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EVALUTION OF SOME BIOCHEMICAL MARKERS IN SERUM AND SEMN IN MALE REPRODUCTIVE WITH HBV-ALFALLUJA CITY

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EVALUTION OF SOME BIOCHEMICAL MARKERS IN SERUM AND SEMN IN MALE REPRODUCTIVE WITH HBV-ALFALLUJA CITY

By Ali Rasool Allawi ALISAWI

April 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

EVALUTION OF SOME BIOCHEMICAL MARKERS IN SERUM AND SEMN IN MALE REPRODUCTIVE WITH HBV-ALFALLUJA CITY

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Master of Science in Chemistry

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Our goal will be to assess the risk of male infertility based on sperm count and some reproductive hormones in individuals with (HBV) infection. The age distribution of 90 newly diagnosed and treated hepatitis B virus (HBV) patients revealed that fifteen (50 in newly diagnosed) patients (84%) were under the age of 35, eight patients (8) were over the age of 35, and seventy-four percent (74%) of the treatment group were under the age of 35. There was a highly significant increase in AST and ALT and an increase in ALP, TBI, and DBI, in comparsion to healthy subject ($P<0.01$). Infertile HBV and control groups evaluated their testosterone, IL-6, and Selenium levels in a univariate method. Il-6 indicators were associated with sperm count, motility and the percentage of healthy spermocyte. All of the research groups' spermiograms revealed a strong negative connection with serum IL-6. Only the activity of sperm of the oligozoospermics and the total count of the healthy control subtect were positively linked to serum selenium. The percentage of sperm and testosterone levels were found to be connected. Unlike previous research, the seminal spermiograms in both groups had a significant relationship with oligozoospermic, asthenozoospermic linear activity, and the fraction of normal sperms.

2022, 34 pages

Keywords: HBV-Infection, Men reroducteive, Male infertility, Sperm infection, Sperm parameters, Azospermia

ÖZET

FELLUCE ŞEHRİNDE HBV ENFEKSİYONU ILE ERKEK ÜREMESİNDE SERUM VE SEMENDEKİ BAZI BİYOKİMYASAL BELİRTEÇLERİN DEĞERLENDİRİLMESİ

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Nisan 2022

Amacımız, hepatit B virüsü (HBV) enfeksiyonu olan bireylerde sperm sayısı ve bazı üreme hormonlarına göre erkek kısırlığı riskini değerlendirmek olacaktır. Yeni tanı konmuş ve tedavi edilmiş 90 hepatit B virüsü (HBV) hastasının yaş dağılımı, on beş (yeni tanı almış 50) hastanın (%84) 35 yaşın altında, sekiz hastanın (8) 35 yaşın üzerinde olduğunu ve tedavi grubunun yüzde yetmiş dördü (%74) 35 yaşın altındaydı. Kontrol grubuna kıyasla AST ve ALT'de oldukça önemli bir artış ve ALP, TBI ve DBI'da bir artış vardı ($P<0.01$). İnfertil HBV ve kontrol grupları testosteron, IL-6 ve Selenyum düzeylerini tek değişkenli bir yöntemle değerlendirdi. Il-6 göstergeleri, sperm sayısı, hareketlilik (doğrusal aktivite) ve normal sperm yüzdesi ile ilişkilendirildi. Tüm araştırma gruplarının spermiyogramları, serum IL-6 ile güçlü bir negatif bağlantı ortaya çıkardı. Sadece oligozoospermiklerin aktif spermleri ve kontrol grubunun sayısı serum selenyumu ile pozitif olarak bağlantılıydı. Sperm yüzdesi ve testosteron düzeylerinin bağlantılı olduğu bulundu. Önceki araştırmalardan farklı olarak, her iki gruptaki seminal spermiyogramlar, oligozoospermik, astenozoospermik lineer aktivite ve normal spermlerin fraksiyonu ile önemli bir ilişkiye sahipti.

2022, 34 sayfa

Anahtar Kelimeler: HBV-Enfeksiyonu, Erkek üreme, Erkek kısırlığı, Sperm enfeksiyonu, Sperm parametreleri, Azospermi

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Ali Rasool Allawi ALISAWI

Çankırı-2022

CONTENTS

LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees celsius
µmol	Micromoles
dL	Deciliter
g	Gram
IU	International unnits
kg	Kilogram
L	Liter
m^2	Square meters
mg	Milligram
mL	Milliliter
ng	Nanogram
µL	Microliter

LIST OF ABBREVIATIONS

A	Absorbance
ADMA	Asymmetric dimethylarginine
ANOVA	Analysis of variance
BMI	Body mas index
CBC	Complete blood counts
DHT	Dihydrotestosterone
DNA	Deoxy neucleic acid
EIZA	Enzyme Immunoassay
IL-6	Inter lukin-6

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1. INTRODUCTION

An infertile case refers to a couple who have been married for one year and have not used contraception or other methods of contraception. According to estimates by the WHO, there are an estimated more than 75 million of infertile in globally, most of whom live in developing countries. 17.2% of Iranian couples are infertile. Percent of Iranians were found to be infected with HBV, while 0.16 percent of Iranians were found to be infected with HBV. Chronic hepatitis can cause cirrhosis, hepatic cancer, and even death if left untreated (Kumar and Singh 2015).

This virus, which produces acute and long- term hepatitis B as well as liver disease and cancer in some cases., is the most important factor in spreading these diseases in people (HBV). About 2 billion people have contracted the virus worldwide. Three hundred and fifty million of them have a prolonged illness, which allows the virus to spread (Liaw and Chu 2009). Men are twice as likely to limit the disease in Northern Europe, where the proportion is less than 1%. This study was conducted by (Hall 2006). Infected individuals can spread hepatitis B by body fluid including such seminal fluid (sperms), vaginal fluid, and saliva, all of which are present in the majority of infected individuals (Jennison 1987).

Sexual contact can transfer the causative agent of hepatitis B, thus couples should be immunized against the virus before engaging in sexual activity (Scott et al. 1980). Even in the case of infertility treatment, if a problem with the liver is discovered during the operation, hepatitis B is treated. However, in this particular instance, the virus itself does not serve as a dependable indicator of the need for surgical assistance.

According to current studies, the hepatitis B virus can harm the function of human spermatozoa. Reduced progressive, nonlinear motility and penetration of the oocyte are some of these consequences (Zhou *et al.* 2009). HBV can also incorporate sperm cells' DNA and cause mutations; as a result, it is believed that hepatitis B virus can be transferred vertically down the germline from one generation to the next.

1.1 Objective

Evaluation of HBV infection has been studied for its implications on sperm parameters, hormone alterations, and immune system abnormalities.

