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BIOCHEMICAL CHANGES IN HEPATITIS B VIRUS AMONG AL-ANBAR PATIENTS

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Abstract: Hepatitis B virus (HBV) infection is a global health challenge, and clearance or persistence of HBV is mostly determined by host immune responses,. A case-control study was conducted during June-October 2022 on 20 HBV patients and 20 matched control. The results revealed that most patients were males 11(55.0%), while female patients accounted for 9(45.0%). Four liver-function tests (LFTs); total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were assessed. Significantly increased levels of ALP, ALT and AST were observed in HBV patients compared to normal healthy control, while TSB showed no significant variation.

1-1 Introduction

Infection with the hepatitis B virus (HBV) is a problem for world health. According to estimates, 257 million people worldwide have the virus, which causes an estimated 887,000 deaths annually From cirrhosis and/or hepatocellular cancer (1,2) In Iraq, a community-based research found that the prevalence of hepatitis B surface antigen was 1.6%. (HBsAg). Also, it was observed that the frequency of anti-hepatitis B core antigen (HBc) and anti-HBs antibodies was 9.7 and 17%, respectively (3). As a result, it was determined that Iraq has a low to intermediate degree of HBV endemicity Infection with the hepatitis B virus (HBV) is a problem for world health. According to estimates, 257 million people worldwide have the virus, which causes an estimated 887,000 deaths annually from cirrhosis and/or hepatocellular cancer (1,4) in Iraq, a community-based research found that the prevalence of hepatitis B surface antigen was 1.6%. (HBsAg). Also, it was observed that the frequency of anti-hepatitis B core antigen (HBc) and anti-HBs antibodies was 9.7 and 17%, respectively (Tarky et al., 2013). As a result, it was determined that Iraq has a low to intermediate degree of HBV endemicity.

1-2 The aim of our study

Based on the above introduction, the purpose of this study was to identify HBV in people living in Anbar Governorate. A correlation was also made between this hepatitis and the sex and age of the patients.. In addition to studying the relationship between the disease and liver enzymes

1-3 Viral Hepatitis

Alcohol intake, drug use, pollutants, and metabolic abnormalities can all result in hepatitis, an inflammation of the liver. Nevertheless, many viruses are also a key issue in the illness (1). Hepatitis A virus (HAV), HBV, HCV, HDV, and HEV are the five viruses that specifically cause viral hepatitis. Hepatitis A virus (HAV), HBV, HCV, HDV, and HEV are the five different viruses that cause viral hepatitis. While they have various disease trajectories and diverse modes of transmission, they all have the ability to replicate in hepatocytes in common (3)

1-4 Hepatitis B Virus

Hepatitis B virus (HBV) was originally found by Blumberg and his colleagues in 1965, and the association between HBV and acute hepatitis in cases of blood transfusion was revealed in 1968. (4). Prior diagnostic investigations were based on immunological and serological techniques, but since the discovery of the HBV particle and the cloning of the HBV genome, the development of molecular-based techniques has expanded the area of diagnosis (5). Afterwards, it was discovered that HBV infection is a significant global health issue, with Asia and Africa being of special concern. It is estimated that more than 350 million individuals worldwide have a chronic HBV infection (6)

1-4-1 Virion Structure

The relaxed-circular (rc) genome of the hepatitis B virus is partially double- stranded and enclosed (diameter: 42–47 nm), and it is covalently coupled to viral polymerase (Figure 1-1). Little amounts of cellular lipid and the three hepatitis B surface proteins (HBS) known as big (LHB), medium (MHB), and small (SHB) make up the membrane (7). Two further sub-viral particle types may be found in the serum of infected people: tiny, spherical particles with a diameter of around 20 nm and longer, variable-length filamentous particles. These non-infectious sub- viral particles that lack genomic DNA are larger than infectious viral particles and resemble the viral envelope in composition (8). One core protein is present in several copies across the nucleocapsid. The N-terminal 149 - 151 amino acids, out of the total 183 to 185 amino acids (depending on the genotype), are in charge of the nucleocapsid's self-assembly. The first stage is the production of homodimers joined by disulfide bridges, despite the fact that the procedures in its assembly were poorly defined. During the production of genomic DNA, nucleotide diffusion is made possible via holes in the nucleocapsid. The pre-genome polymerase complex is packaged into the nucleocapsid by the core protein's C-terminal amino acids (9).

