

Some hematological and biochemical changes on patients with chronic liver diseases and cirrhosis among random samples of Iraqi people.

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Abstract:

Chronic liver diseases tend to be progressively destroyed in liver tissue. Infections are symptomatic or have only mild nonspecific symptoms as long as cirrhosis is not present. The most frequent complaint of those patients are fatigue, less common manifestations are nausea, weakness, myalgia, arthralgia and weight loss.

Materials and Methods :This study included ninety one individuals, their ages ranged from 12 to 73 years (45 male and 46 female) attending the Gastroenterology and Hepatology Center and Baghdad Teaching Hospital during a period starting 2010 to April 2011 to investigate the presence of HBV, HCV and cirrhosis antibodies in serum samples included in this study. Biochemical analyses also were carried out which included estimation of level of total serum bilirubin, aspartate aminotransferase AST (GPT) and alanine amino transferase ALT (GOT). Hematological tests were also carried out which included prothrombin time (PT) and Partial thrombin (PTT). Specimens of liver biopsy were taken from patients with cirrhosis of the liver who have been subjected to surgery collected from patients with liver cirrhosis for pathological studies.

Results :The results suggest that infection with chronic liver diseases and cirrhosis increased with age. The infection was also more frequent among female than male compared with healthy individuals. Elevation in values of GPT, GOT, total serum bilirubin, PT and PTT were observed among the infected individuals. The main pathological changes in liver fibrosis biopsy was regenerated nodules and presence of inflammatory cells, bile ducts, vessels within the fibrous septa and dense inflammatory cells infiltrate in fibrotic bands of infected liver.

Key words: cirrhosis ,prothrombin ,bilirubin ,fibrosis, septa

بعض التغيرات الدموية والبايوكيميائية على المصابين بأمراض الكبد المزمنة وتشمع الكبد في عينة عشوائية من الافراد العراقيين

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المستخلص :

أن أمراض الكبد المزمنة هي مجموعة من الامراض التي تؤدي إلى تدمير تدريجي لأنسجة الكبد. وتكون الإصابة اما بدون أعراض أو بأعراض غير محددة طالما لم تصل الإصابة الى مرحلة تليف الكبد ، حيث يلاحظ ان الاعراض الأكثر شيوعاً له ذا المرض هو التعب ، اما الاعراض الأقل شيوعاً فتشمل الغثيان، الضعف، والألم العضلي والألم المفصلي إضافة الى فقدان الوزن.

وقد شملت عينة الدراسة على واحداً وتسعون فرداً تراوحت اعمارهم بين 73-12 سنة 45 ذكراً و 46 انثى للمدة ما بين كانون الاول 2010 ولغاية نيسان 2011 للتحري عن وجود الاجسام المضادة لفايروس التهاب الكبد الفايروسي نوع ب وج إضافة الى الاجسام المضادة لمرضى تشمع الكبد مقارنة بمجاميع السيطرة (0)

تم عمل تحاليل بايوكيميائية لكافة عينات الدراسة والتي تضمنت تقدير مستوى كل من البيلروبين الكلي والاسبرتيت امينوترانسفيريز والالانين امينوترانسفيريز و بعض تحاليل الدم والتي شملت كل من الوقت اللازم لتخثر البروثرومبين والوقت اللازم لتخثر الثرومبين الجزئي وتم ايضاً اخذ عينات من مرضى تليف الكبد والذين يتم متابعتهم بعد اخضاعهم لعمليات جراحية.

وقد تم التوصل من خلال هذه الدراسة الى ان الإصابة بكل من التهاب الكبد الفايروسي نوع ب وج إضافة الى مرض تشمع الكبد تزداد بازدياد عمر الفرد كما لوحظ ازدياد نسب الإصابة بين الاناث دون الذكور مقارنة بالاشخاص الاصحاء ، وتم ايضاً تسجيل زيادة ملحوظة لمستوى البيلروبين الكلي وانزيمي الاسبرتيت امينوترانسفيريز والالانين امينوترانسفيريز إضافة الى الوقت اللازم لتخثر البروثرومبين والوقت اللازم لتخثر الجزئي للثرومبين في مصول الاشخاص . كما بينت الدراسات النسجية لمقاطع الكبد المتليفة تكوين عقيدات ووجود خلايا التهابية في القناه الصفراوية والاووعية الدموية ضمن الحواجز الكبدية وتكتف الخلايا الالتهابية المترشحة في الحزم المتليفة لخلايا الكبد .