1.1.1 Specific

Aim: An investigation of the prevalence of male infertility among individuals with Tb disease in AL-Falluja was conducted,

1.1.2 Secondary

1. To investigate the relationship between sperm parameters (vitality, activity, morphology) and testosterone levels in patients with HBV infections.
2. Patients with HBV infections were studied to determine the relationship between sperm parameters (vitality, activity morphology) and several clinical pathological factors such as age, gender, body mass index (BMI), and immunological tests such as IL6 and hs-CRP.
3. To characterize the relationship between sperm parameters (vitality, activity, and morphology) and lipid profiles, liver function, and zinc levels in individuals with HBV infections.

2. LITERATURE REVIEW

2.1 Hepatitis

Hepatitis is a medical disorder in which the liver becomes inflamed and becomes infected. Many distinct types of clinical-pathological problems that can occur in the liver as a result of viral, toxic, metabolic, pharmacological or immunologic liver damage are grouped under the name "hepatocellular carcinoma". Viral hepatitis is a disorder in which the body becomes inflamed as a result of an infection. It has the greatest impact on the liver. Aside from that, there are two kinds of infections: acute and chronic. Acute infection occurs when the virus is still relatively fresh and spreads swiftly, whereas chronic infection occurs when the virus has been present for a lengthy period (Neuman *et al.* 2006).

There are five known hepatitis viruses, two of which are HAV and one of which is HEV. Most of these viruses originate in the digestive tract and can cause both sporadic infections and epidemics of hepatitis. It's one of the most common hepatitis viruses. For a few days or a few weeks, they can induce acute viral infections (AVH). Hepatitis B and C viruses (HBV and HCV) are responsible for chronic hepatitis in persons. Conducted a study, These viruses have many similarities in terms of structure, epidemiology, transmission methods, incubation period, clinical symptoms, natural history, diagnostics, prevention, and treatment options. They also share many similarities (Chan *et al.* 2011).

People who have acute viral hepatitis typically recover on their own within a few weeks with no long-term consequences. Acute hepatitis caused by HBV, HCV, or HDV infection can strike at any time. Researchers (Nasiruddin *et al*. 2017) conversely, these viruses are also capable of causing a long-term infection in a subset of those infected. Long-term liver damage, such as cirrhosis or hepatocellular carcinoma (HCC), may be linked to this infection, and these studies are similar to (Seo *et al.* 2014).

2.1.1 Defination of HBV chronic infection

Hepatitis B virus infection is still a leading source of disease and mortality among the worldwide people, despite recent breakthroughs in treatment and prevention techniques. (Papatheodoridis *et al.* 2017).

Virus of HBV is the major reason for chronic disorder in many organ espicially liver cancer in several nations, including the United States and the United Kingdom. 2 billion people have been afflicted with HBV in worldwide. Also HBV surface antigen (HBsAg) can be found in 350 to 400 million persons worldwide, including those who have had the virus in the past or have it today (Stuart *et al.* 2018).

HBV contain a cyclic double-stranded DNA virus that causes hepatitis B infection. It infects hepatocytes and produces changes in the liver that are harmful to the body's overall health and function. It is a member of the orthohepadnavirus family of the Hepadnaviridae, which is related to the hepadnaviridae (Urban *et al.* 2010). DNA and the enzyme DNA polymerase are found in its nucleus, which is necessary for viruses to multiply in the body and replicate in the nucleus. The virus has a protective protein covering outside the virus. The virus, also referred to as the Danish particle, travels through the bloodstream with a large amount of the virus's protein on its surface, which is likewise on the surface of the virus's surface (known as hepatitis B surface antigen, HBsAg). The only thing that can make you sick is another human being (Stuart *et al.* 2018).

HBx protein, HBsAg, HBeAg, and HBV polymerase encode in open reading frames. It's the same for each one. Virus nucleocapsids are liberated when HBV enters the cell's cytoplasm. All virus genes are transcribed from the nucleus using the template provided by the single-stranded circular DNA (cccDNA) maintained. Reverse transcriptase is required for viral spread. Viruses have 3.5-kilobase transcripts, where this enzyme comes into play (pre-atomic RNA). Interaction with envelope proteins, such as those present on offspring viruses, can release RNA from viral capsids. It is also possible to transfer RNA to the nucleus to replenish the pool of DNA (Stuart *et al.* 2018).

2.1.2 HBV transmission

There is a virus that causes hepatitis B, and it can spread worldwide. According to estimates, and over 300 million of people are infected with chronic hepatitis B. (CHB). More than 2 billion people have indeed been infected worldwide, according to the latest estimates (Ott *et al.* 2012). Infection of HBV is the second most harmful human carcinogen after cigarettes in terms of mortality rates among those infected, with up to a quarter succumbing to the disease That's what it says.

Hepatitis B affects a large percentage of the world's population, with over a billion individuals infected (defined as more than 2 percent). More than half of them are in areas characterized by a high frequency of hepatitis B. The graphic shows that 2.3. According to (Cohen *et al.* 2011)

Hepatitis B virus is found in Iraq, according the World Health Organization, with an average frequency of 3.2 percent, according to the World Health Organization. The frequency of HBsAg in the overall people has grown significantly over time, and this trend is expected to continue (Ataallah et al. 2011). The increasing propagation of the hepatitis B virus may be one possible explanation for the increase in prevalence observed. According to figures from the Iraqi Ministry of Health, the disease spread to even more cities in 2016, with Dohuk having the most number of infections and Muthanna got the least number of infections, according to the Ministry of Health (Hussein *et al.* 2017).

2.2 The Male Reproductive Tract and Semen with HBV

2.2.1 Spermatogenesis

Sperm, semin fluid, prostatic fluid, and protein generated from of the male sex system make up semen (male reproductive organs). There are seminiferous tubules in the testicles that create sperm, which develops and is then deposited in the endometrium of

the testicles. Mature sperm move through the vas deferens after being stimulated. With seminal vesicle fluid and prostate fluids from the prostate gland, they form a concoction they enter the uterus via the urethra after passing via the penis and urethral valve (Peate and Nair 2015). It's clear from this. By secreting mucus, the bulbourethral gland, commonly referred as the cooper gland, helps to increase sperm production (Flint *et al.* 2015, Riva et al 2012).

The seminiferous tubules of the testicles are responsible for the production of sperm. Sperms are immature germocyte that develop from the basement membrane of the seminiferous tubules. Sperm are created by the spermatogenic organs of the man. After mitosis, they form primary spermatocytes, which are the cells that produce sperm in the uterus. The blood-testis barrier (BTB), which is responsible for separating the blood from the testis, comprises Sertoli cells. The formation of spermatocytes and secondary spermatids follows. They pass via the BTB and into the seminiferous tubules, where they develop into tail sperms that deposit in the lumen of the tubes. They pass through the BTB and into the ovaries (Shinohara *et al.* 2003).