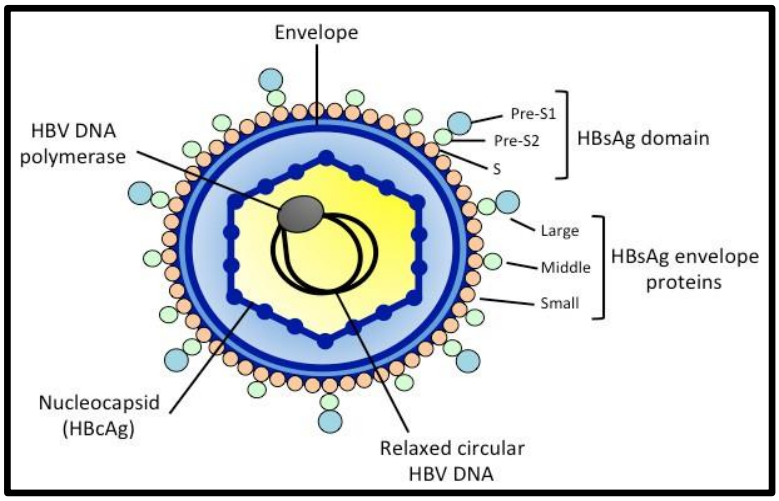


Figure 2-1 Hepatitis B viral schematic is shown in FigureIt demonstrates the virion's structure, which consists of a partially double-stranded DNA genome surrounded by a lipid envelope including large (L), medium (M), and small (S) HBsAg, as well as a capsid made up of the hepatitis core antigen (HBcAg) (10)

2-2-2 Replicative Cycle

The visions are secreted non-cytopathically through the hepatic cell secretory route, where the HBV virus replicates and assembles only in host hepatocytes (Figure 1-3). The nucleus receives the nucleocapsid so that the genome's rcDNA may be released. The nucleoplasm converts rcDNA into covalently closed circular DNA (cccDNA), which is then encased in histones to create an episomally chromatinized structure. Following that, it serves as a starting point for the transcription of all viral transcripts, which are then translated into viral proteins

(11)The pregenomic RNA within the viral capsid is reverse transcribed into fresh rcDNA in addition to encoding capsid protein and viral polymerase. To maintain a supply of cccDNA, the DNA bearing cytoplasmic nucleocapsides is either recycled into the nucleus or encapsulated and released by the endoplasmic reticulum (12) In addition to 42 nm in diameter complete infectious virions, infected liver cells also create an abundance of 22 nm genome-free, non-infectious sub-viral spherical or filamentous particles (13). While viral replication is not necessary for viral genome integration, it is one of the primary mechanisms for hepatocyte transformation and may potentially play a key role in the development of hepatocarcinogenesis. (13)

