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HEPATITIS B AND RENAL FUNCTION OF PATIENTS WITH CHRONIC HEPATITIS B IN FALLUJAH DISTRICT, IRAQ

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ABSTRACT : Chronic Hepatitis B (HBV) is the leading cause of morbidity and mortality worldwide with about 248 million people having HBV infection. Presentstudy focused on the kidney function in patients with chronic hepatitis B. The aims of this study are: to estimate of the electrolytes in patients with chronic hepatitis B individuals. One hundred sixty patients' samples and thirty healthy individuals were investigated in this study. The concentrations of Sodium, Potassium and Magnesium were determined using inductively Coupled plasma- Mass spectrometry (ICP-MS). Alanine amino transferase (ATL) and Aspartate amino transferase (AST) activities, total serum bilirubin (T.S.B), urea, Creatinine and amount of albumin were measured using standard laboratory methods. Viral load (HBV PCR) was also determined in all the examined patients. Results from this study showed that low serum albumin and higher serum T.S. B, S.ALT, S.AST urea and S. Creatinine with chronic hepatitis B compared to those of healthy individuals. The findings of this study show that the concentration range of Sodium element between (136.54±3.107mmol\l) to (140.51±3.598 mmol\l) was significantly lower in HBV infected patients as compared to those in healthy controls (142.82±3.37 mmol\l). Magnesium concentration range between (1.592989±.31mg/l) to (1.7319±.32468 mg/l) was significantly lower in HBV infected patients as compared to those in healthy controls(1.7477±0.17 mg\l). While, potassium concentration range between (4.61877±0.73 mmol\l) to (5.51846±0.736 mmol\l) was significantly higher in HBV infected patients as compared to those in healthy controls (4.4552±0.38 mmol\l). Positive and negative significant correlation relationships between the renal functions were reported between some groups. There are significant differences between some the renal functions in some groups and insignificant in other groups.

Key words : Chronic Hepatitis B, failure renal, electrolytes in patients.

INTRODUCTION

Viral hepatitis is a major human health problem worldwide (Jafri *et al*, 2006). The hepatitis B infection (HBV) was discovered in 1968. It is transmitted through different modes including the use of contaminated syringes, especially among drug abusers, blood transfusions, sexual intercourse with HBV-infected patientsand from infected mothers to their embryos and newborns (Bosch *et al*, 2005). Hepatitis B carriers are defined as persons with positive hepatitis B surface antigen (HBs Ag) for more than 6 months (Alavian*et al,* 2006). Carriers of HBV have expanding danger of creating cirrhosis and hepatocellular carcinoma (HCC) (Jules *et al,* 2008). Albeit most carriers won't develop hepatic complexities from chronic hepatitis B, it is assessed that15% to 40% will develop genuine sequence amid their lifetime (Das *et al*, 2010). The World Health Organization estimates that more noteworthy than two billion individuals worldwide are infected with HBV; 360 million have chronic infection and are at high hazard for hepatocellular carcinoma and cirrhosis of the liver (World Health Organization, 2013). In Iraq, studies showed that the prevalence of HBV infection has decreased from 4.1% to less than 1% in the period of seventies to nineties (Ataallah *et al*, 2011; Al-Juboury *et al*, 2010).

Alanine aminotransferase (ALT) is "found in kidney, heart, muscle and greater concentration in liver compared with other tissues of the body". ALT is absolutely cytoplasmic catalyzing the transamination response. Aspartate amino transferase (AST) exist two different isoenzyme forms which are genetically distinct, the mitochondrial and cytoplasmic form (Mauro *et al*, 2006). The serum albumin level isn't a dependable marker for hepatic protein synthesis in the acute liver. Albumin synthesis is influenced by liver illness as well as by nutritional status, hormonal balance, and osmotic pressure. The liver is the main site of synthesis of albumin (Rosalki *et al*, 1999).

The kidney is "essential in the urinary system, also perform homeostatic functions, such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure". they serve the body as a natural filter of the blood and evacuate wastes, which are transferred to the urinary bladder (Cotran *et al*, 2005).I t was proposed that HBV infection antigenemia may play a noteworthy part in the development of particular forms of glomerulonephritis and this can run an indolent but relentless progressive clinical course (Kar *et al*, 1989). Some scientists report that hepatitis B infection can prompt critical changes in renal function and subsequently cause renal sickness including membranous glomerulonephritis and membranous proliferative glomerulonephritis (Lai *et al*, 1991). Moreover, wide utilization of hepatitis B vaccination has been seen to diminish the frequency of HBV-related renal illnesses in children thereby giving proof of the likely pathogenic role of Hepatitis B virus (Xu *et al,* 2005).