Introduction:

Chronic liver diseases are tend to be progressively destroyed in liver tissue. Infections are symptomatic or have only mild nonspecific symptoms as long as cirrhosis is not present (1).

It can cause serious destructive diseases of the liver and it contributes greatly to the worldwide burden of the disease which may generally develop over many years during which individual

patients will pass through a number of disease states(2). It is the main cause of morbidity and mortality in the world .The pathological features of chronic hepatitis are common to both HBV and HCV infection which include piecemeal necrosis, portal inflammation, periportal fibrosis lobular inflammation, necrosis and regeneration (3)

The most frequent complaint of patients is fatigue, less common manifestations are nausea, weakness, myalgia, arthralgia and weight loss(4).Morphologically, the spectrum of chronic viral hepatitis (B and C) ranges from mild portal inflammation with little or no evidence of liver cells necrosis on to wide spread inflammation, necrotizing and fibrosing condition(2,4)

Cirrhosis of the liver refers to scarring of the liver which results in abnormal of liver function as a consequence of chronic liver injury(5). In cirrhosis, liver cells die and are progressively replaced with fibrotic tissue, this leading to nodule formation(6).Symptoms of cirrhosis vary, depending on severity of the disease, liver inflammation can be totally symptomless. Mild cirrhosis may not exhibit any symptoms at all and progression of inflammation to cirrhosis can occur without the knowledge of the patient (7).

Materials and methods:

The present study extended from December 2010 to April 2011. A total of ninety one serum samples and liver biopsy were collected from individuals attending Center of Gastroenterology and Hematology at Baghdad Teaching Hospital . Their ages ranged from 12-73 years. Information sheets were filled out for each individual included age, sex, occupation and medical history.

Approximately 5 ml of venous blood was obtained from individuals by anticubital vein .Clotting time measured at 37C° with the presence of tissuarthromboplastin, PT (in sec.) were measured then converted into PT (%). The remaining samples were transferred immediately into plain plastic tubes and were placed in a 37 ° C water bath for 10 minutes and centrifuged at 3000 rpm for minutes to ensure complete separation of serum.

The enzyme linked immunosorbent assay test based on sandwich principle used according to(8)supplied by Randox(UK) which included monoclonal antibodies specific for HBsAg,HCvAg and

cirrhosis Ag have been bound to the surface of each microplate well, the positive control, negative control and samples are added to the microplate wells. The microtiter plate wells were thoroughly washed removing unbound other components of the sample. A standardized preparation of horseradish peroxidase (HRP) conjugated antibody specific for HBsAg, HcV Ag and cirrhosis was added to each well to "sandwich" the antibodies immobilized during the first incubation following a wash to remove any unbound HRP conjugate, a TMB (3,3', 5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a 10-minute incubation period. The enzyme-substrate reaction is terminated by the addition of sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm. Only those wells containing HBsAg, HcV Ag and cirrhosis Ag and HRP conjugate will exhibit a change in color. Total serum bilirubin also measured colorimetrically according to (9) supplied by Biolab. (France).

Liver enzymes activity which included AST(GPT) and ALT(GOT) were measured according to the method of (10) using kit supplied by Randox(UK)

Liver biopsy were collected from fibrotic patients and the specimens then fixed in 10% buffered neutral formalin and processed then paraffin then stained with Haematoxylin and Eosin stain(11) and examined under light microscope.

Statistical analysis of data

The data was analyzed by percentage and unpaired t-test expressed as mean \pm standard deviation (SE) and P value was considered as statistically significant in < 0.01 and 0.001 according to the method described by (12)

Results

The results are illustrated in table 1, 2, 3 and figure 1.