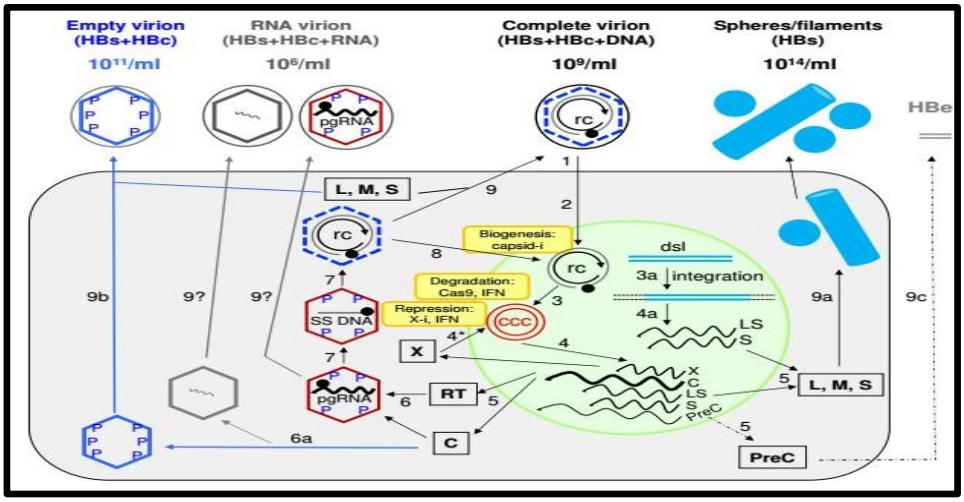


Figure 2-2: Schematic illustration the replication cycle of hepatitis B viruses. 1. Binding virus to and entering the host cell (large rectangle). 2. Intracellular trafficking and the transmission to the nucleus (large circle) of relaxed circular DNA (rcDNA). 3. Conversion of rcDNA to cccDNA, or double stranded linear DNA (dslDNA) penetration into host DNA (3a). 4. and 4a. Transcription for the synthesis of viral RNAs (wavy lines), including C mRNA for both the core and polymerase proteins (reverse transcriptase, RT); LS mRNA for the L envelope protein; S mRNA for the M and S envelope proteins; X mRNA for the X protein transported to the nucleus to induce cccDNA transcription (4*); and Pre C mRNA for the Pre Core protein. The C mRNA is a pgRNA, too. 5. Translation to synthesize viral proteins. PgRNA-(and RT-) assembly which contains capsid, or alternatively empty capsids (6a). 7. PgRNA reverse transcription to synthesize (−) SS DNA strand, and rcDNA afterwards. 8. Capsid nuclear recycling containing progeny rcDNA to form more cccDNA (amplification of intracellular cccDNA). 9. Envelopment of the rcDNA-containing capsid and secretion of complete virions, or alternatively, secretion of empty virions (9b) or spheric and filamentous subviral particles containing HBsAg only (9a). Processing of the PreCore protein and secretion of HBe are depicted in 9c. The secretion of putative RNA virions is not yet resolved (14).

2-2-3 Immunopathogenesis

More than 95% of persons with HBV suffer acute, self-limiting illness and subsequently get rid of the virus. In contrast, early childhood chronic infection affects around 90% of those who are exposed to HBV. Yet, the result of the HBV infection is determined by both the virus's specificity and the host's antiviral immune responses (15). t is commonly accepted that viral genome heterogeneity, viral titers, and suppression of viral components against the host immune system are linked to recurrent infection and liver damage. The malfunction of innate immune cells (NK cells, monocyte/macrophages, and NKT cells), as well as adaptive immunity cells (antigen-presenting cells, T cells, and B cells), is a major role in the inability to effectively remove viruses from the body and the advancement of hepatic inflammation (16).

2-2-4 Innate Immune Response

The initial line of defense for the body against HBV infection is the innate immune response. IFN-/ development and NK cell activation are characteristics of the early stages of HBV infection. IFN-/ is mostly produced by infected plasmacytoid and dendritic cells, whereas IFN- is mainly produced by NK and NKT cells. Dendritic cells are crucial for triggering immunological responses because of their capacity to process and display antigens. In the presence of HBV or its antigen (HBsAg), dendritic cell

maturation is disturbed and tolerance may develop (17). Due to their capacity to generate IFNs, plasmacytoid dendritic cells are essential in anti-HBV immune responses. HBV has been shown to be able to affect plasmacytoid dendritic cell activity and the connections between these cells and NK cells, which reduces anti-HBV immune responses and increases the development of chronic infection (18).

Monocytes are a crucial cellular component of innate HBV defense as well. Inflammatory cytokines like IL-17 are produced by these cells as a result of the heightened inflammatory circumstances, which helps to activate monocytes. It has been demonstrated that the CD16+ monocytes generate more pro-inflammatory cytokines (19). It has been discovered that individuals with HBV had higher levels of monocytes in their peripheral blood, as well as higher levels of CD16+ monocytes in their livers. The liver's generation of monocytes has increased.

2-2-5 Adaptive Cellular Immune Response

Studies on humans and chimpanzees have revealed that, unlike other viral infections, HBV infection is difficult to detect in the early infection period and that HBV-specific T-cell responses have been shown to be significantly diminished. The disease may progress symptomatically or asymptomatically after infection with HBV (20). The host's age and immune system condition are only two examples of the many variables that affect viral clearance. Adults, who normally have higher developed immune systems, may be able to completely remove the HBV infection.. et, the persistent infection is typically recognized in kids and people with weakened immune systems. The collaboration of elements of the endogenous, humoral, and cellular immune systems is necessary for an efficient management of the virus since inflammation and the ensuing liver damage are primarily mediated by inducing numerous immunological responses (21). Immune cells produce a variety of cytokines during acute HBV infection in the early stages of the illness, including IFN/, IFN-, and TNF-. Cell-mediated immunity, such as CD4+ assistant T-cells and CD8+ cytotoxic T-cells, are activated by the innate and adaptive immunological components and are crucial for the clearance of acute HBV infection. These cells take role in the elimination of viruses, but they are also linked to eventual liver injury (22).

