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Full Length Research Paper

Some immunological and biochemical aspects for Hepatitis B virus detection

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Abstract

The study was carried on 1200 subjects suspected to be hepatitis B virus infected, admitted to AL-Hilla Teaching Hospital /Central Public Health laboratory, to investigate some sero- immunological and Biochemical assay. Biochemical assay of liver function enzymes reveals enhancement in both Alanine aminotransferase (ALT) as well as Aspartate aminotransferase (AST) levels in all patients sera in comparison with normal healthy control group, However both enzymes appear to be high in male than female The highest mean value of ALT in infected subject occur in the male with age group (51-60)years old, which reach to (608.8)U/L. While the highest mean value in female occur within age group (31-40)years old , which reach to (380.3)U/L. ELISA assay for detection of hepatitis B virus surface antigen as well as IgM- anti hepatitis B-core antigen and anti-HBeAg were used in this study for detection of HBV and its immunological response in infected subjects . Result revealed that only 48 out of 1200 subjects had HBsAg positive (4%) with high frequency in male(2.1%/), than female(1.3%) group. On the other hand the highest percentage of IgM-anti HBcAg was detected within age group (31–40) years old which reach to(77.7%) whereas anti-HBeAg reach its high percentage within age group (51–60) years old which reach to (15.3%).The study concluded that male are more susceptible to infection with HBV than female and chronisity of the disease is directly associated with age increase.

Keywords: Hepatitis B, AST, ALT, HBeAg, HBcA.

INTRODUCTION

Hepatitis is a general term meaning inflammation of the liver. Originally know as "serum hepatitis" (Barker, 1996). The disease caused by a variety of different hepatitis viruses such as A, B, C, and E. Since the development of jaundice is a characteristic feature of liver disease , a correct diagnosis can only be made by testing patients sera for the presence of specific anti-viral antigen or antibodies (Robinson, 1995; Mahoney et al., 1999; Hollinger, et al 2001). Hepatitis B is a serious global infectious disease and a major cause of morbidity and mortality (Hu KQ, 2002; Alavian et al., 2007; Bhattacharya, et al 2007). HBV endemicity has varied widely worldwide. It has been estimated that HBV is highly endemic in all of Africa (except Tunisia and Morocco), some parts of South America, Alaska, northern Canada and parts of Greenland , eastern Europe, the eastern Mediterranean area , south-east Asia , China , and pacific Island (except Australia, New Zealand and Japan) (Ander, 2000). In the Middle East, Saudi Arabia, Jordan, Oman, and Palestine are areas classified as of high endemicity, Iraq, Kuwait, and Bahrain have low endemicity (Gutierrez et al., 2004). Hepatits B virus (HBV), an enveloped virus, a 42 nm particle containing a partially double stranded, circular molecule of HBV DNA. The HBV genome is 3.2 kb in lengh (Lau et al., 2003). Composed of a 27nm nucleocapsid core (HBcAg), surrounded by an outer lipoprotein coat (also called envelop) (HBeAg), containing the surface antigen (HBsAg) (Gitlin, 1997; Ganem et., al 2001). There are four partially overlapping open reading frames encoding the envelope (pre-S/ S),

