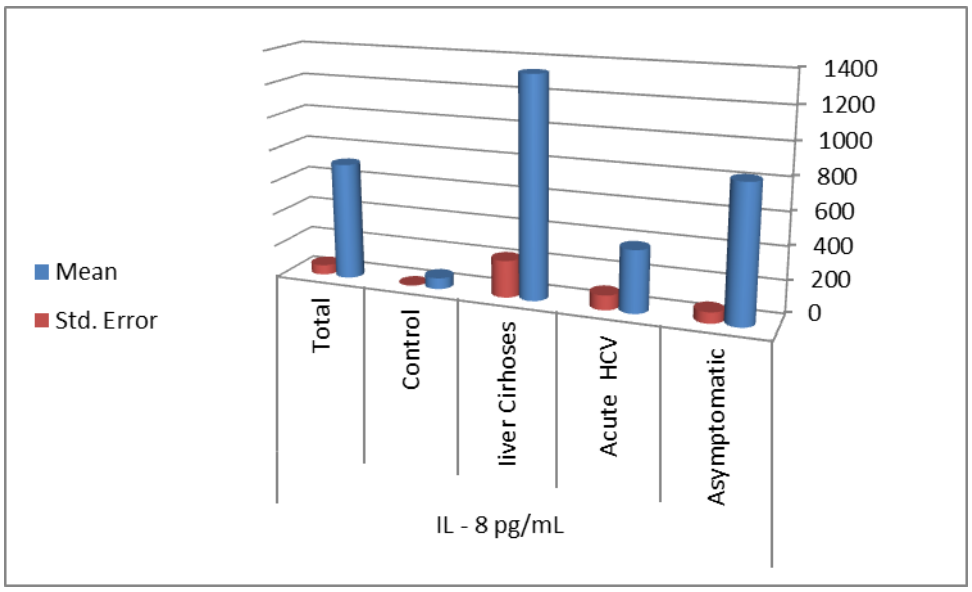
According to F- test the differences between HCV- positive cases is non-significant ($p>0.05$),while there is signficant differences ($p<0.05$) between patients and dialysis patients . This result is agreed with (7) who estimated that 180 million people worldwide (3% of the world population) infected with hepatitis C virus (HCV) and presented with clinical signs. The prevalence of HCV infection among HD patients varies from country to country and from center to another, ranging between 2.9% and 68%, it is higher in developing countries(8).Repeated blood transfusion in thalassemic patients is necessary for their survival; however, such transfusions increase the exposure not only to HCV but also other blood borne viruses(9).

Serum cytokine IL-8:-

The results of cytokineproduction were calculated by using the equationfrom the standard curve in the same assay.
IL-8 concentration was specially increased in patients with chronic liver disease(1334.50±225.50)pg/ml and asymptomatic patients (825.821±66.957) pg/ml respectively than acute HCV disease (379.20±88.959)pg/ml as compared with control groups (67.50±1.490)pg/ml as shown in table (2) and figure (2) .analysis of variance test revealed that there was a high statistically significant differences among HCV and healthy control groups ($p<0.05$).The recent study showed an increase in IL-8 in chronic liver disease patients which same that mentioned by (10) who found thathepatic IL-8 isdetected at low maintenance levels during acute HCV infection,although marked increases in serum and hepatic levelshave been observed in HCV-infected patients with progressiveinflammation and cirrhosis as compared to healthy controls.The most pronounced increase inIL-8 isobserved in patients with a higher degree of neutrophilinfiltration, cirrhosis and impaired liver function.

Table (2): The Concentration of IL-8 in patients and controls.