Table (1) Age of patients with chronic liver diseases and cirrhosis compared with healthy individuals. (N=91, Mean \pm SE)

Groups	Age
Healthy people	31.12±1.86
Patients	48.36± 1.94

Significant at P<0.01

Table (1) shows significant increases in the age of patients with chronic liver diseases and cirrhosis(48.36± 1.94) than healthy individuals (31.12±1.86) at P<0.01.

Table (2) Distribution of chronic liver diseases and cirrhosis by gender

Type of disease	Sex	
	Male	Female
Cirrhosis n=(18)	7 (38%)	11(61%)
CLD & Cirrhosis N=(7)	2(28.6%)	5(71.4%)
CLD n=(41)	20(48.7%)	21(51.3%)
Negative control N=(25)	12 (48%)	13(56%)
Total n= 91	N=41(45%)	N=50(55%)

Table (2)Shows the distribution of chronic liver diseases and cirrhosis among males and females individuals It was clear that the infection was higher in females than male patients infected with cirrhosis . The prevalences were (38%),(61%) respectively. High infection was noticed in female than male infected individuals with chronic liver diseases and the prevalence's were (51.3%), (48.7%) respectively. The infection was also higher among female than male with both chronic liver diseases and cirrhosis(71.4%, 28.6% respectively) when compared with other healthy individuals.

Table (3): Biochemical and hematological analysis of all groups studied. (N=91, Mean±SE)

Group	TSB(μmol/L)	AST(UL)	ALT(UL)	PT(sec)	PTT(sec)
Healthy people	0.86±0.08	13.80±0.74	12.36±0.74	11.88±0.20	30.46±0.53
Patients	16.52±1.49	16.52±1.49	34.08±2.40	15.98±0.49	41.26±1.25
t-value	10.50644	8.733564	8.630173	7.68584	7.930877

Significant at P <0.001

TSB: total serum bilirubin, GPT: aspartate aminotransferase, GOT: alanine amino transfere

PT: prothrombin time, PTT: partial thrombin

Table (3) shows elevation in values of Total serum bilirubin(TSB,16.52±1.49)As compared with healthy people.86±0.08 Levels of AST(GPT,16.52±1.49) ,ALT(GOT,34.08±2.40) was also in high level compared with other healthy group(13.80±0.74, 12.36±0.74 respectively),

PT(15.98±0.49) and PTT(41.26±1.25) was also elevated in values in patients compared with their levels in healthy group(11.88±0.20, 30.46±0.53 respectively) ,the mean differences of all these results were significant at P < 0.001

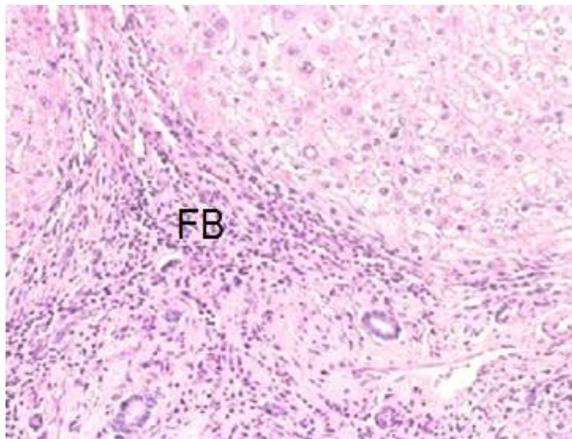
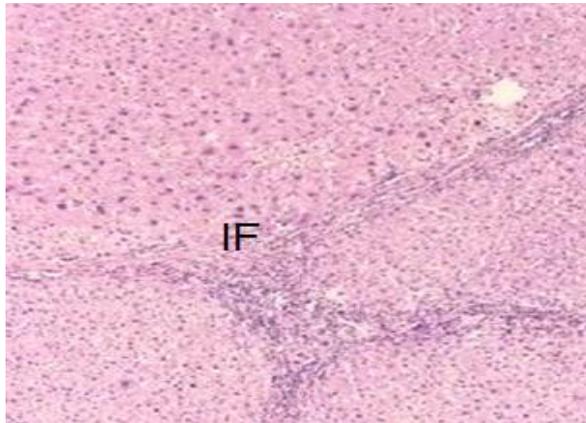