Been proposed between the outcome of HBV infection and the response of CD4 + T cells. A high virus-specific CD8 + T cell response during the acute stage has been linked to viral clearance, and CD8 + T cells are essential for controlling and curing HBV infection. High viral load rates were linked to a lack of CD8 + T cells, whereas their presence in peripheral blood was linked to lower viremia and liver damage (23). Figure 1-4 shows the cells involved in the immune response to HBV.

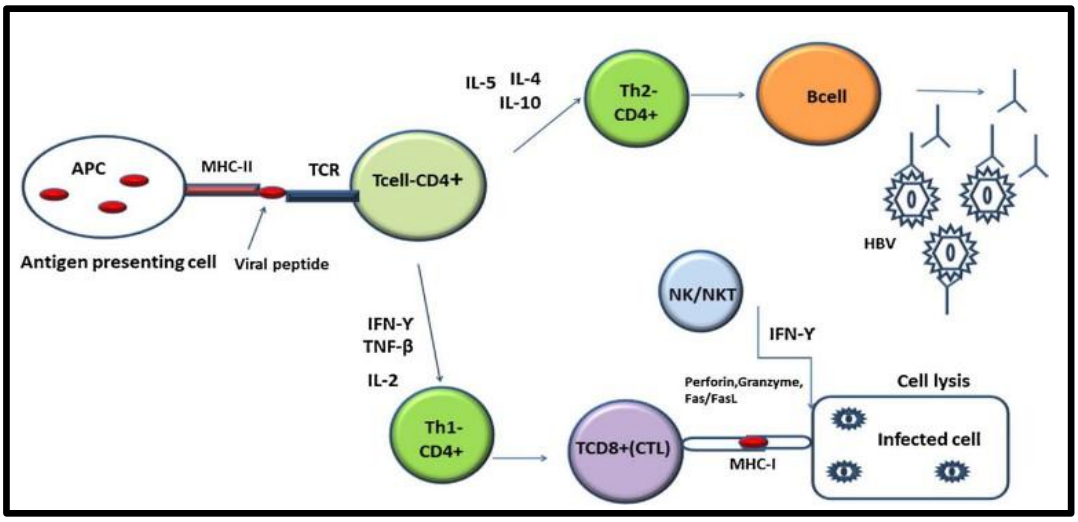


Figure 2-3: Different types of immune system cells involved in preventing infection with the hepatitis B virus. Antigen presenting cells (APC) accumulate viral antigens, which are introduced by MHC class II and MHC class I molecules to T-helper cells. Activated helper CD4 + T cells release various cytokines that are associated with B cell activation, NK cells, and NKT cells, and cytolytic T cell generation (CTL). Along with unique anti-virus antibodies, cellular responses can kill infected cells and neutralize virus particles (23).

3.1 Subjects

The WHO and European Association for the Study of the Liver (EASL) standards for HBV testing were followed. (Lampertico et al., 2017; WHO, 2017b). As a consequence, the HBsAg (hepatitis B surface antigen) antibodies found in 20 patient samples were identified.The patient's sera were also tested for total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase. (AST). 20 healthy blood donors (13 men and 7 women) were utilized as a control sample. Antibody profile serum status for them at the Central Blood Bank was negative.

3.2 Materials

Appendices provide a list of the apparatus, chemicals, biological materials, and kits that were utilized to complete the current investigation.

Apparatus and equipment used in this study (Table 3-1)

Equipment	***Company***	**Origin**
Centrifuge	Gemmyco	Taiwen
Micropipettes(different size).	Brand	Germany
Spectrophotometer	Optema	Japan
Freezer ELISA reader ELISA Washer Cotton Gloves White tube	Ishtar Human Human Jlassco Jlassco Jlassco	Iraq Germany Germany India India India
Spectrophotometer	**Unico**	**USA**

3.3 Blood Collection

Each participant had five milliliters of venous blood extracted, which was split into two aliquots. Serum was isolated from the first sample (3 ml), which was placed in a straightforward tube. The samples were spun for 15 minutes at 3000 rpm in a temperature-controlled centrifuge after being chilled for 15 minutes at 4°C to promote clotting. Prior to measurements of anti-virus antibodies and liver- function parameters, the separated serum samples were aliquoted into Eppendorf tubes and stored frozen at -20 C.