Group	NO.	Serum level of IL-8		
		Mean ±SE	Minimum	Maximum
asymptomatic	73	825.82±66.95	180.00	2200.00
Acute HCV	9	379.20±88.95	115.00	894.00
Chronic liver disease	2	1334.50±225.50	1109.00	1560.00
control	10	67.50±1.49	60.00	75.00
total	94	713.21±59.65	60.00	2200.00



Fig(2) The mean of IL-8 in HCV infected patients

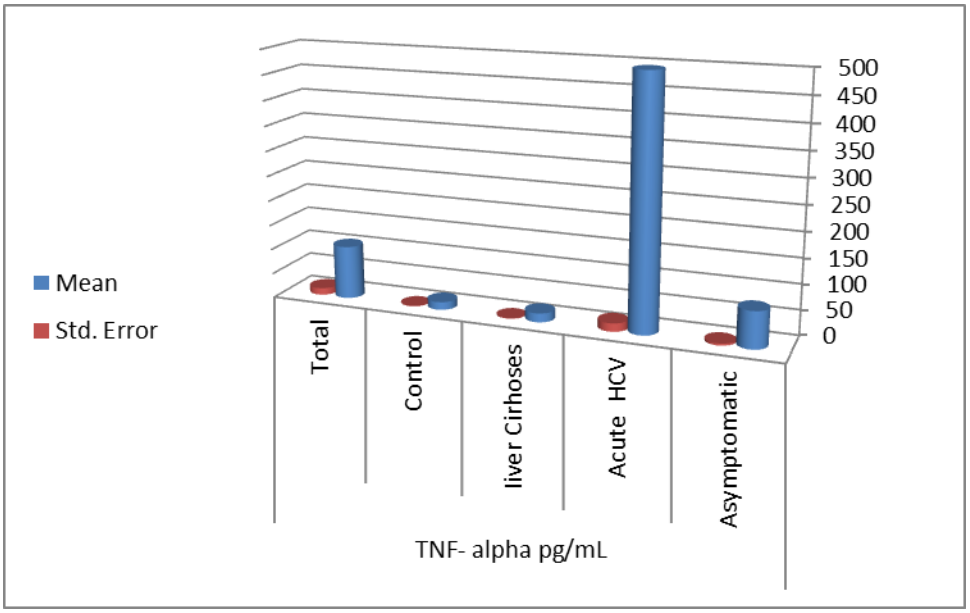
Serum cytokine TNF-α:-

Present study showed that all patients with HCV contain higher level of TNF-α than healthy control group ,TNF-α concentration was increased specially with acute HCV patients (494.66± 16.53)pg/ml ,asymptomatic patients(73.53± 4.00)pg/ml and liver cirrhosis patients (17.81± 0.940)pg/ml respectively as shown in table (3)and

figure (3) .Analysis of variance test revealed that there was high statistically significant differences among acute HCV , asymptomatic, liver cirrhosis , and control groups ($p<0.001$) .

Table (3): The Concentration of TNF-α in patients and controls.

Group	NO.	Serum level of TNF-α		
		Mean ±SE	Minimum	Maximum
asymptomatic	73	73.53±4.00	27.29	220.41
Acute HCV	9	494.66±16.53	403.00	599.00
Liver cirrhosis	2	17.81±0.94	16.87	18.75
Control	10	15.66±1.05	7.08	19,58
Total	94	713.21±59.65	60.00	2200.00



Fig(3).The mean of TNF-α in HCV infected patients

Tumor necrosis factor -alpha (TNF-α) is a cytokine, produced primarily by activated monocytes and lymphocytes, that possesses pleiotropic properties(11,12). It participates in the induction of the immune response to infectious agents and has been shown to exert direct antiviral effects(13,14).Although low levels of TNF-α can contribute to cell protection, excessive amounts may cause cell damage(12,15).Neutrophil chemotaxis may be enhanced further by an increasein vascular permeability mediated by TNF-α, thus contributingto increased levels of IL-8 within the liver. This increase in hepatic and peripheral IL-8 correlatespositively with an increase in TNF-α and advancing fibrosis asindicated by histological activity index and serum levels ofalanine aminotransferase(10).In chronic hepatitis C, inflammation is an inherent part of thedisease process, and cytokines, such as TNF-α, represent key regulatorsof the chronic inflammatory processes involved. Indeed, liver injuryin HCV infection is believed to be caused by host immune responses,not by viral cytopathiceffects. (16). TNF-αhas been involved in the pathogenesis of adiversity of liver conditions including viral hepatitis(17,18) Increased production of TNF-αby peripheral blood mononuclear cells (PBMC) has been observed in fulminant viral hepatitis,(19) in chronic hepatitis B and in chronic non-A non-B hepatitis.(20,18) Recently, raised serum TNF-αlevels have been shown in chronic hepatitis C virus (HCV) infection.(21).It has been recently shown that production of TNF-αin the liver takes place not only in non-parenchymal cells but also in hepatocytes.(22).Although TNF-αmay be generated during theinflammatory reaction that follows the immune recognitionof viral antigens, the mere intracellular presence of viral compounds may also stimulate TNF-αgeneexpression as a built-in defense program of the cell to activate neighboring leukocytes or macrophages(23) or to undergo apoptosis, thus limiting the spread of infection.(24).