Figure (1):Cross section in liver showed fibrosis of periportal area of liver and nodules formation with dense inflammatory cells infiltrate in fibrotic bands

FB: fibrotic band

Histopathology, liver tissues revealed pathological changes represented by regenerated nodules and fibrosis of periportal area of liver connecting with each other resulting nodules formation (figurer1)



Figure(2)Cross section in liver showed dense inflammatory infiltrate in the fibrous septa segregated liver parenchyma IF: inflammation of fibrous septa

Discussion

Infections of HBV, HCV may cause chronic liver cells damage associated with host responses of inflammation and liver regeneration that continues for many years, this pathological process especially leading to cirrhosis (13).

The present study showed increases in chronic liver diseases and cirrhosis especially with older age because of lack in immunity system of older patients infected with such diseases (6).

The other study by ⁽¹⁴⁾ also documented that cirrhosis appears to accelerate after age 45years, which agreed with our study. They suggested that any changes in the immune response to the hepatitis B and C infection may cause increased fibrosis after age 45 years.

More frequent of chronic liver diseases and cirrhosis were noticed two word, among females than males individuals. This may be due to stimulation of cells divisions by both estrogen and androgen in females and elevated levels of these hormones may lead to abnormal

cell growth such as women's' uterine fibroids, liver diseases, ovarian cysts, endometriosis, breast cysts and breast cancer(15). Since the liver is the principal organ which removes these hormones, its failure to remove them efficiently often leads to their accumulation in the body and is a major cause of the above diseases in women, about 15% leading causes of death and about 10 % in men only(15).

Elevation in values of AST, ALT, total serum bilirubin ,PT and PTT were also recoded among patients with chronic liver diseases. The results were in agreement with (16) who documented that sustained increase \ concentrations of aminotransferases and aspartate aminotransferase together with the presence of HBs Ag and HCV Ag is regarded as indicative of chronic hepatitis and liver function tests.Elevation in the level of total bilirubin in the patients is due to problems in the gall bladder itself which can seriously affect the liver(16).

A complicated set of symptoms typical of liver problems can also be a result during hepatitis, which is the inflammation in the liver brought on by infection or due to the ingestion of some types of poisonous substances and metabolic substances(17).

Stefanetal (13) mentioned that cirrhosis is an indolent disease and most patients remain a symptomatic until the occurrence of decompensating characterized ascites, spontaneousperitonitis, hepatoencephalopathy, or visceral bleeding portal hypertension because liver inflammation can be totally symptomless. Progression of inflammation to cirrhosis can occur without the knowledge of the patient. Therefore, most of the carriers are contagious but some are not.

The present study also showed abnormal prothrombinic(PT) activity and partial prothrombin(PTT). Impaired Blood clotting may be due to poor protein production in the liver of patients compared with healthy peoples.Disturbance in prothrombinic activity also has been observed by (18).In clinical study of liver diseases (hepatitis and cirrhosis) they also recorded normal prothrombinic activity with some patients and referred that to a normal of plasmas leveling in blood samples of some patients with liver diseases(19).

In cirrhosis, liver cells die and are progressively replaced with fibrotic tissue leading to nodule formation although numerous pathophysiologic mechanisms of injury exist, the final common

pathway is persistent wound healing, resulting in hepatic parenchymal fibrosis(17).

Jorge and Herrera (20) mentioned that liver biopsy plays no role in determining the severity of liver cirrhosis. Factors that are taken into account to determine the severity of cirrhosis include PT and PTT and the level of serum bilirubin which, when it accumulates , causes jaundice.

Recommendation

Additional laboratory tests like prothrombin time, partial thrombin test and liver function tests should be performed to the patients if any liver abnormality suspected.

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