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RESEARCH ARTICLE

RELATION BETWEEN (EPITHELIAL NEUTROPHIL-ACTIVATING PEPTIDE 78) OR (CXCL5) SERUM LEVEL AND CHRONIC LIVER DISEASES.

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Manuscript Info	Abstract
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Background and aims:- CXCL5 is a small cytokine belonging to the CXC chemokine family predominantly expressed on epithelial cells . This cytokine stimulates the chemotaxis of neutrophils having angiogenic properties. Small interfering RNAs or antibodies against CXCL5 can suppress tumor growth.CXCL5 is considered a therapeutic target in liver diseases. The aim of our study was to assess CXCL5 serum levels in patients with chronic liver diseases.
Materials and Methods:- CXCL5 serum levels were measured in 60 patients with chronic liver disease and 20 apparently healthy controls.
Results:- Serum CXCL5 levels in patients with liver cirrhosis were lower than in healthy controls, and correlated with the hepatic biosynthetic capacity, Child-Pugh and MELD scores. In patients with typical clinical complications of cirrhosis, CXCL5 levels were found to be decreased. CXCL5 levels were correlated with clinical presentation and laboratory parameters.
Conclusions:- serum CXCL5 levels are lower in patients with chronic liver disease, suggesting that CXCL5 might be involved in the pathogenesis of chronic liver disease. CXCL5 could serve as an additional biomarker for hepatic necroinflammation and fibrosis.

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Introduction:-

Hepatic cirrhosis is an endstage disease and can be induced by several etiologies including viral hepatitis, biliary disease, autoimmune disorders and chronic alcohol abuse.[1]

Approximately 550 million people worldwide are chronically infected with hepatitis B (HBV) or hepatitis C (HCV) virus. The clinical route of HBV and HCV infection is variable ranging from chronic mild hepatitis to liver cirrhosis and hepatocellular carcinoma.[2]

Egypt commands the world in hepatitis C infection cases*.* Schistosomiasis and hepatitis C virus (HCV) co-infection is common in Egypt, Hepatitis B virus is also encountered.[3]

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Although significant progress has been made to understand the molecular mechanism for advancement from acute liver injury to fibrosis and cirrhosis, there is no antifibrotic therapy actually available for patients with chronic liver disease. The mainstay is to treat the underlying liver disease. If this can not be performed and the patient progresses to decompensated liver cirrhosis, liver transplantation will be the only treatment option.[4]

Following an acute liver injury, hepatocytes are damaged and undergo necrotic or apoptotic cell death. This is joined with a massive inflammatory reaction, which is partly mediated by occupant kupffer cells or infiltrating inflammatory cells such as monocytes and neutrophils.[5]

Portal hypertension is the central cause of morbidity and mortality in liver cirrhosis. Complications of portal .[8] hypertension in cirrhotic patients include gastric and esophageal varices, ascites, portal hypertensive gastropathy, hepatorenal syndrome, hepatopulmonary syndrome and portopulmonary hypertension.[6]

Ascites is the most prevalent complication of cirrhosis and is connected with a poor quality of life, increased risks of other complications of cirrhosis such as; spontaneous bacterial peritonitis and hepatorenal syndrome with its high mortality rate the moment it is diagnosed.[7]

In those without underlying liver disease , HCC rarely occurs Chronic hepatitis B (CHB) represents approximately 50% of the implied etiologies for the expansion of hepatocellular carcinoma (HCC) worldwide.[9]

Hepatic stellate cells and other fibrogenic cell types are stimulated by chemokines and cytokines such as TGF-B1 to generate large amounts of extracellular matrix proteins. Several chemokines have been involved in the pathogenesis of liver fibrosis. For example the CCR2 ligand MCP-1 is produced by kupffer cells and HSCs, which enhances hepatic fibrosis by recruitment of macrophages that are associated with HSC activation.[10]

CXCL5 is well known for its function as chemoattractant and activator for neutrophils mainly during acute inflammatory responses , and it shares structural and biological features with IL-8.[11]

CXCL5 is produced by a diversity of cell types including monocytes, neutrophils, epithelial cells, fibroblasts and smooth-muscle cells, and its gene expression is generated by proinflammatory stimuli such as TNF, IL-1B and lps.[12]

Expression of CXCL5 is also increased in atherosclerosis but is not associated with neutrophil infiltration. Instead CXCL5 has a protective role in atherosclerosis by directly commanding macrophage foam cell formation.[13]

CXCL5 belongs to the CXC chemokine family and had been shown to have promitotic effects on hepatocytes. The aim of my study was to assess CXCL5 serum levels in patients with chronic liver disease.