In addition to the follicle-stimulating hormone (FSH) and testosterone generated by Granulosa cells in the interstitial fluid of Sertoli cells with in germline cell layer seminiferous tubules, the anterior pituitary glands also produce luteinizing hormone (LH) and estrogen. The endocrine system glands are responsible for the production of Follicle-stimulating hormone (Costabile 2013)

2.2.2 Immune systems in the testicles

There is a blood-testis barrier that protects the testicles from the body's own immune system (BTB). For nourishment and hormones, the BTB serves as a buffer between spermatogenic germ cells cells and post-meiotic germinal cells in the interstitium because of its physical location (such as spermatocytes & spermatids). Immune reactions can occur when antigens from post-meiotic germinal cells are exposed outside of the BTB (Cheng and Mruk 2012).

The BTB prevents anti-sperm antibodies from forming, causing male infertility. Sertoli cells can decrease immune responses to transitory antigens by producing immunosuppressive cytokines and prostaglandins Only Sertoli cells with gap joints, adhesion junctions, and desmosomes coexist in the BTB, leading to dynamic ultrastructure that allows germinal cells to flow into the lumen (Cheng and Mruk 2012).

BTB regulates drug entry into the lumen by segregating the interstitial blood vessels, lymphatics, and neurons. In spermatogenesis, the proliferation and death of germinal cells are timed to ensure that a critical number of germinal cells and Sertoli cells are maintained. In the mature mammalian testis, apoptosis kills up to 75% of the germinal cells. Apoptotic and damaged germ cells are phagocytosed by Sertoli cells before they induce inflammation (Nakanishi and Shiratsuchi 2004). When the antigen is present, testicular resident macrophages generate anti-inflammatory cytokines such as IL-3, IL-4, and IL-6 to counteract the detrimental effects of immune cells on sperm production (Hedger *et al.* 2011). Leydig cells create androgens necessary for sperm production. Several androgens, like testosterone and luteinizing hormone, are well-known for keeping BTB stable.

2.2.3 Exposure to HBs in sperm cells

The male sperm plasma membrane is crucial to a woman's ability to conceive. Membranes are critical to cell metabolism, sperm motility, acrosome response, and sperm-oocyte interactions. Evidence suggests that reactive oxygen species (ROS) are responsible for sperm destruction. After 3 hours of exposure to 25 g/mL of HBs, ROS-positive cells rose significantly (P 0.01). HBs monoclonal antibody and ROS absorber NAC were used to incubate sperm cells. A rise in reactive oxygen species was observed in sperm cells after exposure to HBs, according to these findings. HBs exposure also resulted in ROS formation, and this was dose-dependent, as we found out (Schuh *et al.* 2004).

Recent studies have shown an enhanced loss of motility and an adverse effect on sperm functions when the cells are incubated in the presence of HBV and HBV Abs According

to current studies, HBs Ab greatly reduced ROS generation in sperm cells and enhanced sperm function, which is at odds with this statement. Distinct HBs MAb could have played different roles, explaining the discrepancy. One side of HBs MAb inhibits HBs biological activity directly, by decreasing reactive oxygen species in sperm cells (Garolla *et al.* 2013)

In numerous investigations, the reduction of sperm motility was accompanied by an increase in gamete fusion and the ability to execute acrosomal exocytosis, which was both observed, both caused by an increase in the membrane's permeability due to sperm lipid peroxidation A lipid peroxidation comes from infection by HBV, therefore, after being treated for three hours to HBs at a 50 g/mL concentration, it considerably increased MDA levels than the control group in this study ($P<0.05$). As HBs concentrations rose, so did MDA levels. These data suggested that his exposure may cause sperm cell lipid peroxidation (Gadella and Harrison 2000).

2.2.4 Apoptosis of sperm cell

Apoptosis is widely recognized to function in physiological and pathological processes Men's infertility and seminal quality issues such as mobility, survival, and sperm abnormalities have been linked to a malfunctioning apoptotic system The connection between HBs exposure and sperm cell apoptosis in a cell culture model was investigated using annexin-V staining, TUNEL for DNA fragmentation measurement, and fluorescently tagged caspase inhibitors for caspase activation detection. This study showed an after 3 hours of exposure to 25 g/mL HBs, a statistically meaningful change in the proportion of annexin V-positive or PI-negative cells was observed ($P<0.01$) (Shukla *et al.* 2012).

HBs exposure maybe leads to sperm cell death. The presence of caspases indicates the onset of early-stage apoptosis. When apoptosis is induced, it can convert a class of inactive pro-form proteins known as caspases into active enzymes through cleavage. This work investigates the effects of HBV exposure on sperm cell caspase activation. Caspases-3, eight and 9-positive cells were found in much higher numbers in cells that

had been exposed to HBs compared to cells that had not been exposed to the substance. Discovered that HBs had a dose-dependent influence on the activation of caspases in sperm cells. Apoptosis is a cell death marked by DNA fragmentation in many different cell types.

2.3 Clinical Pathological of Male Infertility with HBV

According to sperm preparation, men infected with HBV had spermatozoa that were less motile than healthy men. Two of the 15 HBsAg-positive subjects studied had severely low sperm counts, while the remaining 12 showed a moderate but statistically propotional correlation increase in asthenozoospermia in compartion to healthy subject. As a result of the spermatozoa, the incidence of necrosis or apoptosis increased (Cito *et al.* 2021).

Demonstrated no differences in sperm viability across groups, indicating that this could not be the source of the lower motility observed in this study. According to one idea, HBV infection results in a reduced in the mitochondrial membrane potential of sperm, which has been demonstrated in vivo in a person (Lee *et al.* 2010).

Greater virus loads decreased sperm motility. When detectable viral load is high, there's a better probability of discovering virus in the sperm, but it's possible to find the virus in the sperm even if plasma viral replication is minimal (Zhou *et al.* 2009).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Instruments

Table 3.1 contains a complete list of all of the instruments used in this inquiry, as well as the names of the companies that made them.

Table 3.1 A list of instruments and the firms that create them is provided below

NO.	INSTRUMENTS	COMPANY
1	ELIZA units	Biotek-USA
2	Spectrophotometer	Cecil CE 7200-France
3	Centrifuge	Eppendorf-Germany
4	Micropipette	Gilson-France
5	Oven	Kablkolb , Germany
6	Refrigerator	National-Japan
7	Microscope	Optic

3.1.2 Chemical kits

List of all of the kits that were used in this investigation with Table (3.2).

Table 3.2 Kit used and company

NO.	TEST KITS	COMPANY	ORIGIN
1	ALB	Linear Chemicals	Spaın
2	ALP	Human	Germany
3	ALT	Randox	Unıted Kingdom
4	Testosterone	Human	German
5	AST	Randox	United Kingdom
6	Selenuim, zinic	Linear Chemicals	Spain
7	GGT	Human	Germany
8	IL-6	MyBioSource	USA
9	Lipid profile	Linear Chemicals	SPAIN

3.2 Study Subjects

Fifty newly diagnosed HBV cases and fifty acute infections after 3-6 months of HBV infection are included in the study. Fifty healthy volunteers (not infected) came to the Gastroenterology and Hepatology Teaching Hospital Baghdad Health Ministry for data collection December 2021 to February 2022. Blood samples were obtained from the venous system. As a result of centrifugation, the fractions were separated and frozen at 70°C. Aspiration blood samples (10 mL) were taken in the morning, and 8 mL was put in a gel tube and left to coagulate for 10 minutes at room temperature before centrifugation at 1800 xg for 5 minutes. As a result, The current study included fifty healthy volunteers of a similar age.