MATERIALS AND METHODS

Sampling

The study was carried out in Fallujah teaching hospital for the period from 1-5-2013 to 3-11-2013 at viral hepatitis consultation clinic in the hospital. One hundred sixty patients were included in this study. The inclusion criteria wereAll of patients were HBs Ag positive andall of them HBcIgGAb positive.

That means the patients had chronic hepatitis B infection. Sera samples were collected from these patients were examined for profile viral tests which include HBS Ag¸ HBSAb, HBc Ag (IgM-IgG) HBe Ag, HBeAb and also for viral load by polymerase chain reaction (HBV PCR) with unit (copies/ml). In addition (HCV and HIV) tests were done to all patients. According to the results of these profile tests, the patients were categorized in to five groups.

The healthy control groups (n=30) were selected based on: non-alcohol drinkers,with no smoking habits, no history of viral hepatitis and they are HBS Ag negative with HBcAb (IgG) negative.

Eight ml of blood was collected from the veins of both the patients and the control groups. Six ml of blood kept in sterile tubes without adding any anticoagulant agent and two ml of blood kept in a tube containing anti-clotting agent. Patients did not take any antiviral treatment prior to this study. Collected blood samples were placed in sterile place and allowed to clot. The blood samples were centrifuged and then the collected serum was stored in plastic vials at -20^0C until further analysis.

Sample analysis 1

1-Determination of urea and creatinine : Urea and creatinine were determined by strip (Microlab 300), and then they were measured by using reflotron plus devices.

2- Determinations of ALT and AST and S. Albumin : -ALT, AST and S. Albumin were determined by using kit, and then they were measured by using spectrophotometric analysis. They were measured at wave lengths (540 nm, 540nm, 630nm), respectively.

Materials 1 : A 150 µL aliquot of serum samples were thawed at room temperature for 20 minutes and pipette into pre-cleaned polyethylene tubes. 150 µL of optima grade nitric acid and 100 µL of trace select grade hydrogen peroxide were then added to the samples. The tubes were tightly capped, centrifuged for 10 minutes at a speed of 4400 r/min in a centrifuge. Samples were then placed in a hot block digester and digested at 95°C for 90 minutes with the tube caps loosened. Following digestion, the samples were diluted to 150 µL(Gang *et al*, 2012).

Procedure : Standard multi-elements solutions including Na, K, and Mg were prepared by dilution high purity 1000mg L^{-1} stock solutions with deionized water and nitric acid. The prepared solutions were kept in a dark room to reduce contamination. High purity water was used to prepare the samples. ICP-MS responses and the experiments were performed using different concentration levels.

Sample analysis 2 : The digested serum samples were analyzed for selenium using Inductively Coupled plasma- Mass spectrometry (Agilent Technology, Japan). The analytical calibration method was accomplished with aqueous standards in 0.5% (v/v) HNO_3. Fresh calibrations were made each time before analysis. ICP-MS responses, the experiments were performed using different concentration levels. Instrument was calibrated using aqueous standards of elements 1, 100, 300, 900, 1000, 3000, 9000,18000 and 27000 µg/L.

Statistical analysis

Our study as well as most other studies uses statistical analysis to determine the relationships between variables. The analysis is performed by using the statistical package for the social sciences (SPSS, version 22) for windows. Continuous variables were expressed as mean ± standard deviation (SD). Pearson correlation test was used to correlate between different variables among the studied groups. Pearson's correlation and analysis of variance (ANOVA) were conducted.

RESULTS AND DISCUSSION

Prevalence of Chronic Hepatitis B

Samples the detail of patients and healthy group are shown in Table 1. The male patients were higher in number 84, while female patient were 76 patients from urban area were 60 patients (37.5%), while patients from rural area were 100 patients (62.5%) (Table 2). This difference in statistically showed that increasing of the incidences of the disease in rural areas compared to the urban regions. This increasing of incidence can be attributed to lack of the health awareness and not to follow the instructions to avoid the spread of diseases. In addition, the people in rural areas did not immunize themselves by taking hepatitis B vaccine.