Patients and Methods:-
Ethical consideration: Written informed consents were obtained from all participants, The study was designed as a case controlled prospective study after approval by the ethical committee of Benha Faculty of Medicine.

Selection of subjects:-
A total of 80 individuals were included in this study, who were categorized into the following groups:
Group 1:- sixty patients with chronic liver disease, who were selected from The Internal Medicine Department of Benha University Hospital between November 2014 and June 2015. Inclusion criteria were both males and females chronic liver disease patients aged more than18 years while exclusion criteria were presence of Diabetes Mellitus, heart diseases or renal diseases.
Group 2 : Twenty apparently healthy subjects working as control group with normal ALT, negative HBV, HCV and HIV markers and with no history of liver disease.

Blood Sampling:-

Peripheral blood was collected from each individual fulfilling antiseptic measures. The serum samples were separated by centrifugation at 3000 rpm for 10 min. then sera were stored at - 20°C till analysis. 1.8 ml of blood for each 0.2 ml trisodium citrate were used to separate plasma for PT and PTT measurement. K2EDTA blood samples were used for CBC.

A) Standard parameters of liver and kidney functions as well as complete blood count and hepatitis markers were measured at Benha university hospital lab.
B) Estimation of scores:

Child stage:- The score employs five clinical measures of liver disease. Each measure is scored 1-3, with 3 indicating most severe derangement. [14]

Measure	1 point	2 points	3 points
Total bilirubin, μmol/L (mg/dL)	<34 (<2)	34-50 (2-3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8-3.5	<2.8
Prothrombin time, prolongation (s)	<4.0	4.0-6.0	> 6.0
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I-II	Grade III-IV

Chronic liver disease is classified into Child-Pugh class A to C, employing the added score from above.

Class	A	B	C
Points	5-6	7-9	10-15

MELD score: MELD uses the patient's values for serum bilirubin, serum creatinine, and the international normalized ratio for prothrombin time (INR) to predict survival. It is calculated according to the following formula: $10 \times (0.957 \log(s.creat) + 0.378 \times \log(T.Bil.) + 1.12 \log(INR) + 0.643)$ [15]

Measurement of serum CXCL5 concentrations by ELISA:-
This was done using SunRed kit,provided by Biogene(e Mail:sunredbio@msn.cn).

Test principle:*

The kit uses adouble-antibody sandwich enzyme-linked immunosorbent assay (ELISA). To assay the level of Human Epithelial neutrophil activating peptide 78 in samples, (ENA-78/CXCL5) was added to monoclonal antibody enzyme well which was pre-coated with(ENA-78/CXCL5) monoclonal antibody, incubation; then, (ENA-78/CXCL5) antibodies labeled with biotin, and combined with Streptavidin-HRP were added to form immune complex; then incubation and washing were carried out again to remove the uncombined enzyme. Chromogen Solution A& B then added, the color of the liquid changed into the blue, and at the effect of acid, the color finally became yellow. The chroma of color and the concentration of the Human (ENA-78/CXCL5) of sample were positively correlated.

Clinical complications:-
Typical clinical complications due to portal hypertension, e.g. splenomegaly, ascites, hypertensive gastropathy or esophageal varices, were evaluated by complete abdominal ultrasound and upper gastrointestinal endoscopy.

The medical history of gastrointestinal bleedings (variceal bleeding, hematemesis, melena, hemorrhoidal bleeding, intra abdominal bleeding and other serious bleedings) and the current systemic bleeding tendency unrelated to portal hypertension (epistaxis, prolonged post traumatic bleeding, gingival hemorrhage, spontaneous hematoma and hypermenorrhea / menorrhagia) were assessed by a standardized interview in all patients.

Statistical analysis:-
The clinical data were recorded on a report form. These data were tabulated and analyzed using the computer program SPSS (Statistical package for social science) version 16. The significance of difference was tested using one of the following tests:- **Student t test**: Used to compare mean of variables in two groups of quantitative data. **ANOVA test (F value):**Used to compare mean of more than two groups of quantitative data. Inter - group comparison of categorical data was performed by using **fisher exact test (FET). Correlation coefficient (r test):** used to find relationship between two variables within the same group.