3.2.1 Exclusion criteria

1. Another type of infections.
2. Infection with the human immunodeficiency virus
3. Alcoholic and nonalcoholic fatty liver disease
4. Coronary artery illness
5. A family history of hepatitis
6. Diseases of the urinary tract, such as rheumatoid arthritis, diabetes, obesity, and cancer, are all examples of chronic kidney disease.

3.3 Design of Study

This project is case control.

3.4 Sample Specimen and Prepration

There were five milliliters of blood drawn from infertile men and healthy controls. Then, the blood was placed in a tube lacking anticoagulants and left out at room

temperature for 30 min. The blood samples were centrifuged at 1000–2000 x g for about 10 minutes. It was stored at (–20 C) until it was required.

3.4.1 Collection of semen and seminal plasma preparation

It must have been necessary to collect sperm specimens from both groups. After 72 hours of abstinence, masturbation was employed to collect all specimens, which were evaluated around one hour of collection. The concentration of sperm, the percentage of motile & healthy cells, and the time period that had passed since the sperm had been frozen were all evaluated by the process of semen analysis. The semen cutting fluid can be used to measure sperm concentration after semen samples have been diluted with formaldehyde (40%) and distilled water. In the Neubauer holding chamber, which was filled with diluted material, the sperm were counted under a microscope. Sperm motility can be assessed by determining how many spermatozoa reached the limit of the measuring cylinder (sum of grade a and b sperms). To liquefy the samples, they were subjected to a 15-minute centrifugation procedure at 15000 x g. From the supernatant, seminal plasma was carefully extracted and put to Eppendorf tubes. To prevent it from freezing, the semin plasma was kept at a temperature of -20°C.

3.4.2 Determination of ALP, GOT, GPT, by enzymatic colorimetric

Magnesium and zinc, an appropriate substrate concentration, plus 2-amino-2-methyl-1-propanol as a buffer have been used to determine alkaline phosphatase ALP. IFCC recommends this assay, which is explained in details paragraphs by the IFCC. ' Use a regular procedure when doing a colorimetric assay. Phosphatases break down p-nitrophenyl phosphate from phosphate and p-nitrophenol when magnesium and zinc ions are present in the reaction. High levels of p-nitrophenol synthesis suggest active ALP. The rise in absorption at 409 nm is used to determine it.

The GOT/AST transaminates L-aspartate and L-ketoglutarate into acetate and L-glutamate in the test reaction. The enzyme malate dehydrogenase is required for the

conversion of oxaloacetate to malate and NAD to NAD when malate dehydrogenase is present. At 340 nm, the device measures the rate at which the absorbance changes over time. The AST activity of a sample is directly related to the rate of change in absorbance.

GPT/ALT transaminates L-alanine and L-ketoglutarate to pyruvate as well as L-glutamate in the test reaction. Pyruvate gets oxidized to NAD and NADH is converted to lactate when LDH is present. Absorbance changes at 340 nm are tracked over time by the system. When the sample contains a high level of ALT activity, the rate at which absorbance changes decreases.

3.4.3 Estimation of testosterone, IL-6 by ELISA

Testosterone ELISA, standards, quality controls and samples incubated in pre-coated microplate wells using polyclonal antibodies against testosterone and interleukin-6 (IL-6). After washing, HRP-labeled monoclonal anti-hormone, IL-6 antibody is incubated at room temperature for an additional 60 minutes with collected testosterone, IL-6. The conjugate can bind to the substrate solution after a second washing procedure (TMB). Adding an acidic solution to the mixture will stop the reaction once we have determined much of the yellow constituent has been absorbed. Absorption increases in direct proportion to testosterone and IL-6 levels. Absorbance measurements are plotted against normal concentrations to create a standard curve, and the unknown chemicals' concentrations are calculated.

3.4.4 Determination of ZINC and selenium in plasma and semen

Basic principle: The amount of zinc present in the sample influences the intensity of the color produced by the chromogen-zinc reagent reaction.

- Selenium Assay Kit. Cat# abx298910.Supplier: abbexa.

- Selenium Assay Kit is an assay kit designed for the detection and quantification of Selenium concentrations.

3.5 Statistical Analysis

Case-control research was used to conduct this investigation. The Student's t-test can be used to detect not whether groups are statistically significantly different. Significative results are those in which the P-value falls below 0.05. In this experiment, we used Pearson's correlation coefficients to analyze the relation between independent variables.

4. RESULTS

4.1 Clinical Pathological Study

1. The study looked at risk factors for the beginning of hepatitis B virus infection in the first of three study phases.
   - Men in the control group (N=50) who had no illness
   - Newly diagnosed men with HBV (N=50) were included in the study.
   - Acute infection after 3-6 months of illness 40 (treatment group).
2. Part two is an analysis of the biochemical composition of the blood of each of these individuals.
3. These groups' blood biochemistry essays are the third portion of this section in seminal fluid.

4.1.1 Age and HBV studeied

Table 4.1 shows how the ages of 90 people with newly diagnosed and treated hepatitis B virus (HBV) show that 84% of those in newly diagnosed groups were under the age of 35, while only 16% were over the age of 35, and 74% of those in the treatment group were under 35, as shown in this study's age distribution (Figure 4.1).

Table 4.1 Age mean SD and age frequency 35 in men without infection, newly diagnosed, and treated

GROUPS		**Number %**	**MEAN ± SD**	**SIG.**
Healthy Control group men without infection (NP = 50)	Age <35	33 (65%)	33.68 ± 3.30 (Range 23-35)	0.001
	Age >35	17 (35 %)	42.9 ± 4.05 (Range 36-44)	
	Total	50	34.06 ± 3.25	
Newly diagnosed men with HBV (N= 50)	Age <35	42 (84%)	32.66 ± 6.82 (Range 26-35)	0.0031
	Age >35	8 (16%)	40.75± 7.55 (Range 38-51)	
	Total	50	35.22± 7.11	
Treatment group (N= 50)	Age <35	37 (74%)	29.38 ± 4.48 (Range 24-33)	0.002
	Age >35	13 (26%)	58.16 ± 9.03 (Range 36-44)	
	Total	50	29 ± 11.30	