3.4 Methods

3.4.1 Serological Diagnosis of Hepatitis B Virus Infection

Anti-HBV antibodies in patient and control samples were qualitatively assessed using enzyme-linked immunosorbent assay (ELISA) kits. My BioSource Company (USA) produced the kits, and the manufacturer's instructions were followed

3.4.2 Anti-HBsAg Antibody Assay

The assay employs a qualitative sandwich ELISA technique. The microtiter plate provided with the kit was pre-coated with anti-HBsAg antibody. Standards and samples are pipette into the wells with a HRP-conjugated HBsAg antibody. Subsequent a wash to rub any unbound reagent, a substrate solution is added to the wells and the color appears in proportion to the amount of HBsAg. The color appearance is stopped and the intensity of the color is measured

Assay procedure

1. In accordance with the kit's instructions, reagents and samples were first let to sit for at least 15 to 30 minutes at room temperature (18 to 25 oC).Aliquots of 100 µl of serum samples, positive control, and negative control were placed in the appropriate wells. For 60 minutes, the plate was covered and incubated at 37
2. °C. Then each well received 50 µl of HRP-conjugate reagent. For 30 minutes, the plate was covered and incubated at 37 °C..
3. Each well cleaned five times with washing buffer at the conclusion of the incubation. The dish was placed on blotting paper and tapped to remove any leftover liquids after the last washing cycle..
4. Fill each well with 50 µl of chromogen A and 50 l of chromogen B after washing. The plate was covered and kept at 37 degrees Celsius in the dark for 15 minutes. 50 µl of stop solution were added to each well to stop the reaction. Within ten minutes after the reaction's termination, the absorbance of each well was measured at a wavelength of 450 nm.

3.4.3 Liver Function Tests

The sera of patients and controls were quantitatively tested for total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase using ready-to-use kits (Linear Chemicals, Spain) and following manufacturer's instructions. (AST). The four kits all used the same approach. (colorimetric method). The TSB was provided in terms of mol/L whereas the APL, ALT, and AST were published as units per liter (U/L).

4-1 Age and Gender of Hepatitis B Virus infection Patients Age

The age mean ± SD (standard deviation) of hepatitis B virus (HBV) infection patients was 42.2 ± 14.7 years, while control had a higher age mean (44.2

± 12.9 years). However, there was no significant difference between the two means (p = 0.208)

4-1-1 Age groups

Distributing HBV patients into age group revealed that > 40 year age group had the highest frequency (50.0%), while the age group < 30 year recorded the lowest frequency (20.0%). Among control, the age group > 40 years had the highest frequency (40.0%), followed by the age group 30-40 year and < 30 (30.0%). There was no significant difference between patients and control regarding gender distribution (p = 0.519) .

(Table 4-1- Distributing HBV patients into age group)

Age Group (year)	HBV patients (N = 20)		Control (N = 20)	
	N	%	N	%
< 30	4	20.0	6	30.0
30 – 40	6	30.0	6	30.0
> 40	10	50.0	8	40.0
Statistical analysis	Pearson Chi-Square = .622[a]		*p* =0.733	

4-1-2 Gender

Most of HBV patients were males (55.0%), while female patients accounted for 45.0%. Almost approximated frequencies were reported in control (65.0 and 35.0%, respectively). There was no significant difference between patients and control regarding gender distribution (p = 0.519)

Table 4-2: Hepatitis B virus infection patients and control distributed according to gender.

Gender	HBV patients (N = 20)		Control (N = 20)	
	N	%	N	%
Male	11	55.0	13	65.0
Female	9	45.0	7	35.0
Statistical analysis	Pearson Chi-Square=*0.0417*		*p* = 0.519	

4-1-3 Gender and Age

There was no significant difference between age means of HBV patients and control (p = 0.226) distributed according to gender. The age mean was 41.7 ± 14.6 year in male patients and 45.4 ±13.8 year in female control.

The results validated the matching between HBV patients and control in terms of age and gender. However, it was observed that individuals at the age range < 40 – 50 year were at a greater risk to be infected with HBV. In addition, males were also observed to have an increased risk to develop HBV infection. The male: female ratio was 1.7:1 among patients. Consistent with such observations, previous Iraqi studies also reported that most HBV patients was at the age less than 50 years, and male patients were more frequently observed than females (24,25,26). In addition, Arabian populations, as well as other worldwide ethnicities, reported similar findings (27,28,29). Accordingly, male gender and reproductive age might be considered as

risk factors for HBV infection. In a recent study, the association between several sociodemographic and medical variables and HBV infection was examined in 314 HBV-infected patients and 557 healthy individuals. Among the sociodemographic parameters, higher age, being male, lower economic status, and Family history of hepatitis, dental treatment history, and hospitalization were further risk factors (30). In our conservative society, multi-partner sexual behavior is mostly recognized in males of low educational status during their active age of sexuality. This may justify the high occurrence of HBV infection in males and age lower than 50 years.