Serum liver markers

Viral load (HBV PCR) of the patients with chronic hepatitis B was measured from the present study revealed low serum albumin and higher serum S.ALT and S.AST in patients with chronic hepatitis B compared to those of healthy individual's (Fig. 1). In addition, HBV-DNA was significantly higher in patients with chronic hepatitis B (Tables 3 and 4).

Table 1 : List of Symbols and details for study groups.

Symbols	Details	Numbers
1A	Patients HBe Ag (+ve) normal liver function PCR= (>100000)	36
2A	Patients, HBe Ag (+ve) abnormal liver function PCR= (>100000)	28
1B	Patients HBeAg (-ve) PCR= (>100000)	30
2B	Patients HBeAg (-ve) PCR=4000-100000	36
3B	Patients HBeAg (-ve) PCR=N0N	30
C	healthy individuals	30

Hepatitis B and renal function

Hepatitis B is ubiquitous not only in liver, but it also has been seen to cause persistent infections in many other human organs. However, the evidence of a putative HBV association with other organs needs to be investigated. Liver has been the sole target for research on hepatitis B virus for the past 40 years. Scientists and clinicians had been more interested in knowing the genotypes, their differences in biological properties; the prevalence of hepatitis B virus mutants in various geographic regions; in addition to the clinical outcomes and response to antiviral treatment in different population groups (Baig *et al*, 2007).

There were no significant differences in serum creatinine, urea and Sodium in patients and control group as shown in Table 5, Fig. 2.

This perception might be clarified in view of an earlier that recorded no change in plasma urea and creatinine levels level in patients suffering from renal tubular acidosis with no glomerular lesion (Mayne *et al*, 1996). Ventataseshan *et al* (1990) observed that extra-hepatic complication of hepatitis B infection disease will just show in chronically sick patients.

The findings of this study show an increase in mean serum Potassium concentration in patients with chronic hepatitis B compared to healthy individuals (Table 5, Fig 2). The serum potassium concentration might increase along with deteriorating renal function. Subsequently, the elevated potassium levels might stimulate Potassium excretions. As a result, a new steady state develops without medical complications (Gennari *et al*, 2002). There will also be a decrease in glomerular filtration capacity as a renal complication of HBV disease. The DNA of HBV infection was also found in the close-by renal tubules, where pee is concentrated (Lai *et al*, 1991).

Table 2 : Age, gender and residence distribution of groups for patients with chronic hepatitis B and healthyindividuals.

Variables	1A		2A		1B		2B		3B		Healthy individuals	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Urban	5	4	4	3	4	5	8	10	9	7	11	5
Rural	15	12	11	10	9	12	8	10	8	6	10	4

Table 3 : Serum levels T.S.B, ALT and AST of different groups for HBV patient and healthy individuals.

Parameters	1A	2A	1B	2B	3B	Healthy individuals
T.S.B (mg/100ml)	0.8161±0.1263	0.8664±0.21257*	0.8453±0.1973	0.885±0.3*	0.8760±0.2*	0.699± 0.063
ALT(IU/L)	20.4389 ±6.743*	38.3893±17.5	31.8567±15.16	23.7667±11.336	32.3±12.7	23.662±5.4*
AST(IU/L)	26.93±15*	52.46±18.4	36.3567±16.337	23.828±9.138	30.66±14.4778	19.759±7.2*
ALT\AST	0.9±0.414**	0.8267±.49**	0.95±.43**	1.21±0.7**	1.236±0.61**	1.37±0.64*
Albumin (gm/dL)	3.98±0.673*	4.1719±0.432	4±0.3735*	4.1769±0.69145*	4.0533±0.59	4.38±.3357

