Estimation of Ceraluplasmin (CER) and Alpha- 1 Antitrypsin (AAT) levels among hepatitis patients in acute and chronic state

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الخلاصة

يعتبر فيروس التهاب الكبد الفيروسي هو المسبب الاساسي والاعتيادي للإصابة الفيروسية للكبد ، وينتشر هذا المسبب بكثرة في الاصابة البشرية ، له اكثر من خمسة انواع معروفة ومنها A،B،C،D، 0E يختلف مستوى كل من السيرولوبلازمين والفا – 1- انتي تربسين في حالة الاصابة الحادة او المزمنة لهذا المرض ، وقد يكون مستواهما ضمن الحد الطبيعي او المرتفع جزئيا في حالة التهاب الكبد الفيروسي الحاد ، بينما ينخفض المستوى في حالة الاصابة المزمنة بالمرض .

الهدف من هذه الدراسة هو ايجاد العلاقة بين الترابطية بين هذه المتغيرات البايوكيميائية والانواع المختلفة لهذا المرض .

اشتملت الدراسة على 36 شخص مريض بالتهاب الكبد الفيروسي نوع B و30 شخصا مريض بالتهاب الكبد الفيروسي نوع (C) وبأعمار تتراوح بين 22-55 سنه وتم استثناء جميع الاشخاص المصابين بتشمع الكبد من هذه الدراسة في حين كان عدد الاشخاص الاصحاء 24 شخصا اللذين تم اعتمادهم كمجموعة سيطرة .

استخدمت طريقة Single R adial lmmunodiffusion assay لتحديد مستوى كل من المتغيرين اعلاه في مصل دم الاحصاء والمصابين واجريت الدراسة في مختبر الصحة العامة في بابل للفترة من حزيران لغاية ايلول 2007.

اتضح من الدراسة انه لا توجد زيادة معنوية في مستوى الفا -1- انتي تربسين والسيرولوبلازمين لمرضى المصابين بالتهاب الكبد الفيروسي الحاد نوع B،C في حين كانت هناك زيادة معنوية واضحة في المرض من نوع المزمن للمتغير الفا وانخفاض واضح في مستوى السيرولوبلازمين لنفس النوع من المرض مقارنة مع مجموع الاصحاء .

Abstract

Hepatitis Viruses are a common cause of viral hepatitis, it has a wide distribution among peoples, it has more than five common types: Hepatitis A, B, C, D, E. The level of Ceraluplasmin and alpha- 1 antitrypsin differences in acute and chronic hepatitis, it may be normal or mild increased in acute hepatitis while is decreased in chronic hepatitis patients. And the aim of this study to find out the relationship between the frequency of hepatitis and the concentration of immunochemical protein (such as Ceraluplasmin and Alpha -1 antitrypsin) present in patients during the acute and chronic periods of hepatitis infections.

The total number of 36 patients with hepatitis (B) and 30 patients with hepatitis (C) viral infection with an age range (22- 55 years) were enrolled in this study. Patients who had liver cirrhosis was excluded from this study. With a total number of 24 age matched apparently healthy control group was taken to be compared with case studies. Assessment of serum levels of Ceraluplasmin and α-1 Antitrypsin were done by the Single Radial Immunodiffusion Assay to both case study and control group in Babylon public health laboratory from June to September 2007.

The our result shows no significant (N.S) increased in level of up-1 Antitrypsin among acute patient in both types (B and C) While there is a highly significant (H.S.) result for chronic patients also in both types (B and C). Ceraluplasmin level show (N.S.) decreased among acute patient while there decreases (H.S.) In both groups of

patients (B and C) after compared with a control sample. This result with agreement with many universal results.
Keywords: Hepatitis B and C; Ceraluplasmin , Alpha -1 antitrypsin, Single Radial Immunodiffusion Assay.

Introduction

Hepatitis B virus infection described as the second carcinogen factor after smoking by the World Health Organization (WHO) is a global public health problem (1).It was reported that 15 – 40 % of HBV infected patients would develop cirrhosis, liver failure, or hepatocellular carcinoma and 500 000 to 1.2 million people die of HBV infection annually (2).

Because of the high HBV –related morbidity and mortality, the global disease burden of HB is substantial. There are three main strategies have been approved to be effective in preventing HBV infection. They are behaviour modification, passive immunoprophylaxis and active immunization. The implementation of the mass HBV immunization program is recommended by the WHO since 1991 (3).

