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Immune Parameters [Immunoglobulins and Complements] and PHA-Changes Induced Lymphatic Transformation in Patients with Hepatitis

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Abstract:
With regard to illness and death, HB is one of the foremost ailments in global healthcare facilities especially in Asia. As proposed in the previous studies, it has been believed that the pathogenicity of HB depends on immune response. The main purpose of this study was to assess the level of Complement (C3) and Cytokines (C4), T lymphocytes proliferation, phagocytic cell percentages, IgA, IgM and IgG in adult Iraqi patients who contracted Hepatitis B Virus (HBV). With the help of enzyme-linked immunosorbent test (EIISA) kits, 75 patients of hepatitis B virus had HBsAg as well as the other indicators of HBV in their serum. In a lymphocyte transformation assay, whole blood was mixed with RPMI-1640 medium (which does not contain FCS) at a ratio of 1:15 (v:v). Mean transformation of lymphocyte in the healthy carriers was found to be significantly low ($P<0.05$) compared to the control group. Also, IgA was found to be raised in acute hepatitis B infected individuals ($P<0.05$). IgG level showed a highly significant increase ($P<0.05$) in healthy carriers. Thus, comparing with the patients, no statistically significant changes of IgM levels were observed ($P>0.05$). Complement C3 Levels in patients of acute, chronic hepatitis B and control groups recorded (140.3 ± 10.09), (97.84 ± 8.64) and (108.82 ± 9.12) respectively. The results of C3 levels that there (Mean ± S.D) was no significant difference ($P>0.05$) in C3 levels between patient's groups and control group. Complement C4 Levels in patients of acute, chronic hepatitis B and control groups recorded (25.37 ± 3.81), (27.65 ± 5.83) and (36.22 ± 7.19) respectively. C4 levels show a significant decrease in C4 levels of AHB and CHB groups, when compared with and control group.

Keywords: Immunoglobulins, Complements, PHA, Lymphatic Transformation, Patients, Hepatitis, Immune Parameters