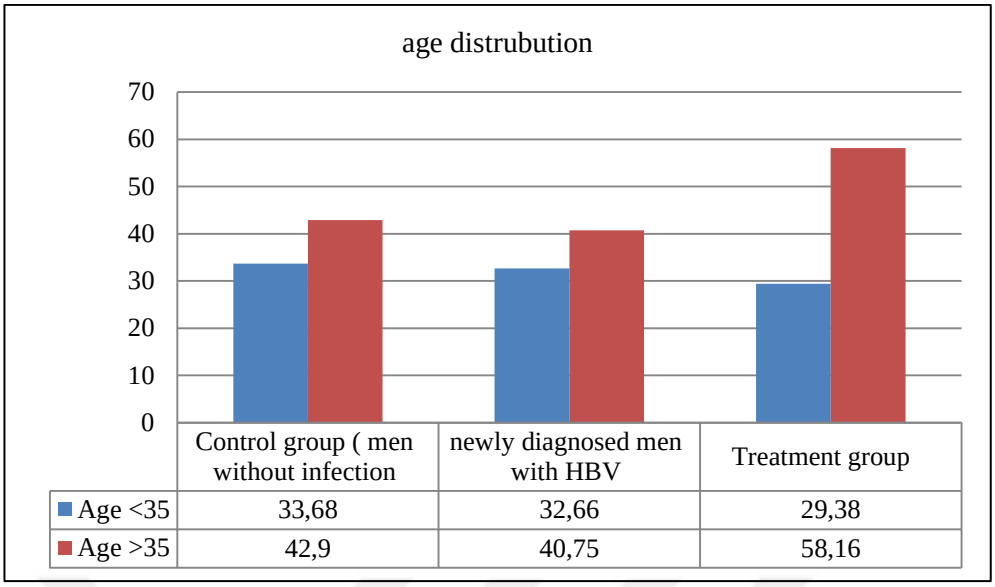


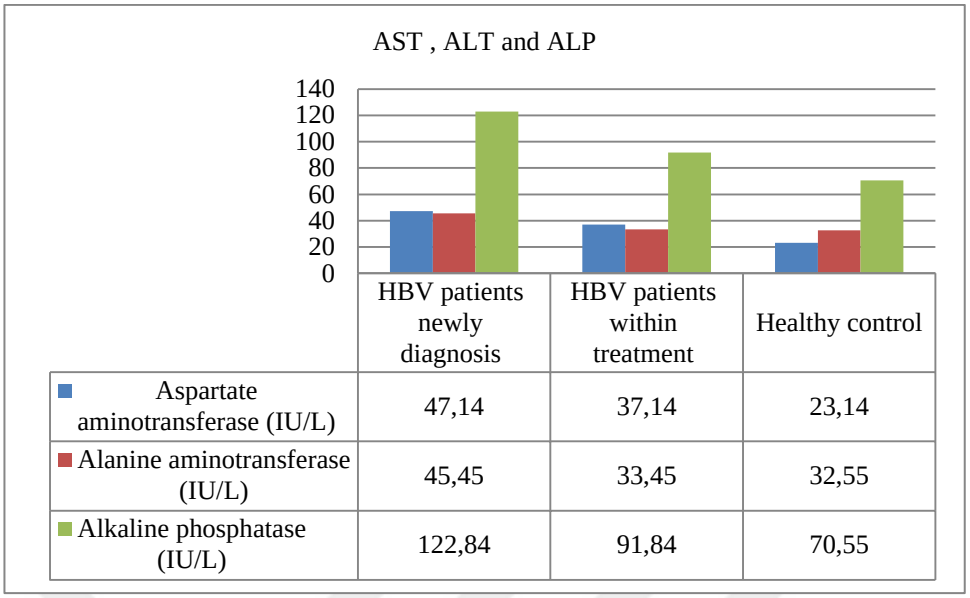
Figure 4.1 The age range of men throughout the control, HBV (newly diagnosed), and therapy groups

4.1.2 Liver function test

Participants with hepatitis B had greater transaminases (AST) & ALT levels than those without the condition. Patients with hepatitis B had considerably higher AST/ALT ratios than healthy individuals. Table 4.2, Figure 4.2, and Figure 4.3 demonstrate substantial increases in hepatitis B serum AST and ALT and ALP, TBI, and DBI, as shown significantly (P<0.01).

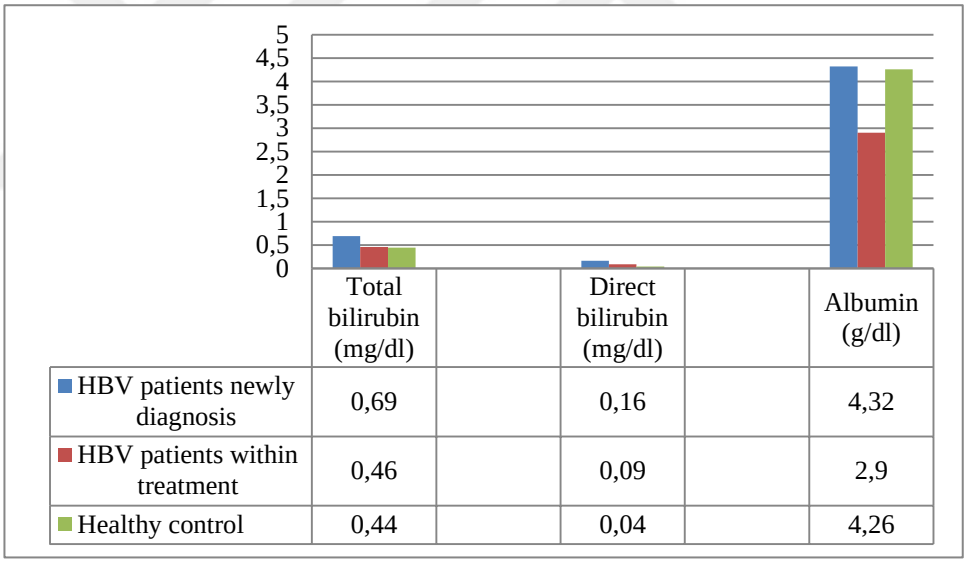
Table 4.2 Mean SD of transaminase enzyme and alkaline phosphatidase, serum bilirubin, and directly bilirubin in patients with chronic hepatitis B and control subjects

PARAMETARS	**HBV PATIENTS NEWLY DIAGNOSIS (N= 50)**	**HBV PATIENTS WITHIN TREATMENT (N= 50)**	**HEALTHY CONTROL (N= 50)**	**P-VALUE**
	Mean ±SD			
AST (IU/L)	47.14±16.60	37.14±16.60	19.14±5.63	0.0001*
ALT (IU/L)	45.45±19.52	33.45±19.52	30.55±6.18	0.001*
ALP (IU/L)	122.84±42.59	91.84±42.59	67.55±10.0	0.002*
Serum bilirubin (mg/dL)	0.69±0.69	0.46±0.69	0.38±0.27	0.0001*
Direct bilirubin (mg/dL)	0.16±0.17	0.11±0.16	0.04±0.06	0.0001*
Albumin (g/dL)	4.12±0.42	2.88±0.50	3.99±0.29	0.002*
Student's t-test at the 0.01 threshold yields a significant difference between two independent means.				