Hepatitis C, a viral disease, is the most common blood-borne infection in the United States, affecting more than 4 million Americans. Approximately 36,000 cases of acute hepatitis C infection occur each year in the United States and 85 percent of those with acute hepatitis C develop a chronic infection(4,5). Chronic hepatitis C is often asymptomatic but may cause progressive liver injury. Chronic hepatitis has significant morbidity and mortality as it can lead to cirrhosis of the liver as well as hepatocellular carcinoma (HCC). Approximately 15 -25 percent of patients with chronic hepatitis develop cirrhosis(6,7) .The time frame between infection and the development of cirrhosis is affected by several factors, including the use of alcoholic (8), and viral co- infection with HIV or hepatitis B (9,10),male sex, and older age at infection(11). The prognosis of those with HCV related cirrhosis often depends on the development of two complications, hepatic decompensation and HCC.

Serum Ceraluplasmin may be a suitable measure of hepatic dysfunction in the absence of cirrhosis or other protein mal-synthesis. Allow serum Ceraluplasmin level may be a prognostic indicator of a lack of response to therapy with interferon and ribavirin (12).

Material and methods

Patient selection:-

• The total number of 36 patients with hepatitis (B) and 30 patients with hepatitis (C) viral infection with an age range (22- 55 years) were enrolled in this study. Patients who had liver cirrhosis was excluded from this study.

• The total number of 24 age matched apparently healthy control group was taken to be compared with case studies.

• Assessment of serum levels of Ceraluplasmin and α-1 Antitrypsin were done by the Single Radial Immunodiffusion Assay to both case study and control group in Babylon public health laboratory from June to September 2007.

Screening of hepatitis:-

• Serum sample was taken for detection of (HBs –Ag and Anti- HCV antibody) for confirmation of hepatitis infection in the case study and for exclusion of hepatitis infection in the control group by ELISA technique according to the principle and procedure of BIOMERIEUX Hepanostika, Micro Elisa system (13).

Single Radial Immunodifusion (SRID):-

Principle:-

Equal volumes of reference sera and sample are added to the wells in agarose gel containing monospecific antiserum, the sample diffuse radially through the gel and the antigen form precipitin ring with the monospecific antiserum. The result can be calculated easily from the table of diameters provided with plates (14).

Procedure of SRID:-

1. All Endo plates, reference and test sera were brought to room temperature .
2. 5 micro litre of serum and control were dispensed into the wells of plate by using a suitable pipette (sample should be mixed gently before use).
3. Then wet cotton was put in the plate centre to avoid affairs dehydration.
4. The plate was tightly closed and incubated at room temperature (23 $^{+/-}$ 2 ^{0}C) on a level surface for equal or more than 48 hours.
5. After incubation, the diameter of each ring was measured with 0.1 mm precision using a suitable viewer.
6. The results were directly evaluated by using the results of the control samples and a reference table that is provided with RID plates (14).

Results and discussion

The most important clinical application of the Ceraluplasmin (CER) test is to in the diagnosis of Wilson's disease, where typically, concentrations of Ceraluplasmin are reduced and concentration of dialyzable copper is increased . Unless treated with copper chelators ,the disease is always progressive and fatal.

Alpha1-antitrypsine (AAT) is responsible for 90% of serum antitrypsin activity, although it is relatively inactive towered trypsin. Its main function is to neutralize lysosomal elastase released upon phagocytosis of particles by polymorphonuclear leukocytes, a relatively small molecules, can pass from capillaries into tissue fluid, bind protease, and pass back into the intravascular fluid.

In this study Ceraluplasmin and alpha1-antitrypsine were measured in both cases, chronic and acute hepatitis B & C. The results indicate that these variables can be used as indicator for these two types of hepatitis. Among all patients under study, standard error, standard deviation and significance values were calculated using T-test statistical method.

Table (1) The level of Alpha – 1 antitrypsin among hepatitis (B and C) patients

D.S of AAT	HBV		HCV		Control
	Acute	Chronic	Acute	Chronic	
Mean +/- S.E.	145.7 13.4	87.37 8.66	147.59 15.24	102.6 12.24	219.5 9.00
N.	36	36	30	30	24
Sig.	Ac. and Con.	Ch. and Con.	Ac. and Con.	Ch. and Con.	
	H.S.(0.000)	H.S.(0.000)	H.S.(0.000)	H.S.(0.000)	
	Ac. And Ch. B		Ac. And Ch. C		
	H.S.(0.000)		H.S.(0.008)		

This Table show H.S. Decrease in alpha – 1 antitrypsin level among acute and chronic HBV patients compared with control , H.S. Decrease show between acute and chronic HCV patients compared with control. There is no significant difference between Acute (B and C) and chronic (Band C) patients, but there are H.S. Decrease in chronic stage compared with acute stage for both types (B and C).