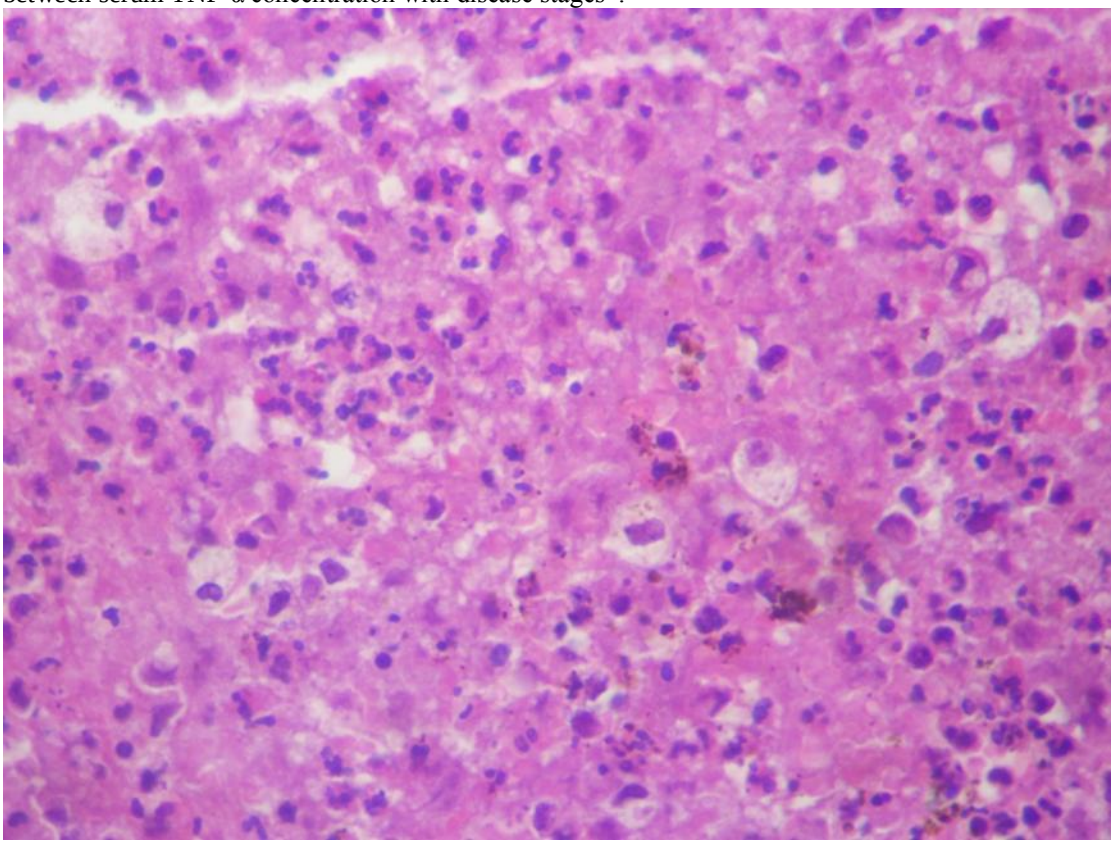
Histopathological examination: From results showed one biopsy was reveals an acute HCV and two biopsies were showed chronic HCV . Histological section of liver obtained from acute HCV patient reveals marked hepatitis .