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core (precore/core), polymerase, and X proteins, replicates through an intermediate reverse transcription step (Maini et al., 2000). The three main modes of transmission are via blood, during sexual intercourse, and perinatally from mother to newborn, People have been infected by improperly sterilized syringes, needles, or scalpels and even by tattooing or ear piercing. Hepatitis B infection is common among patients and staff of hemodialysis units. Health care personnel (medical and dental surgeons, pathologists, nurses, laboratory technicians, and blood bank personnel) have a higher incidence of hepatitis (Levinson, 2006). The liver is one of the heaviest organs in the body weighting about (1.2-1.5kg) and it has traditionally been divided into the left and right lobes, by the falcifrom ligament, fissure of the ligamentum teres and fissure of the ligamentum venosum; the right and left hemi livers are further divided a total of eight segments, in accordance with subdivisions of the hepatic and portal veins; each segments has their own hepatic artery branches and biliary tree (Nicholas et al., 2006). Acute infection with hepatitis B virus associated with acute viral hepatitis an illness begins with general ill-health , loss of appetite , nausea, vomiting, body ach, mild fever , dark urine and then progresses to development of jaundice . Acute hepatic injury can be recognized by the presence of jaundice or non-specific symptoms of acute illness accompanied by elevation of AST and /or ALT activities. AST also sometimes termed SGOT and ALT , also sometimes termed SGPT are widely distributed in cells throughout the body (Zheng et al., 2008). AST is found primarily in heart , liver, skeletal muscle, and kidney, while ALT is found in liver and kidney , with lesser amounts in heart and skeletal muscle (Carol, 2005). Chronic infection may be either asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years, this type of infection dramatically increases the incidence of hepatocellular carcinoma (Can, 2005). Diagnosis is confirmed by demonstration in sera of specific antigen and/ or antibodies. Three clinical useful antigen –antibody systems have been identified for hepatitis B: Hepatitis B surface antigen (HBsAg), antibody to HBsAg(anti-HBsAg), antiHBcore antigen (HBcAg) (anti-HBc IgM and anti-HBc IgG), hepatitis B envelope antigen (HBeAg) and antibody to HBeAg (anti-HBe) (Robinson, 1995). Biochemical assessment of liver function include: bilirubin, ALT, AST, alkaline phosphatase, total protein, albumin, globulin (Hollinger et al., 2001). PCR tests have been development to detect and measure amount of HBV DNA, called the viral load , in clinical specimens. These tests are used to assess a person′s infection status and to monitor treatment (Zoulim, 2006).

MATERIALS AND METHODS

Samples Collection and Processing

One thousand two hundred blood samples of 5ml volume each were collected aseptically using sterial syrings from subjects admitted to AL-Hilla Teaching Hospital / Central Public Health laboratory.

Each blood sample was collected in sterile plan tube, labled then all samples were incubated at room temperature till clotted , sera sample were collected seperoly after centrifugation in Kokusan (Japan), centrifuge at 3000 r.p.m for 10 min, each sample was tested for Hepatitis – B – surface antigen (HBsAg) detection (Teknika Organon combany, USA) as well as GOT and GPT liver function test (Randox combany , USA). The remaining sera sample were distributed in 0.5 ml aliqutes in sterile eppendorf tubes (3 tubes for each patients) and stored at –20C° for further tested, tested includiong Anti□Hepatitis B core antigen (HBcAg) ELIZA detection (DRG combany, USA) only 48 subjects aging from (11- 60) years old gave positive HBsAg result. However, information required were fixed according specified formula, including subject name, age, gender, and resident . The study includes fifty samples obtained from healthy subject a control group, that classified to five group according to age in similar way of patients group (11 - 20), (21 - 30), (31 - 40), (41 - 50), and (51-60) years old.

Statistical analysis:Data were analyzed statistically using complete randomized design(CRD), LSD and X2 test (Naizi ,2004).

RESULTS AND DISCUSSION

The current study shows the significant differences in the ratio of enzyme –linked liver function (GPT), where the elevate level of the enzyme occur within age group (31-60) years old in most viral hepatitis (B) infected subjects . The mean value difference depend on gender of the infected subject , the highest mean value of infected subject occur in the male within age group (51-60)years old, which reach to (608.8IU/L)U/L. While the highest mean value in female occur within age group (31-40)years old, which reach to (380.2I U/L). There are a significant differences ($P<0.05$) in enzyme value between male and female and among age groups Table (1).

The serum levels of alanine aminotransferase are elevated in acute hepatitis as levels of other enzymes released by damaged liver cells , the levels of alanine aminotransferase are higher than those of aspartic aminotransferase (Zuckerman et al., 2005). When hepatocytes are damaged , they may leak enzymes into the blood, where they can be measured as indicators of cell damage, Alanine aminotransferase (ALT) is one such enzyme, it is markedly elevated in hepatitis and from other causes of acute liver damage , when the

Table 1. Alanine amino transferase concentrations in sera of hepatitis B virus infected patients .