	HBV patients newly diagnosis	HBV patients within treatment	Healthy control
Aspartate aminotransferase (IU/L)	47,14	37,14	23,14
Alanine aminotransferase (IU/L)	45,45	33,45	32,55
Alkaline phosphatase (IU/L)	122,84	91,84	70,55

Figure 4.2 Distrubution of AST, ALT and ALP in studeied groups



	Total bilirubin (mg/dl)		Direct bilirubin (mg/dl)		Albumin (g/dl)
HBV patients newly diagnosis	0,69		0,16		4,32
HBV patients within treatment	0,46		0,09		2,9
Healthy control	0,44		0,04		4,26

Figure 4.3 Distribution of bilirubin and albumin in studied groups

4.1.3 Measurement of IL-6 and testosterone in serum and seminal plasma of ınfertile and control groups with HBV

HBV infertile patients and healthy men were tested for IL-6 levels in their seminal plasma or serum. Data from Table 4.3 shows that azoospermic males had considerably higher concentrations of IL-6 in both semin and blood.

HBV infertility patients and controls had lower testosterone levels in their seminal plasma and sera. Find out it went wrong based on the student's test results. As seen in Table 4.3, testosterone concentrations were considerably lower among those with azoospermia.

Table 4.3 Infertile male sexual seminal and plasma IL-6 and testosterone levels were compared to those of healthy subject.

PARAMETERS	SUB GROUPS	HBV. PATIENTS NEWLY DIAGNOSIS	HBV. PATIENTS WITHIN TREATMENT	HEALTHY CONTROL
IL-6	Control	2. 8 ± 0.54	3.38 ± 0.54	3.8±0.88
	Azoospermia	1.37 ± 0.52	1.99 ± 0.46	-
	Asthenozoospermia	2.38 ± 0.72	3.38 ± 0.66	-
	Oligozoospermia	2.14 ± 0.81	2.8 ± 0.67	-
	P- value	0.001*	<0.0001	
Testosterone	Control	4.19 ± 1.50	4.55 ± 1.67	5.38±1.99
	Azoospermia	0.89 ± 1.20	2.09 ± 0.99	-
	Asthenozoospermia	1. 2 ± 1.2	1.92 ± 1.41	-
	Oligozoospermia	1.49 ± 1.20	2.4 ± 1. 5	-
	P- value	0.002	<0.0001	3.8±0.88
Selenium	Control	55.11±22.7	66.57±20.0	89.11±22.7
	Azoospermia	43.19±16.65	47.23±18.8	78.22±19.7
	Asthenozoospermia	48.13±17.45	49.13±17.15	-
	Oligozoospermia	40.87±13.99	39.17±9.09	-
	P- value	0.001*	0.001*	0.13

4.2 Correlations Among Seminal Plasma and Serum IL-6, Testosterone and Selenium Levels

Blood IL-6 is significantly correlated with seminal IL-6, but not with seminal testosterone (excluding azoospermics) or seminal catalase (except oligozoospermics).

In the normal group, serum testosterone has a substantial negative association with only seminal IL-6, a significant association between testo and selenuim in the control and oligozoospermic groups, and a thers no significant correlation with seminal testo and selenuim.

However, only seminal IL-6 from oligozoospermic men correlates negatively with serum selunium, while seminal testo from azoospermic and asthenozoospermic men correlate positively. This table summarizes the findings (Table 4.4).

Table 4.4 Testosterone, IL-6, and Selenium levels in infertile HBV and control groups were studied

GROUP	**SERUM**	**IL-6**		**TESTOSTERONE**		**SELENUIM. ***	
	Seminal fluid	Correl -ation	Significant sighn	Correl -ation	Significant sighn	Correl -ation	Significant sighn
Normal Azoo. * Astheno. * Oligo. *	IL-6	0.77 0.39 0.53 0.50	0.001 0.011 0.047 0.001	-0.63 -0.35 -0.23 -0.22	0.001 No Sig No Sig. No Sig.	-0.25 -0.37 0.16 -0.45	No Sig. No Sig No Sig. 0.048
Normal Azoo. Astheno. Oligo.	Testost-erone	-0.57 -0.32 -0.45 -0.46	0.01 0.03. 0.033 0.05	0.08 0.25 0.11 0.16	No Sig No Sig. No Sig. No Sig	0.24 0.33 0.19 0.33	No Sig. No Sig. No Sig. No Sig.
Normal Azoo. Astheno. Oligo.	Selenium	-0.53 -0.52 -0.44 -0.29	0.01 0.01 0.06 No Sig	0.17 0.09 0.22 0.26	0.01 No Sig. No Sig. No Sig	0.25 0.43 0.40 0.32	No Sig. 0.039 0.033 No Sig

4.3 The Correlation between Serum and Seminal IL-6, Testosterone and Selenium Levels with Some Spermiograms

There was a correlation between sperm concentration, motility (directional activity), and the proportion of normal-appearing sperm in the different study groups. Except for the asthenozoospermic group's count, seminal IL-6 and the measured spermiograms of the research groups had a substantial negative correlation. The research groups' spermiograms were strongly correlated with the levels of seminal testosterone, save for the asthenozoospermic group's average sperm percentage. Count, motility and the percentage of normal sperms were all correlated with the IL-6, TESTOand selenuim markers in the study groups, as shown in Table (4.5. and 4.6).

Table 4.5 The correlation between seminal fluid parameters in HBV groups

Seminal fluid parameters in HBV groups								
Groups			Sperm count			Activity of sperm		Normal sperm
			r	p	r	p	r	p
HBV. PATIENTS NEWLY DİAGNOSİS	l IL-6 (µmol/L)	Control Astheno. * Oligo. *	-0.52 -0.25 -0.60	0.001 N.S. 0.003	-0.61 -0.63 -0.43	0.0019 0.002 0.03	-0.65 -0.52 -0.57	0.003 0.02 0.02
	TESTO (ng/dl)	Control Astheno. * Oligo. *	0.35 0.39 0.53	0.04 0.049 0.032	0.47 0.65 0.68	0.05 0.001 0.001	0.60 0.23 0.53	0.002 No Sig. 0.01
	Selenium (mg/dL)	Control Astheno. * Oligo. *	0.40 0.47 0.37	0.04 0.053 No Sig	0.41 0.28 0.65	0.05 No Sig 0.001	0.40 0.54 0.29	No Sig. 0.01 N.S.
HBV. PATIENTS WITHIN TREATMENT	IL-6 (µmol/L)	Control Astheno. * Oligo. *	-0. 23 -0.25 -0.60	0.13 N.S. No Sig	-0.11 -0.63 -0.43	0. 19 0.002 0.03	-0.05 -0.52 -0.57	No Sig 0.02 0.02
	TESTO	Control Astheno. * Oligo. *	0.15 0.19 0. 33	0.11 0.19 0.02	0.1 0.03 0.48	0.15 0. 1 0.019	0.60 0.23 0.53	No Sig No Sig. 0.01
	Selenium (mg/dL)	Control Astheno. * Oligo. *	0.37 0.47 0.37	0.033 0.053 No Sig	0.31 0.28 0.65	0.024 No Sig 0.001	0. 04 0.44 0.11	No Sig. 0.01 No Sig
HEALTHY CONTROL	l IL-6 (µmol/L)	Control Astheno. * Oligo. *	0.55 0.31 0.42	0.001 0.053 No Sig	0.36 0.28 0.65	0.048 No Sig 0.001	0.330 0.54 0.29	0.037. 0.01 No Sig.
	TESTO (ng/dl)	Control Astheno. * Oligo. *	-0. 53 -0.15 -0.10	0.013 No Sig. No Sig	-0.44 -0. 39 -0.40	0. 001 0.015 0.033	-0.55 -0.49 -0.57	0.022 0.02 0.02
	Selenium (mg/dL)	Control Astheno. * Oligo. *	0. 50 0.10 0. 33	0.011 0.011 0.02	0.41 0.09 0.48	0.025 0. 09 0.019	0.59 0. 3 0.53	0.001 No Sig. 0.01