Results:-
CXCL5 serum concentrations are lower in patients with advanced liver disease
We evaluated 60 patients with chronic liver diseases. Serum CXCL5 levels in patients with chronic liver disease were significant lower than in healthy controls($p<0.043$; Fig. 1). CXCL5 plasma levels decreased with the stage of cirrhosis according to the Child-Pugh classification (Fig. 2). The level of decompensation was also assessed by the MELD scoring system, and CXCL5 showed a negative correlation with the MELD score ($r= -0.418$, $p=0.001$; Fig.3). There was a significant correlation between CXCL5 serum levels and the hepatic biosynthetic capacity as assessed by various laboratory parameters such as albumin and prothrombin time (Table 1).
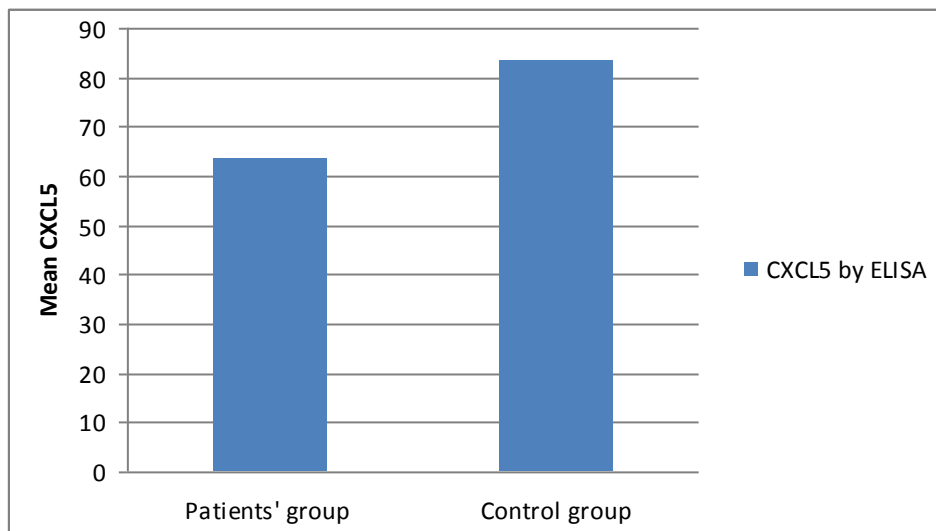

Fig. 1:- Comparison between patients' group and control group as regards serum CXCL5.
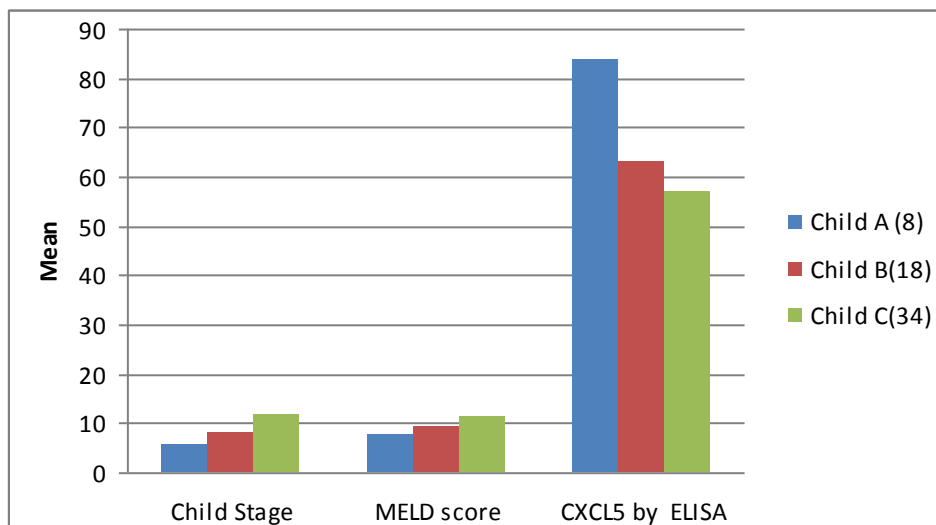