Table 4-3 Hepatitis B virus infection patients and control distributed according to age , gender and Blood group

-Blood group and Rh	HBV numbers	HBV percentage (%)	Blood donors numbers	Blood donors percentage (%)
A	5	25.0	4	20.0
B	4	20.0	3	15.0
AB	4	20.0	5	25.0
O	7	35.0	8	40.0
Rh positive	15	75.0	16	80.0
Rh Negative	5	25.0	4	20.0
Total	20	100.0	20	20
Statistical analysis	Pearson Chi-Square=*0.575*			*p* =0.989

Hepatitis B infection routes through exposure to blood or body fluids that contain blood. Less than 0.01 ml of these secretions can cause infection. ABO blood groups are one group of agglutinogens (antigens), which are carbohydrate-forming compounds that are determined by genes and present on the outer envelope of erythrocytes (31,32)]. It has long been noted that blood group antigens have a role in determining the relationship between disease and different groups (33,34), the type O blood group and its relationship with duodenitis, which recorded double the infection number if compared with group A, B, while blood group A recorded the highest infection number within the tumors of the digestive system, on the other hand, compared with blood group O (35).

4-2 Serological Diagnosis of HBV Infection

The sera of HBV patients were qualitatively screened for three antibodies; anti-HBc (hepatitis B core antigen) IgM, -HBc IgG and -HbsAg (hepatitis B surface antigen) antibodies. The results revealed that all patients were seropositive for anti-HBc IgG and -HbsAg antibodies, while they were seronegative for anti-HBc IgM antibody. Such profile is consistent with the interpretation of HBV serology in the diagnosis of chronic HBV infection ((13) Lamper. Accordingly, the infection in present sample of HBV patients was considered chronic. It is worth to mention that all participants of control sample were seronegative for these antibodies.

4-3 Liver-Function Tests

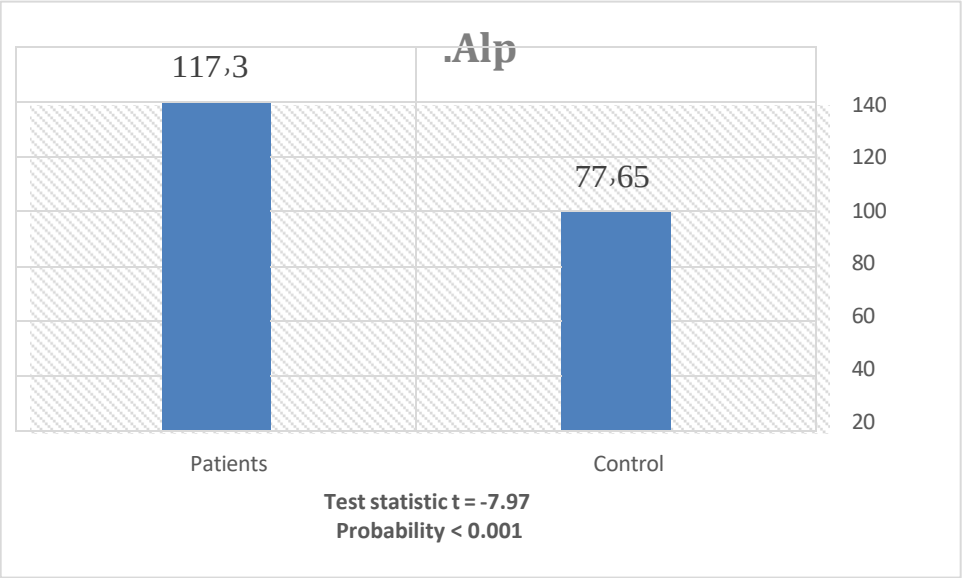
Sera of HBV patients and control were quantitatively assessed for four liver- function tests (LFTs); alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The serum level of these parameters was given as median and range, because normality testing (Kolmogorov-Smirnov

and Shapiro-Wilk tests) revealed a non-normal distribution. Therefore, non- parametric tests were applied to assess significant differences between medians (36).