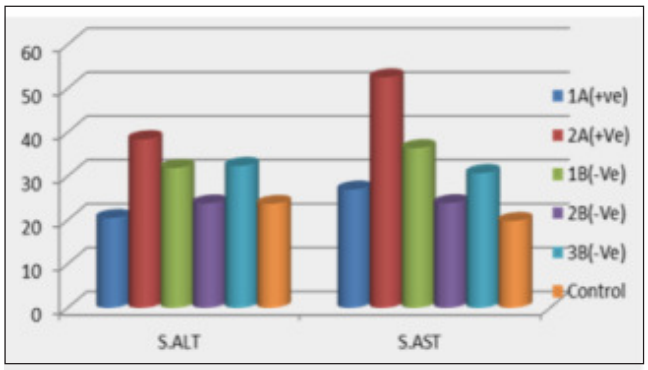
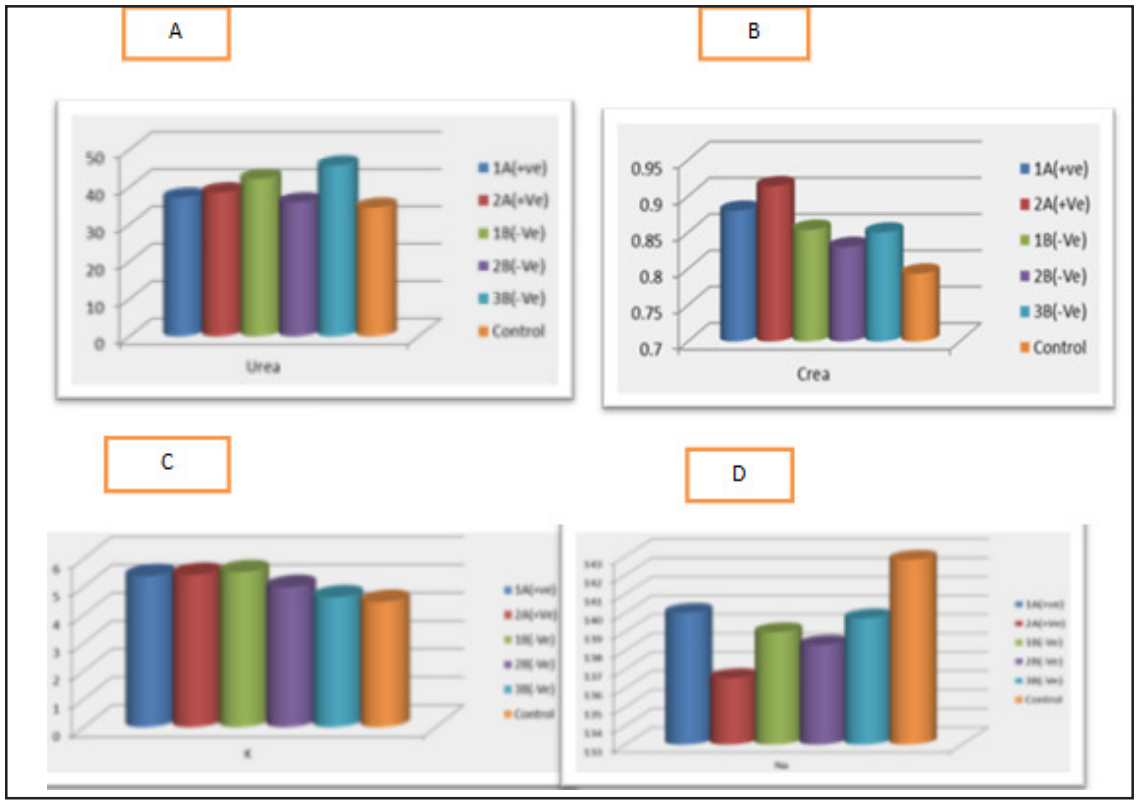


Fig. 1 : Serum levels ALT and AST of different groups for HBV patient and healthy individuals.

While serum sodium concentration recorded a significant decrease compared to healthy individuals as in Table 5, Fig. 2), this decrease may be due to impaired renal capacity and its inability to excrete solute free water (Agrawal *et al*, 2008). If found that hyponatremia and hypernatremia represented disorders of water balance, or due to impaired renal water excretion and antidiuretic (Cotran *et al*, 2005). Hyponatremia resulting from the impairment of solute-free water excretion is commonly accompanied by portal hypertension (Adrogué *et al*, 2000). In recent years, hyponatremia has attracted interest as a possible prognostic factor for liver cirrhosis. Currently, many on-going studies are examining the