Acute hepatitis:-
The portal area and the hepatic lobule are shown with infiltration of lymphocytes ,plasmocytes and even neutrophilic ,eosinophilic granulocytes (fig.4).

Chronic hepatitis:-
Section from hepatic tissue show affected lobular architecture by aggregation of lymphocytes in portal area , with small quantity of plasmocytes but rarely neutrophilic granulocytes ,also showed marked portal and peripheral fibrosis with ductularproliferation are identified (fig.5).
Histological changes of liver in HCV positive patient show conformity with clinical signs , cytokines results . Where most of chronic HCV showed liver fibrosis and most of acute HCV patient showed severe lobular acute inflammatory cells infiltration , this results was similar to that obtained by Wan et al ., (2009)(25) .Who mentioned that cytokines are key mediators of inflammation ,apoptosis, necrosis and fibrosis and they are activity involved in the regeneration process of liver tissue after injury . It has been hypothesized that successful treatment of hepatitis C depends on a complex balances between pro and anti- inflammatory responses.

Conclusions:-

The data of this study strengthen the possibility that IL-8 play a role in progress of liver changes then cirrhosis and higher serum TNF-α concentration was found in acute HCV patients sera with a clear cut quantitative correlation between serum TNF-α concentration with disease stages .



Figure(4) : The portal area and the hepatic lobule are infiltration with lymphocytes and neutrophil (H&E 400E)(400X).

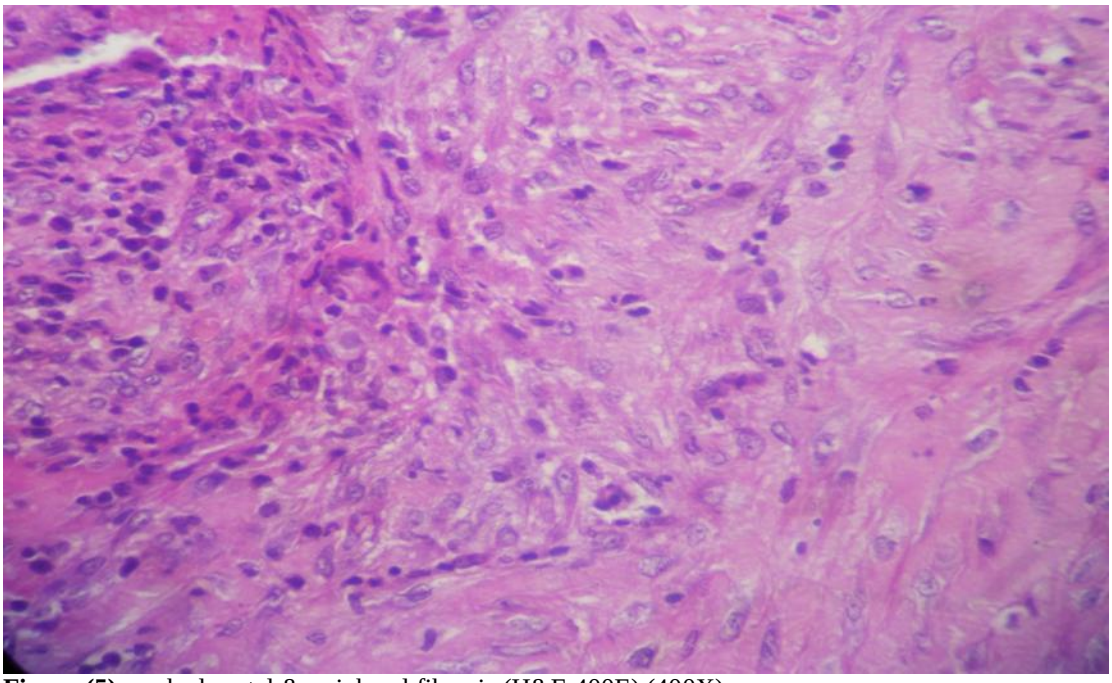


Figure (5):marked portal &peripheral fibrosis (H&E 400E) (400X).

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