Testosterone and selenite levels in the research groups were connected with the number of sperms, motility (linear correlation activity), and the average sperm percentage. They found that there was a strong correlation between the seminal spermiograms, as well as the straight interaction of asthenozoospermic sperms and the ratio of healthy male gametes between the control and asthenozoospermic groups, compared with findings from other studies. Serum IL-6 had a strong negative correlation with the spermiograms of all research groups.

Table 4.6 The correlation between serum fluid parameters in HBV groups

Serum parameters in HBV groups								
Groups			Sperm count		Activity of sperm			Normal sperm
			r	p	r	p	r	p
HBV. PATIENTS NEWLY DİAGNOSİS	l IL-6 (µmol/L)	Control	-0.35	0.044	-0.36	0.02	-0.46	0.021
		Astheno. *	-0.28	0.033	-0.19	0.31	-0.43	0.05
		Oligo. *	-0.40	0.020	-0.24	0.044	-0.55	0.01
	TESTO (ng/dl)	Control	0.45	0.001	0.36	0.037	0.19	No Sig
		Astheno. *	0.29	0.044	0.12	No Sig	0.39	0.044
		Oligo. *	0.10	No Sig.	0.34	N.S.	0.41	0.02
	Selenium (mg/dL)	Control	0.51	0.01	0.17	No Sig	0.10	N.S.
		Astheno. *	0.30	0.04.	0.13	No Sig.	0.12	N.S.
		Oligo. *	0.33	0.018.	0.55	0.01	0.07	N.S.
HBV. PATIENTS WITHIN TREATMENT	IL-6 (µmol/L)	Control	-0. 3	0.03	-0.11	0. 19	-0.13	No Sig
		Astheno. *	-0. 42	0.001.	-0.50	0.001	-0.42	0.01
		Oligo. *	-0.60	No Sig	-0.43	0.03	-0.37	0.02
	TESTO	Control	0.15	0.11	0.1	0.15	0.60	No Sig
		Astheno. *	0.19	0.19	0.03	0. 1	0.23	No Sig.
		Oligo. *	0. 33	0.02	0.48	0.019	0.53	0.01
	Selenium (mg/dL)	Control	0.37	0.033	0.31	0.024	0. 04	No Sig.
		Astheno. *	0.47	0.053	0.28	No Sig	0.44	0.01
		Oligo. *	0.37	No Sig	0.65	0.001	0.11	No Sig
HEALTHY CONTROL	l IL-6 (µmol/L)	Control	0.55	0.001	0.36	0.048	0.330	0.037.
		Astheno. *	0.31	0.053	0.28	No Sig	0.54	0.01
		Oligo. *	0.42	No Sig	0.65	0.001	0.29	No Sig.
	TESTO (ng/dl)	Control	0.35	0.05	0.16	N.S.	0.28	No Sig.
		Astheno. *	0.19	No Sig.	0.30	0.023.	0.42	0.05
		Oligo. *	0.10	No Sig.	0.04	No Sig.	0.51	0.01
	Selenium (mg/dL)	Control	0. 39	0.020	0.41	0.025	0.59	0.001
		Astheno. *	0.19	No Sig	0.09	0. 09	0. 18	No Sig.
		Oligo. *	0. 40	0.005	0.37	0.009	0.49	0.02

Serum tests were positively associated with spermiograms in all groups except the asthenozoospermic and oligozoospermic groups, but no other correlation was established between serum testosterone and spermiograms in those groups. Only the oligozoospermics' active sperms and the control group's count were positively connected to serum selenium in this study.

4.4 Correlation of IL-6 with Other Parameters

For the purpose of determining the association between interleukin-6 and the other biochemical indicators that were tested, the Pearson correlation coefficient was calculated. Table 4.7 shows that interleukin-6 (IL-6) has a statistically significant positive relationship (P0.01) with the enzymes AST, ALT, and GGT. This is in conformity with the findings in table below. (P<0.01).

Table 4.7 Correlation of IL-6 with other parameter

		(IL-6)
		Hebtiteus B virus
AST (IU/L)	Correlation	-0.503**
	P	0.004
ALT (IU/L)	Correlation	-0.433**
	P	0.003
ALP (IU/L)	Correlation	-0.193
	P	0.105
TB (mg/dL)	Correlation	-0.178
	P	0.094
DB (mg/dL)	Correlation	-0.520**
	P	0.005
Platelet count (10^3/ μL)	Correlation	0.368*
	P	0.014
Albumin (g/dL)	Correlation	0.050
	P	0.612
GGT IU/L	Correlation	-0.599**
	P	0.0001
*At the 0.05 level, the correlation is significant. *At the 0.01 level, the correlation is significant		

4.5 Correlation of Selenium with Other Parameters

There was a positive and statistically significant relationship (P0.05) between selenium and all of the markers in Table 4.8, and this was proved to be the case in the experiments.

Table 4.8 Correlation of selenium with other parameters

		SELENUIM (mg/dL)
		Hebtiteus B virus
AST (IU/L)	Correlation	0.301*
	P	0.047
ALT (IU/L)	Correlation	0.288
	P	0.058
ALP (IU/L)	Correlation	0.304*
	P	0.045
TB (mg/dL)	Correlation	0.225
	P	0.142
DTB (mg/dL)	Correlation	0.096
	P	0.535
Platelet count (10^3/ μL)	Correlation	-0.202
	P	0.189
Albumin (g/dL)	Correlation	-0.006
	P	0.968
GGT (IU/L)	Correlation	0.219
	P	0.154
IL-6 (mg/L)	Correlation	-0.127
	P	0.412
At the 0.05 level, the correlation is significant. *At the 0.01 level, the correlation is significant.		

4.6 Correlation of Testosterone with Other Parameters

There really was no relationship found among testosterone and several of the parameters listed in Table 4.9.