Fig. 2:- Comparison between patients' subgroups as regards Child stage, Meld score and serum CXCL5.
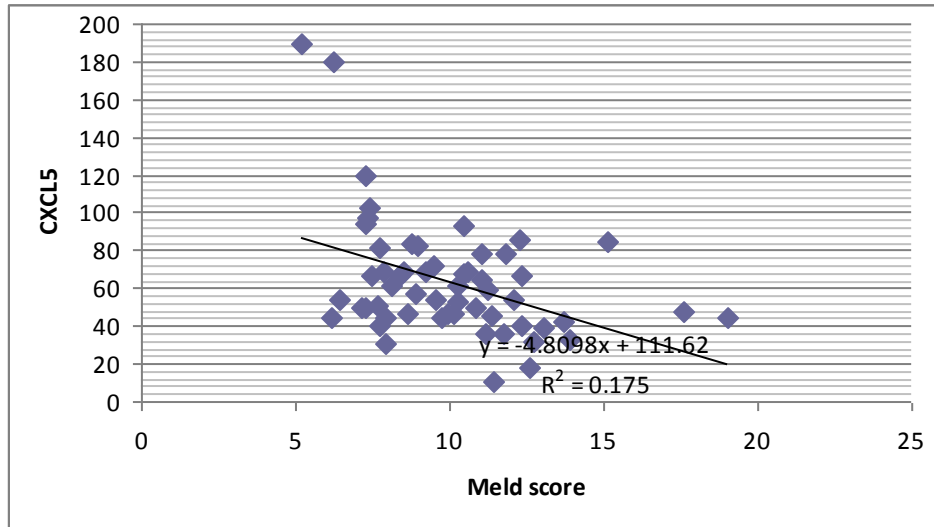
Fig.3:- Correlation between CXCL5 and MELD score among patients' group.

Table 1:- Correlation between CXCL5 and other parameters among patients' group.

CXCL5 ELIZA	r test	P Value
Age	0.094	0.489
S.albumin (g/dl)	0.443	0.001**
S.creatinine (mg/dl)	0.009	0.948
S.T.Bilirubin (mg/dl)	-0.384	0.003**
Platelet count(x1000/mm)	0.357	0.007**
PT (sec)	-0.382	0.004**
aPTT (sec)	-0.319	0.017*
INR	-0.386	0.003**
Child Stage	-0.479	0.001**
Meld score	-0.418	0.001**

CXCL5 is lower in chronic liver disease patients with clinical signs of decompensation
Patients with liver cirrhosis are susceptible to a variety of clinical complications such as splenomegaly, ascites, bleeding tendency, hypertensive gastropathy and esophageal varices. Patients with L.L. oedema & HCC (Fig. 4A), Ascitis & Jaundice (Fig. 4B), showed lower plasma levels of CXCL5. Thus, CXCL5 concentrations correlate well with clinical signs of decompensation.
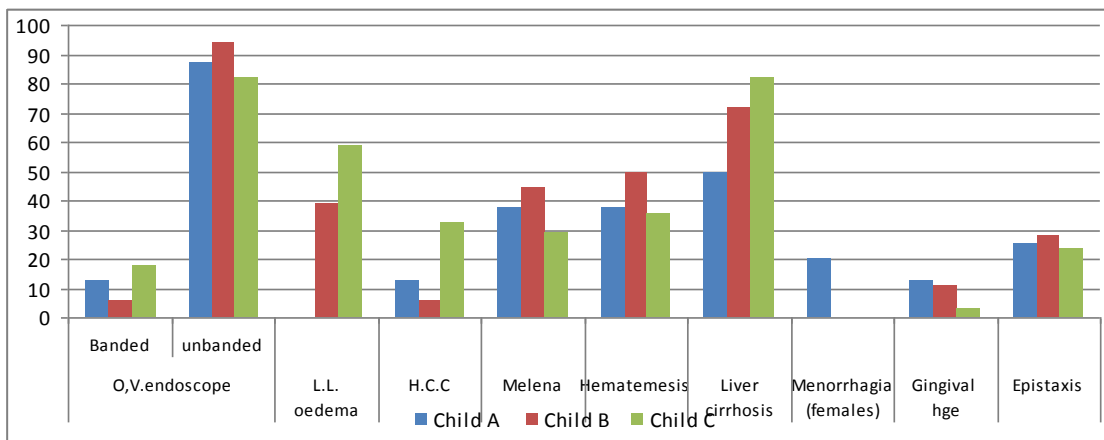