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Introduction

Viral hepatitis B is an infectious disease which mostly affects the liver and is caused by Hepatitis B virus. Living and health experience a tremendous change. The estimated global population with hepatitis B virus is between 350 to 400 million. This global medical society has the social responsibility of improving antiviral treatment research and quality of life in those affected. Another crucial ground in the development of hepatitis B is the cellular immune response T cells dysfunction. The kind of mutation frequently causes immunodeficiency in cellular immunity, the reduction of the immunological response in the organism, the inability to eliminate the virus, and the long- proscribed disease that does not come to its remission [1]. Currently, there are few studies done about the correlation between hepatitis B viral load and T cells and subsets and almost none of them pay attention to the relationship between AIT and this connection. An epidemiological sign of hepatitis B would be an ALT level that is more than two times the upper limit. However, despite the increasing number of people receiving the vaccination of hepatitis B virus (HBV) the virus remains a worthy opponent to human health and survival. Due to the complexity of the virus replication with the host immunity response, the disease outcome after contraction of this virus is variable. Despite the fact that cells or tissues which are protected by innate immunity do not have information about previous infections, they can use it in early stages of a disease. However, HBV disperses it in the body and is hidden from the host's natural immunity system. Hence, the treatment and elimination of the HBV infections that lead to inflammation and destruction of liver needs immunity especially the adaptive immunity- T and B cells. Immunological tolerance is created when HBV remains; hypofunctioning immune cells, exhausted T cells, and increased suppressor cells and cytokines [5, 6]. Even though progress has escalated in the treatment of chronic hepatitis B, retaining an effective cure remains a mystery due to immunotolerance and immunoinflammatory balance in addition to immunological activation and fibrosis. The human immune system can develop many phases after the contraction of hepatitis B virus. Clinically, it's possible to separate HBV carriers from HBV patients depending on symptoms, signs and conditions that may be related to liver function, including normal or elevated ALT levels. While their liver functions translate to being a shade lower than normal, the majority of hepatitis B carriers have no way of appreciating this since they are asymptomatic, their bodies do not display symptoms or signs that indicate they are hepatitis B carriers. The measurements most frequently provided of transaminase ALT levels are either normal or only slightly elevated [7]. However, other signs include the following, jaundice in hepatitis B patients as well as anorexia, and weakness. Functionally, the liver was very much affected; the animal had an elevated ALT level, more than a double of the standard value [8]. Most of the enzymes alanine aminetransferase (ALT) are found in liver cells. The kinds of enzyme activity of ALT in serum can be significantly elevated in the presence of even a small amount of ALT leaks into the blood. It is necessary to point out that screening of different types of viral and toxic hepatitis is impossible without determining the presence of blood serum ALT, which is detectable due to its release into bloodstream by necrotic liver cells. Liver cells contain 1000-3000 times more of alanine aminotransferase than exists in serum. Transaminase, especially ALT, is an acutely sensitive marker of liver cell injury since hepatic enzyme activity in plasma can increase twofold as long as 1% of necrotic hepatocytes [9, 10]. Similarly, according to the Infectious Diseases Branch of Chinese Medical Association and the Liver Disease Branch, a double ALT is deemed as clinical guideline for antiviral therapy. This study shows that the established hepatitis B treatment guidelines were universally acceptable among the liver disease specialists. About 295.7 million people were consistently living with HBV in 2019, and 820000 people died from the conditions related to HBV such as cirrhosis, hepatocellular cancer, and liver failure. Chronic HBV infection natural history can be separated into four phases which are tolerance, immunological activity, inactive carriers and reactivation phases. Liver cell injury is not a direct benefit of an HBV infection and is caused after a period of time. Host's immune response influences the viral replication of HBV; in this way, clinical manifestations of the disease are defined. At present, the available treatments for chronic hepatitis B cytokines are interferons (IFNs), nucleotide analogues [12, 13]. The failure to clear covalently closed circular DNA, an important feature of chronic herpes simplex virus infection and hepatitis B recurrence, is why these drugs do not cure chronic hepatitis B. Hence, WHO has established its target as eliminating viral hepatitis as a public health problem in 2030, and the investigation of a clinical treatment for CHB has emerged as a recent popular topic of discussion. Most cases of chronic hepatitis B are associated with this pathognomic immunological response

Liver inflammation and damage result from host immune response that encompasses both the innate and adaptive immune systems as the fight off the virus. HBV infection is tightly controlled and cleared by innate immunity, although adaptive immunity is essential [14]. Immuninology, the innate immune system produces cytokines and activation signals required for the adaptive immunity to operate. To help to prevent the release of HBsAg and neutralise or engulf the cells infected with the various strains of HBV, HBsAb can complex with it. The aim of this work was to evaluate serum levels of Complement 3 (C3), Complement 4 (C4), Immunoglobulin A (IgA), Immunoglobulin M (IgM), and T-lymphocyte proliferative response, as well as phagocytic cell percentages of adult patients with Hepatitis B Virus (HBV) infection from Iraq.

MATERIALS and METHODS

Patients Groups:

The presence of hepatitis B surface antigen (HBsAg) and other HBV markers in the serum of 75 HBV patients was detected using enzyme-linked immunosorbent assay (EIISA) kits. Their HBV status was thus recorded.

Previous laboratory tests and the clinical examination have led to a clinical diagnosis for these patients.

- **A- Fifty patients with HBV infections:** 1- Twenty-five patients with acute hepatitis B. 2- Twenty-five patients with Chronic hepatitis B. Blood samples had been collected from (25) males and (25) females.
- **B- Healthy adult** (**Control group**) Blood samples had been collected from twenty-five normal adult volunteer, used as control group.

Separated Lymphocytes for Lymphocyte Transformation Assay

A different concentration of PHA (250µg/ml) was used to initially identify the optimal concentration for lymphocyte proliferation, and serial dilution was prepared in the following way: range of concentrations in milligrams per milliliter The RPMI-1640.