Age groups(years)	subjects	ALT concentration M±SD (U/L	
		Male	**Female**
11-20	patients	**375.2*** ± 271.1	**307.4** ± 176.7
	control	15.0 ± 5.6	10.2 ± 4.2
21-30	patients	447.6 ± 224.6	301.6 ± 295.7
	control	12.0 ± 3.8	13.6 ± 5.4
31-40	patients	265.8 ± 187.7	380.2 ± 225.8
	control	11.2 ± 5.5	9.6 ± 4.1
41-50	patients	306.2 ± 231.4	360.3 ± 252.4
	control	10.8 ± 6.3	9.0± 3.5
51-60	patients	608.8 ± 73.5	251.4± 239.1
	control	19.6 ± 4.2	16.4 ± 4.2
		LSD (0.05) = 31.88	

Table 2. Aspartate aminotransferase (AST) in sera of hepatitis B virus infected patients

Age groups(years)	subjects	AST concentration M±SD (U/L	
		Male	**Female**
11-20	patients	**270.6** ± 214.9*	**262.6** ± **178.1**
	control	13.8± 3.4	8.8 ± 2.5
21-30	patients	392.2± 192.8	275.6 ± 150.0
	control	8.0 ± 2.9	7.6± 3.0
31-40	patients	253.8± 181.8	260 ± 202.6
	control	10.6 ± 1.8	11.4± 1.1
41-50	patients	257.8 ± 105.3	367.2 ± 246.4
	control	16.2± 2.5	8.6 ± 2.7
51-60	patients	532.2± 94.3	251.4 ± 239.1
	control	9.8± 1.9	11.8 ± 3.1
		LSD (0.05) 40.88	

liver is injured or inflamed , the levels of ALT in the blood usually rise , therefore this enzyme is most suited for assessing liver disease (Aach et al., 1981). ALT is elevated even before the clinical signs and symptoms of disease such as jaundice appear (Dufour et al., 2000). The ALT elevates higher than AST in acute infection and level of both are usually 500U/L or greater (Decker, 1998). After the acute phase of infection, serum ALT levels fall but remain abnormal (from 50 to 200 U/L) (Perrillo, 2001).

The study also shows the significant differences in the ratio of enzyme –linked liver function (GOT), where the elevate level of the enzyme occur in age group (41:60) years old in most viral hepatitis (B) infected subjects . This study also shows the mean value difference depend on gender of the infected subject, the highest mean value of infected subject occur in the male within age group (51:60) years old, which reach to (532.2 IU/L). While the highest mean value in female occur within age group (41:50) years old, which reach to (367.2 IU/L) Table (2).

AST and ALT reflect hepatocellular injury, Common hepatic causes of elevated levels include viral hepatitis, alcohol, drugs, nonalcoholic steatosis and steatohepatitis (Dufour, 1998). The actual values may differ from laboratory to laboratory, both AST and ALT are released into the blood in greater amounts (George, et al., 2012).

The study shows out of 48 subjects who had been diagnosed as having hepatitis B surface antigen (HBsAg), only 34 subject shows positive to IgM antibody against hepatitis B core antigen (anti-HBc IgM), all age group revealed acute hepatitis B virus (HBV) infection. The highest percentage of IgM anti-HBcAg occur within age group (31:40) years old, which reach to (77.7%), whereas lowest percentage of IgM anti:HBcAg occur within age group(41:50) years old , which reach to (62.5%) Table (3).