Fig.4A:- Comparison between patients' subgroups as regard clinical complications.
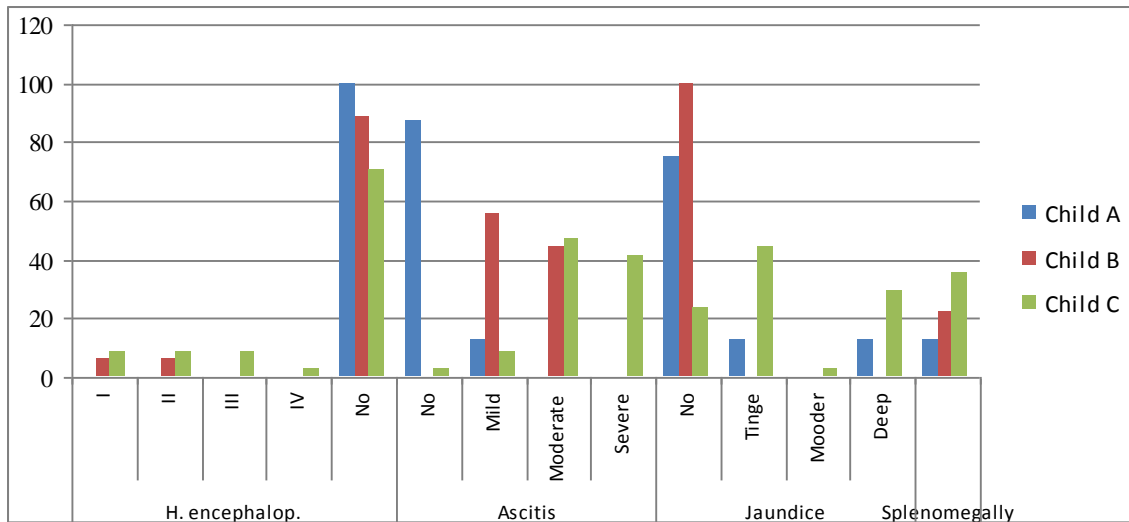
Fig.4B:- Comparison between patients' subgroups as regard clinical complications.

Discussion:-

Cirrhosis is a complication of liver disease which involves loss of liver cells and irreversible scarring of the liver. Hepatitis C, fatty liver, and alcohol abuse are the most common causes of cirrhosis of the liver in the U.S., Liver fibrosis is the scarring process that represents liver response to injury. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension and often requires liver transplantation. Cirrhosis is always developed from fibrosis. Although, fibrosis and cirrhosis are two different distinguished pathological conditions, they are closely related. [16]

The present study was designed to assess CXCL5 serum level in patients with chronic liver disease.

In this study, serum CXCL5 levels were reduced in patients with chronic liver disease compared with the control group.

These results were in agreement with **Frank et al.,**[17] who reported that plasma CXCL5 levels in patients with chronic liver disease were significantly lower than in healthy controls.

CXCL5 (also known as Epithelial neutrophil-activating peptide 78 or ENA-78) belongs to the CXC chemokine family and has been shown to have promitotic effects on hepatocytes.[18]

Patients with hepatic necroinflammation and fibrosis on liver histology showed lower serum level of CXCL5. In cirrhosis, CXCL5 levels were found to be decreased while Intrahepatically, CXCL5 expression was increased in patients with advanced fibrosis and cirrhosis.[17]

The isolation of different cellular compartments from mouse livers suggested that hepatic stellate cells and sinusoidal endothelial cells are the main sources of hepatic CXCL5.[19]

Other cell types than hepatic stellate cells and endothelial cells must express and secrete higher amounts of CXCL5, as systemic plasma levels are lower in patients with chronic liver disease. Neutrophils and monocytes secrete CXCL5 and could be the extrahepatic source for systemic CXCL5.(20)
As the infiltration of blood monocytes and neutrophils into the liver participates in experimental hepatic fibrosis, the reduced circulating CXCL5 levels might be related to an altered distribution or activation of these innate immune cell subsets in patients with advanced liver disease. [5]

Plasma CXCL5 levels are lower in patients with chronic liver disease, suggesting that CXCL5 might be involved in the pathogenesis of chronic liver disease. CXCL5 could serve as an additional biomarker for hepatic necroinflammation and fibrosis.[17]

In the present study, reduction in CXCL5 serum levels in chronic liver disease patients was associated with alteration in the hepatic synthetic capacity.

There was a high statistically significant decrease of serum albumin in patients' group when compaired with control group and in different stages of cirrhosis than in control group while there was statistically highly significant increase of (PT and aPTT) in patients' group than in control group and in different stages of cirrhosis than in control group.