4-3-1 Alkaline Phosphatase (ALP)

Serum median of ALP showed a significant increase in HBV patients compared to control (117.3 vs. 77.65 U/L; $p < 0.001$). The age groups and male and females of patients also showed a significant increased median of ALP compared to the corresponding groups in control. The age groups of patients or controls showed no significant variation between them. However, females of patients and control showed an increased median compared to the corresponding males, but the difference was significant among control only ($p = 0.011$) (Tables 3-5 and 3-6).

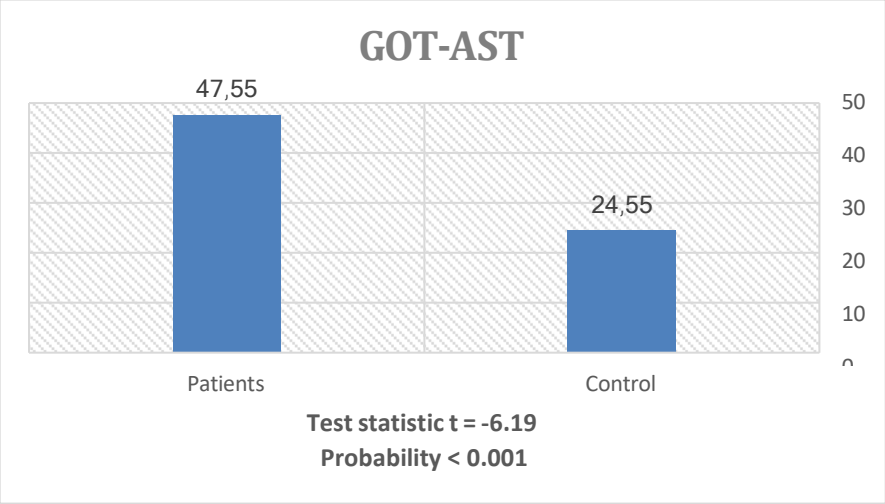
Table 4-4 presentation of alkaline phosphatase in sera of hepatitis B virus infection patients and control.



4-3-2 Aspartate Aminotransferase (AST)

The serum median of AST was significantly increased in HBV patients compared to control (427.55 vs. 24.55 U/L; $p < 0.001$) (Figure 3-6). Patients distributed according to age groups or gender also showed a similar significant increased median compared to the corresponding groups in control. The age groups of patients or controls showed no significant variation between them.

Table 4-5 presentation of aspartate aminotransferase in sera of hepatitis B virus infection patients and control. Error bars represents standard deviation.



4-3-3 Alanine Aminotransferase (ALT)

Serum median of ALT showed a significant increase in HBV patients compared to control (50.15vs. 29.95 U/L; p = 0.001).

Table 4-6 alanine aminotransferase in sera of hepatitis B virus infection patients and control. Error ars represents standard deviation

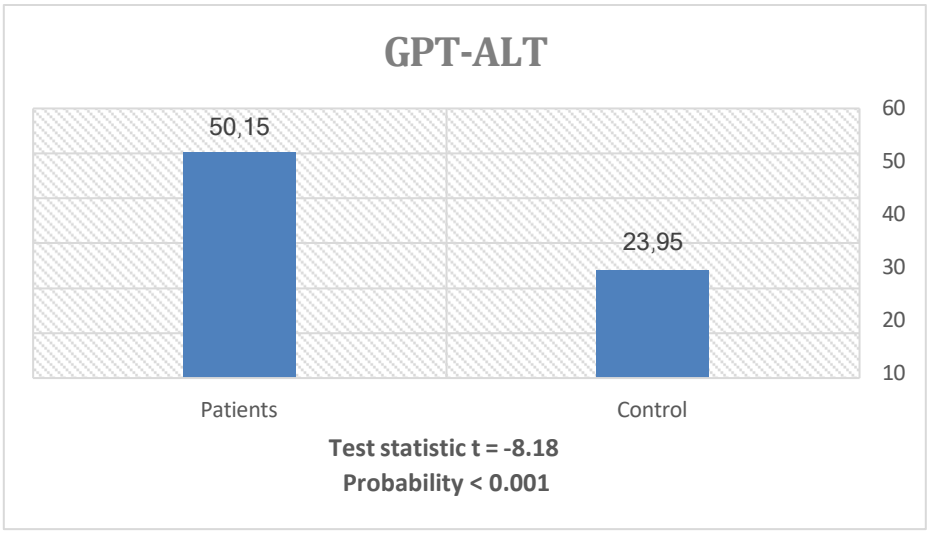
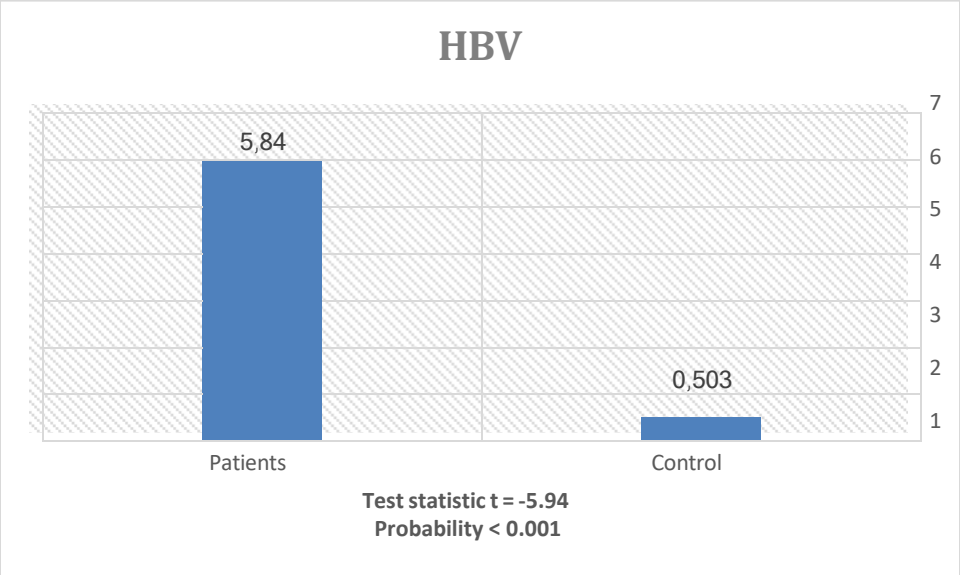