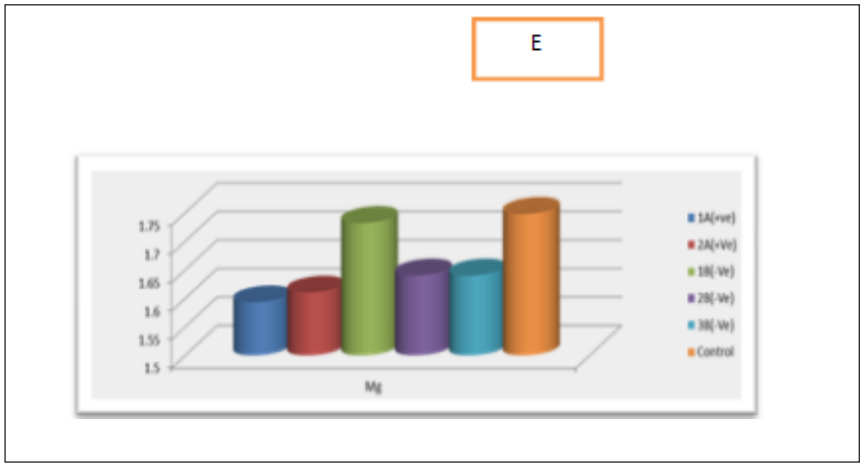


Fig. 2 : A Serum levels B. Urea for HBV patient and healthy individuals.B. Serum levels S. Creatinine for HBV patient and healthy individuals.C. Serum levels Potassium of different groups for HBV patient and healthy individuals.D. Serum levels Sodium of different groups for HBV patient and healthy individuals. E. Serum levels Magnesium of different groups for HBV patient and healthy individuals.

Table 4 : Viral load (HBV PCR) of groups for patients with chronic hepatitis B.

Parameters	1A	2A	1B	2B	3B
Level of HBV DNA (copies/ml)	1510055884	286109934.8	31818160*	33446.188*	NON

Table 5 : Parameters for patients with chronic hepatitis B and group healthy individuals.

Parameters	IA	2A	1B	2B	3B	Healthy individuals
Urea (mg/dL)	37.21±8.470**	38.6±6.664**	42.2467± 6.65*	35.94±10.51*	45.81±9.985**	34.5± 4.76
Creatinine (mg/dL)	0.88±0.160**	.9138±.1398**	.8537±.14779*	0.83±0.138	0.85±0.274**	.7933±.08*
Na+ (mmol/L)	140.51±3.598	136.54±3.107	138.97±3.056*	138.30±3.376	139.71±4.084	142.82±3.37**
K+ (mmol/L)	5.374±.868	5.4199±.8832	5.51846±0.736	4.98±0.954	4.61877±0.73	4.4552±0.38
Mg(mg\l)	1.592989±.31	1.61±.251286	1.7319±.32468*	1.64±0.392	1.64±0.392**	1.7477±0.17

*. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level

pathophysiology of hyponatremia accompanied by liver cirrhosis, and dilutionalhyponatremia has been considered the most plausible pathophysiology (Gines *et al*, 1998).

Magnesium has shown decrease in mean serum concentration in patients with chronic hepatitis B compared to healthy individuals as Table 5, Fig. 2. Magnesium lack causes renal complications. The appearance of different diseases is associated with its depletion in the human body. In radiotherapy and additionally in chemotherapy, particularly in the treatment of malignancies with Cis-platinum, hypomagnesemia is watched. The part of Magnesium in DNA adjustment is fixation subordinate. At high fixations, there is a gathering of Mg restricting which instigates conformational changes prompting Z-DNA, while at low concentration; there is lack and destabilization of DNA. The biological and clinical consequences of irregular concentrations are DNA cleavage leading to diseases and cancer (Koivisto *et al*, 2002). Das *et al* (2011) found the serum magnesium levels significantly diminish in liver cirrhosis patients with the advancement in age. Our results were in contrast to the results of (Abdul Aziz *et al*, 2010) that serum magnesium levels remained unchanged or noted decrease levels.

The relationship among the different biochemical parameters and HBVDNA in HBV e Ag (+ve), HBVeAg(-ve) and healthy individuals were determined by using Pearson product. A negative correlation and positive correlation were observed between variables at 0.05 or > 0.05 level.

CONCLUSION

In this study, we found liver functions (T.S.B, S.ALT, S.AST, S. albumin) were unequal for some patients with chronic hepatitis B. Pearson's correlation showed significant positive and negative relationships for some liver functions. Significant and insignificant differences were reported between the patient's groups and the healthy individual as the control, group also high patient numbers were high viral load (HBV PCR) especially for patients with (HBe) Ag positive group. The higher Potassium concentration and lower Magnesium and Sodium concentrations indicate that complications in Chronic hepatitis B patients may cause malfunction or weakness in renal functions.

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