Correlation studies revealed that, CXCL5 serum concentration seems to correlate with other parameters among patients' group. There was significant positive correlation between CXCL5 and serum albumin and there was significant negative correlation with (PT and aPTT) among patients' group.

These results go in hand with **Essam et al.,**[21] who reported that serum albumin level in patients with chronic liver disease were significant lower than in healthy controls. And with **Mohammed et al.,**[22] who reported that PT, aPTT, INR were significally increased in all patients with liver diseases and platelet count showed significant decrease among cases.

Decrease levels of serum albumin in chronic liver diseases was explained by decreased synthesis by the hepatocytes in addition to water and sodium retention that dilutes the content of albumin in the extracellular space. Other factors likely contribute to the development of hypoalbuminemia, includes an increased transcapillary transport rate.[23]

Patients with decompensated liver cirrhosis have significantly impaired synthetic function. Many proteins involved in the coagulation process are synthesized in the liver.[24]

Liver production of coagulation factors 1, 2, 5, 7, 9 and 10 which is reduced in chronic liver disease. Factors 2, 7, 9 and 10 are further reduced by vitamin K deficiency due to cholestasis.PT can be selectively elevated because factor 7 is the first factor to be depleted in cirrhosis due to its short half-life.[25&26]

Result of the present study showed a high statistically significant decrease of platelet count in patients groups when compaired with the control group with gradual decrease in different stages of cirrhosis and positive correlation with CXCL5.

liver production of plasma CXCL5 is mainly platelet-derived, suggesting the role of platelets in immune responses and inflammation. Measurement of CXCL5 in human blood allowed testable inferences concerning physiology and pathophysiology in quantitative platelet disorders. CXCL5 was reported to be secreted by peripheral blood monocytes and involved in neutrophil activation. Interestingly, CXCL5 also share similar functions in platelet activation and coagulation. As platelet alpha-granules contain a variety of growth factors, cytokines, and chemokines, in addition to hemostatic proteins, platelets might be a source of CXCL5.[27]

Result of present study showed a highly significant increase of serum total bilirubin in patients' group than in control group with gradual increase in different stages of cirrhosis showing negative correlation with CXCL5.

In this study Patients without liver cirrhosis had significantly lower levels of CXCL5 than controls, but higher levels than patients with Child A-C cirrhosis . CXCL5 serum concentrations correlates with Child- Pugh's stage of liver cirrhosis. And also with the disease severity as assessed by the MELD score.

As regard signs of decompensated liver cirrhosis there was statistically significant increase in the percentages of L.L oedema, HCC, ascites and jaundice in patients' group as regards different stages of cirrhosis and patients with liver cirrhosis who had manifestation of hepatic decompensation showed lower plasma levels of CXCL5.

In consistent with these findings **Frank et al.,**[17] revealed that CXCL5 concentrations correlate well with clinical signs of hepatic decompensation.

Edema in patients with cirrhosis has a multifactorial pathogenesis. The most obvious pathogenic mechanism is portal hypertension causing increased hydrostatic pressure within the mesenteric veins and causing fluid to seep out into the mesentery. In addition, liver dysfunction produces a state of water overload due to hypoalbuminemia and

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decreased aldosterone catabolism. This also plays a role in producing mesenteric edema, ascites, pleural effusions, and subcutaneous edema.[28]

Statistically significant relation between the mean serum CXCL5 level and HCV as cause of cirrhosis was reported in the results of the present work .

Studies conducted at National Liver Institute (NLI),[29 &30] reported that hepatitis C was the most frequent cause of chronic liver disease in Egypt. Hepatitis C antibodies were more common in patients with active schistosomiasis and patients without hepatitis B surface antigenaemia. These studies found that 60% of patients of chronic liver disease were anti-HCV positive and 40% had HCV viremia, as compared with only 6% having HBsAg and 8% having S. mansoni ova, so the predominant cause of chronic liver disease in NLI outpatients was infection with HCV.

 In conclusion, plasma CXCL5 levels are lower in patients with chronic liver disease. Decreased CXCL5 concentrations appears to be a good marker for cirrhosis and its staging, clinical signs of decompensation and synthetic capacity of the liver. further studies are required to determine if CXCL5 supplementation could be used as therapeutic intervention in chronic liver disease and fibrosis.

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