Table 4-7 Positive HBV and negative HBV



The obtained results highlighted the diagnostic potential of LFTs in chronic HBV infection and their order of significance was AST, ALP and ALT. Whereas, TSB failed to show any related significance. Therefore, the biomarker expectation of AST, ALP and ALT is suggested in the present study. Different biochemical markers of diverse clinical and epidemiological potential are augmented when HBV infection is established. These markers can occur independently or in different combinations; this is dependent on the disease stage and its natural history (37). Among these biomarkers are LFTs, which are important in the evaluation of hepatic function and disease severity in HBV infection patients. As in present study, it has been depicted that HBV infection may alter serum levels of certain hepatic enzymes (i.e. ALP, AST and ALT) (38; 39). In this context, marked elevation of ALT in sera of chronic HBV patients with acute flare-up has been suggested (40). The release of AST and ALT into the bloodstream is a consequence of hepatocellular damage due to HBV infection; so an elevation of both enzymes is more frequently correlated with hepatic injury. With respect to ALP, its level has been significantly associated with HBsAg seropositivity (41); an observation that is supported by the present findings, because all HBV patients were seropositive for HBsAg and marked with a significant increased ALP level. A similar conclusion can be drawn for AST and ALT, and an increase in the levels of both enzymes has been reported in HBsAg-positive patients (42). Although, AST, ALP and ALT were associated with HBV risk and their diagnostic values were suggested, they may represent an initial non-specific testing of HBV infection and specific diagnosis of HBV infection must involve the evaluation of further specific HBV serological markers that include certain HBV antigens and antibodies (41).

For TSB, its serum level showed no significant variation between HBV patients and control; therefor such serum protein might not be related to HBV chronicity. Such findings might be expected, because increased levels of TSB are generally associated with acute HBV infection rather than chronic infection (43). It has been suggested that bilirubin might be implicated in the protection of specific kinds of diseases resulting from oxidative damage, and appeared to have the innate capacity to resist oxidative damage that occurs during acute HBV infection (44).

5- Conclusions and Recommendation 5-1 Conclusions

The study reached the following conclusions:

1. The chronic HBV infection tended to be more prevalent in males than in females.
2. The four parameters of liver functions were influenced by the HBV infection, but from the diagnostic view points, alkaline phosphatase (ALP) and aspartate aminotransferase (AST) recorded an excellent area under curve in the receiver operating characteristic analysis; therefore, their diagnostic significance is enhanced.

5-2 Recommendation

he current outcomes recommends future further research studies as follows:

1. More definite conclusion about the effect of the genetic variation infected with different HBV genotypes.
2. Re-evaluation regarding HBV genotypes is necessary by using DNA sequencing this will help to know changes in the geographical distribution of viral patterns over time

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