There is a significant value was appeared in case of AAT patients with chronic hepatitis type B with respect to that normal human control subjects (table- 1). In all types of hepatitis under study the level of alph1 - antitrypsin was reduced in patients than that in normal healthy control. At this tables T-test indicates that there is a significant reduction in α1-antitrypsin in all patient types compared with than in control subjects. But there is no difference in its level between type B and C. Low level of AAT is found in neonatal respiratory distress syndrome, in severe protein losing disorders, and in congenital deficiency (15).

This fact, on line with our finding since hepatitis effect liver cell, the centre of protein synthesis.This effect the level of AAT, and the level depend on liver cell damage.When the M protein of AAT is deficient, the uninhibited enzyme attacks the elastin of the alveolar wall, whose springy recoil after distention with inhaled air normally drives out exhaled air.The liver is also affected most seriously in the ZZ phenotype, where the Z protein accumulates in hepatic cells. Whether Sz ,Ms ,and SS phenotypes are also at risk is not yet clear (16).

Serum Ceraluplasmin (CER) may be a suitable measure of hepatic synthetic dysfunction in the absence of cirrhosis or other protein mal- synthesis.

Table (2) the level of Ceraluplasmin among hepatitis (B and C) patients.

D.S of CER	HBV		HCV		Control
	Acute	Chronic	Acute	Chronic	
Mean +/- S.E.	70.4 4.9	50.8 3.59	75.45 3.92	52.73 4.26	77.8 4.61
N.	36	36	30	30	24
Sig.	Ac. and Con.	Ch. and Con.	Ac. and Con.	Ch. and Con.	
	N.S.(0.280)	H.S.(0.001)	N.S.(0.690)	H.S.(0.008)	
	Ac. And Ch. B		Ac. And Ch. C		
	H.S.(0.006)		H.S.(0.008)		

This Table show H.S. decrease in Ceraluplasmin level among chronic Hepatitis (B and C) patients compared with control , N.S. decrease show between acute (B and C) patients compared with control . There is H.S. Decrease in chronic stage compared with acute stage for both types (Band C).

Using T-test for statistical analysis of the data obtained from the CER level in chronic and acute hepatitis B, for patients with respect to normal volunteers ,there is a significant decrease in CER level. Normally CER is an α2-glycoprotein with a single polypeptide chain containing 6-7 copper atoms per molecule. Because of its copper content, the pure protein has a blue colour, hence the name. It is also originally called copper oxides, it is rapidly catalyses oxidation of Fe^{+2} to Fe^{+3} and this ferroxidase activity is essential to transform Fe^{+2} at the cell surface, before iron can be bound to transferrin (17). In acute hepatitis C, there is nearly no difference between normal and patient level of CER, so it is less important for diagnosis compared to the other values.

A majority of cases present with liver dysfunction; any patient between the age of 5 to 50 years with unexplained liver disease, enlarged liver and spleen, a history of attacks of jaundice, or signs of unexplained brain damage should be screened for CER level; In which typically the plasma CER is reduced (18).

Decreased serum Ceraluplasmin levels may be seen in advanced liver disease due to decrease in synthesis, and may be seen in acute malnutrition. In advance liver disease the other synthesized proteins, particularly albumin are usually low in association with CER, so it may be a suitable measure of hepatic synthetic dysfunction in the absence of cirrhosis or other protein mal- synthesis.

Conclusion

There is a significant reduction in α1-antitrypsin in all patient types compared with than in control subjects. But there is no difference in its level between type B and C. Since Hepatitis effect liver cell, the centre of protein synthesis.This effect the level of AAT, and the level depend on liver cell damage. So liver damage may give an idea about the level of AAT and estimation of this protein is important. And there is a significant decrease in CER level. In acute hepatitis C, there is nearly no difference between normal and patient level of CER, so it is less important for diagnosis compared to the other values.

Recommendation

• Further study with Large sample size should be done to be estimate the level of CER and AAT.

• Further studies are recommended to compare the level of CER and AAT in persistent chronic infection and chronic carrier state.

• Any patient between the age of 5 to 50 years with unexplained liver disease ,enlarged liver and spleen , a history of attacks of jaundice should be screened for CER level; In which typically the plasma CER is reduced .

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