The setting of acute HBV infection, HBsAg typically becomes detectable 4 to 8 weeks after infection. Shortly thereafter, IgM anti-HBc appears in

Table 3. IgM antibody appearance against hepatitis B core antigen (anti-HBc IgM)

Age groups	No. of samples	IgM anti HBcAg Positive (no. and %)		IgM anti HBcAg negative (no. and %)	
11-20	10	7	70 %	3	30 %
21-30	8	6	75 %	2	20 %
31-40	9	7	77.7%	2	22.2%
41-50	8	5	62.5	3	37.5%
51-60	13	9	69.23 %	4	30.76%
Total	48	34		14	

X2 =10.750

Table 4. Apperance of Antibody against hepatitis B virus envelop antigen (Anti-HBeAg).

Age groups	No. of samples	IgM anti HBcAg Positive (no. and %)		IgM anti HBcAg negative (no. and %)	
11-20	10	1	10 %	9	90 %
21-30	8	0	0 %	8	100 %
31-40	9	0	0%	9	100%
41-50	8	1	12.5%	7	87.5%
51-60	13	2	15.3 %	11	84.6%
Total	48	4		4	

X2 =18.532

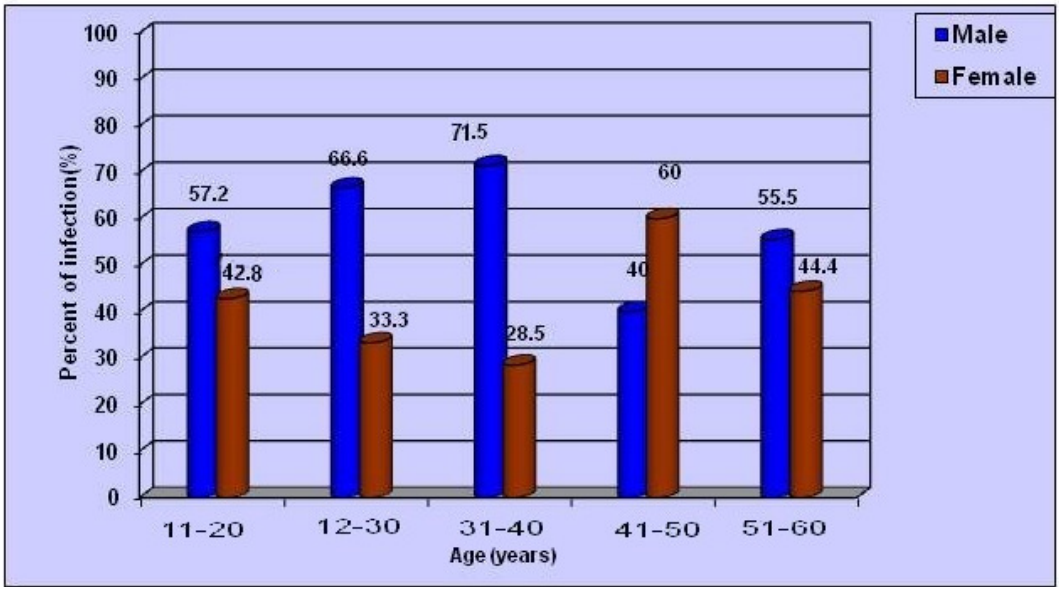

Figure 1. Percentage of IgM antibody against hepatitis B core antigen (anti-HBc IgM) in sera of viral hepatitis B infected subject.

the blood, Thus, the diagnosis of acute hepatitis B is generally made by the simultaneous detection of HBsAg and IgM anti-HBc (Hoofnagle, 1981). I f anti-HBc IgM is negative, the probability of acute infection in HBsAg-positive cases is nil (Smith et al., 1992).