Table 4.9 shows a statistically significant positive correlation between selenium and testosterone ($P < 0.05$).

Table 4.9 Correlation of Testosterone with other parameters

		TESTOSTERONE (ng/mL)
		Hebtiteus B virus
ALT (IU/L)	Correlation	0.066
	P	0.672
AST (IU/L)	Correlation	0.160
	P	0.300
ALP (IU/L)	Correlation	-0.092
	P	0.552
TB (mg/dL)	Correlation	0.054
	P	0.728
DTB (mg/dL)	Correlation	0.013
	P	0.931
Platelet count (10^3/ μL)	Correlation	-0.155
	P	0.314
Albumin (g/dL)	Correlation	0.018
	P	0.908
Gamma-Glutamyl Transferase (IU/L)	Correlation	0.146
	P	0.343
Selenium (mg/L)	Correlation	0.35
	P	0.023*
*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.		

5. DISCUSSION

Many studeied showed a hepatitis B is a severe public health problem that affects people all over the world. Affecting millions 600,000 people die annually from HBV (WHO 2018). Hepatitis B affects both sexes patients (34%) were under 40, 10 (22.7%) were 40-49, and 19 (43.2%) were above 50. Males with infertility and hepatitis B were more likely to be male, linked fibrosis score to age (Tanaka 2006). Like Mohamadnejad et al. Showed, mild testicular affect patients were younger than severe. Neither age nor gender is connected with inflammation or fibrosis stage, according to (Demir *et al.* 2014).

Alanine and aspartate aminotransferase, which come from damaged cells, are called "liver function tests" because they come out of them. Combined, they give a lot more information than if they were looked at alone. There is (Bao *et al.* 2017). The liver cells that make AST are more active than the ALT cells. AST is released into the bloodstream faster than ALT when the liver is damaged. Because ALT has a longer plasma half-life, it will overtake AST in 24–48 hours, especially if the damage keeps going. Most of the time, ALT is bigger than AST. Chronic HBV infection is found when a high ALT level is found in blood tests every few months. Patients with HBV who have high ALT levels are more likely to get sick and have more problems. They say that the AST/ALT ratio changes when there is more fibrosis in the body. Fatty tissue makes it more difficult to break down food, so the AST/ALT ratio rises. After cirrhosis, the ratio usually goes above one is (Bao *et al.* 2017).

Hepatitis B patients had considerably higher levels of alkaline phosphatase in their plasma than did the controls in the current study, and this was associated with the HBV stage at the time of testing. According to Olatunji et al. ALP abnormalities were related to HBsAg positive (Olatunji *et al.* 2017). Previous research had linked ALP to fibrosis progression, and this study's findings confirmed this connection (Olatunji *et al.* 2017).

Total bilirubin and direct were found to be considerably higher in the plasma of HBV than in the healthy subject group in the current study, whereas total bilirubin rose non-

significantly with the sperm parameter especially cunt The TBI was shown to be significantly different between HBV patients and the control group by Yongqiong et al. However the fibrosis stage was non-significantly different (Yongqiong *et al.* 2017).

By Hou et al. The $HBsAg^{+}$ group had higher TBl, IBl, and DBL concentrations than the control group (Hou *et al.* 2020). Lemoine M et al also observed no association between liver and another organ associated with total bilirubin in their. According to Saim D et al., there was no difference in total bilirubin between the groups. The main causes testicular pabnormality because of virus-specific $CD8^{+}$ T cells and nonspecific inflammatory cells in testicular parenchyma due to infalamation factor activation according to their findings (Hou *et al.* 2020).

The proportion of albumin production determines the interchange between intravascular and interstitial compartments, catabolism, and renal or intestinal and sexual testes loss, all of which influence the level of albumin in the blood plasma and the amount of albumin in the blood plasma (Levitt and Levitt 2016).

Albumin levels in the blood are a good indicator of how well a person is doing. People with low albumin levels are more likely to die than those with normal levels HBV patients' ALB levels dropped considerably compared to those in the comparison group However, the fibrosis stage did not significantly reduce. HBsAg viral load was found to be associated with inadequate albumin levels, which was statistically meaningful. in Olatunji Ayodeji et al.'s study. In addition to, have reached the same conclusion (Levitt and Levitt 2016). It differs from the findings of (Wang *et al.* 2020), who observed that serum albumin was an independent predictor of patients with CHB bridging fibrosis/cirrhosis. Mueller et al. Research suggests that ALB can predict severe fibrosis, contrary to this study's findings. In the work many studied, A decrease in albumin production is a result of acidosis (but not acute acidosis) and proinflammatory cytokines such TNF-, IL-6 and-1. The liver, kidney, and muscle are the three primary sites of albumin breakdown (Mueller *et al.* 2018).

IL-6 aids overall survival by increasing several organ regenerations and protecting the hepatic and testicular from the immunological response, alcoholic, and viral infection-induced damage. IL6 signalling is beneficial during fibrosis progression but promotes carcinoma insensitivity to carcinogenic agents despite its crucial involvement in the acute-phase response in the organ hepitite (Taub 2003).

Multiple effects on cell proliferation, differentiation, survival and inflammation are all due to this particular cytokine. One or more of the gp130 subunits are involved in the interaction of IL6 with receptor complexes (Taub 2003).

Immune reactions, alcohol, and viral infection can all damage the testicles, but the presence of IL-6 can also prevent them. In response to chemical carcinogens, IL6 signalling promotes HCC but is helpful in the evolution of fibrosis (Taub 2003).

Sperm volume, pH, density, activation rate, sperm survival rate, and sperm shape were all substantially greater in the average group (group A <P.05) than the sterile patients with or without Viral hepatitis (group B, P.05). HBV infection IL-6 levels and semen quality measures are shown in Table 2 of this study. Thirty male patients with HBV who were unable to conceive were able to link their semen. For male infertility, selenium concentrations in sterile men's sperm can serve as a diagnostic marker because they are connected to IL-6 (r=0.516 and 0.522) and selenium (r=0.522, respectively). Male sexual failure & infertility have been linked to HBV infection by these studies.

Found a negative link between sperm characteristics such as sperm volume, pH, density, sperm activation rate, and sperm survival rate with the IL-6 content in infertile male sperm (Eggert-Kruse *et al.* 2001).

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Testosterone levels are linked to infertility. One of the findings of this study was the correlation between seminal spermiograms, linear activity, and normal sperm percentage.
2. IL-6 had a strong negative correlation with the spermiograms of all research groups. Only the oligozoospermics' active sperms and the control group's count were positively connected to serum selenium
3. This suggests that maintaining high amounts of selenium is beneficial to the health of the testicles.
4. Many cases of HBV occour in younger cases.

6.2 Recommendations

1. Histology studies on the individuals of this study are needed to learn more about how immunological variables reduce testicular abnormalities after infection.
2. In persons with HBV, copperand cobalt should also be screened for.
3. Third, a study in infertility is investigating the link between IL-6 genotype and HBV.

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