IgM anti-HBcAg according the gender revealed highly appearance in male than female. The highest percentage of infected subject occur in the male within age group(31-40)years old which reach to (71.4%), whereas the highest percentage of IgM-anti-HBcAg in

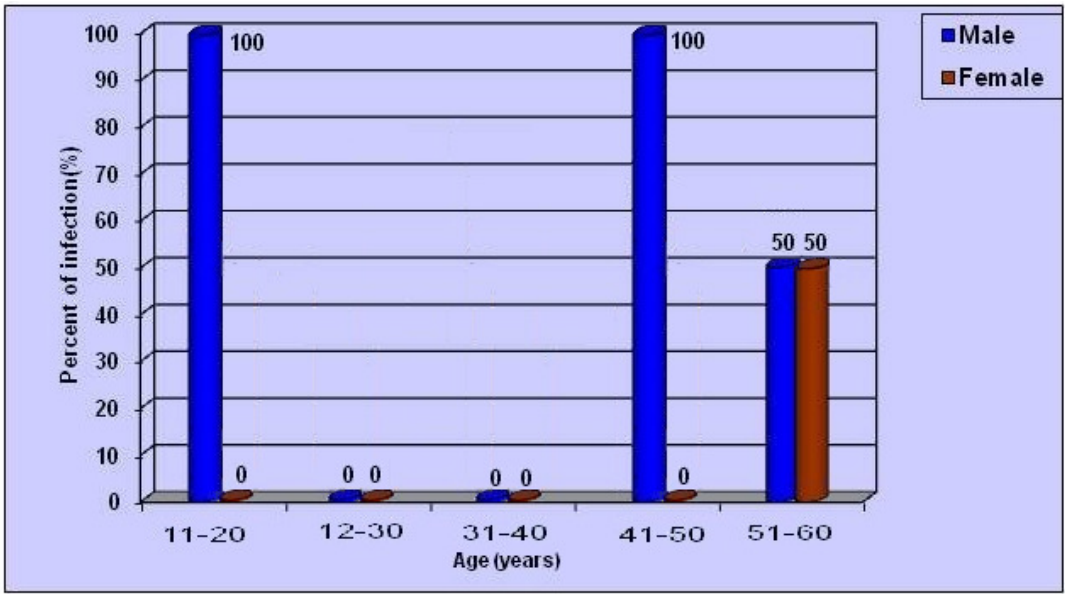

Figure 2. Percentage of antibody against hepatitis B envelope antigen (anti-HBeAg) in sera of viral hepatitis B infected subject.

female appear within age group (41-50) years old, which reach to (60%). These result indicated that most subjects are of the acute phase of infection Figure 1.

Primary infection leads to an IgM and IgG response to HBcAg shortly after the appearance of HBsAg in serum , at onset of hepatitis (Robinson et al., 1994). IgM anti – HBc is present in high titer during acute infection and usually disappear within 6 months (Hollinger, 2001).

This study revealed that the positive anti-HBeAg (chronic infection) was detected within age group (11-20), (41-50) and (51-60) years old, the highest percentage occur within age group (51-60) years old which reach to (15.3%), whereas age group (21-30) and (31-40) years old don't revealed any chronic infection Table 4 .

The evolution of chronic hepatitis B depends upon the geographic location of the host, age and mode of acquisition of virus and predominant type of virus [30]. Antibody to HBeAg is detectable as HBeAg disappears from the serum and the presence of anti-HBe is associated with likelihood of resolution of acute infection. In chronic hepatitis B virus infection the loss of HBeAg and acquisition of anti-HBe tends to be associated with biochemical and histological improvement (Yuen et al., 2001).

The results concerned with anti-HBeAg according the gender show that male show the highest percentage of chronic infection appear in male with age group (11-20), (41-50) years old which reach to (100%). While the highest percentage in female occur within age group (51-60) years old. Whereas age group (21-30) and (31-40) don't revealed any chronic infection Figure 2.

Chronic HBV infection begins when the immune response that normally clears the infection fails to take place or is too weak to be effective, thus, infections are more common in low immunity subjects as a result of poverty (Hoofnagle, 1997). The males are more affected with chronic HBV than females (Yuen, et al., 2001).

CONCLUSION

The study concluded that males are more susceptible to hepatitis B viral infection than female and the biochemical and immunological assay such as AST, ALT anti HBcAg and HBs Ag are useful for detection of infection phase.

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