Transformation of Lymphocytes with the Use of Whole Blood and Culture Method

Complete blood was used in a lymphocyte transformation test in a ratio of 1:15 (v:v) with RPMI-1640 medium that did not contain FCS.

Using the Stain Method for Lymphocyte Transformation Assay

The following procedure had been followed: For each test, use two sterile silicon-coated tubes containing 250 □l of heparinized blood cultured in 2.5 ml of full RPMI-1640 media. One tube (the test) was treated with 250 □l of PHA mitogen, while the other (the control) was left undisturbed. The tubes were incubated at 37°C with 5% CO2 in a humidified environment for 72 hours; daily shaking was required. After the incubation period ended, the cells were centrifuged at 2000 rpm for 10 minutes to cause sedimentation. The sediment cells were treated with 5 milliliters of KCl, a hypotonic solution, and left to incubate at 37 degrees Celsius for half an hour. - ACentrifugation was then performed for 10 minutes at 2000 rpm. After being introduced to the sediment cells, 5 milliliters of fixation solution was refrigerated at 4 degrees Celsius for 15 minutes. The sediment cells were suspended in a colorless solution by washing them three or four times with fixing solution and centrifugation. Next, a single drop of sediment cells was pipetted onto two separate clean slides. The cells were then allowed to dry at room temperature. After 10 minutes, they were stained with giemsa stain. After washing with D.W., the cells were viewed microscopically under an oil immersion lens, and a count of 200 cells was made. Following this equation, we were able to determine the proportion of altered cells:

Transformed cells % = No. of transformed cells /Total no. of cells counted.

Phagocytosis Assay

In a sterile test tube, combine 250 □l of heparinized blood with 106 /ml of s. aureus bacterial suspension in a 1:1 ratio. For 30 minutes while being constantly shaken, the mixture was incubated in a water bath set at 37°C. A single drop of the mixture was used to prepare the smear on the slide. Each tube has its own set of duplicate slides. The following

procedures were performed on the slides: air drying, absolute methanol fixation, 10-minute Giemsa staining, and D.W. washing. The amount of ingested microorganisms by neutrophils and monocytes was determined by examining the slides using an oil immersion lens. Here is the percentage of cells that are phagocytic:

No. of phagocytic cells % = No. of phagocytic cells / No. of 100 cells phagocytic & non phagocytic cells.

Assessing the Concentrations of Immunoglobulins and Essential Components

The levels of immunoglobulins and complement components were assessed using the Mancini method's described single radial immunodiffusion (SRID) in agar. According to the Sanofi method, the following tests can be run on human immunoglobulins (IgA, IgG, IgA) and complement components (C3, C4): The plates were partially opened for 5 minutes prior to commencing in order to remove any moisture droplets. Each plate included 16 wells, and 5 microliters of serum from each person was pipetted into one of those wells. This was done for three different kinds of immunoglobulins (IgA, IgG, and IgM) and two different kinds of complement components (C3, and C4). For 10–20 minutes, the plates were kept open [15]. Afterwards, the plates were covered securely and left to incubate at room temperature (23□2C□) for 48-72 hours. Immunoviewer had previously assessed the widths of the immunoprecipitin rings. 6-The unknown values that can be inferred from the standard curve that is included in the test kits, which shows the relationship between the concentration in mg/dl and the ring diameters in mm.

Statistical Analysis

We utilized analysis of variance (ANOVA) for our statistical analysis, one-way analysis of variance for our group comparisons, and the least significant difference (LSD) for our source identification.

RESULTS and DISCUSSION

Serum antibodies mediate the humoral immune response; antigen attachment to particular membrane immunoglobulin activates B cells to produce antibodies. Glycoproteins called immunoglobulins (Igs) or antibodies (Abs) are found in serum. They are made by plasma cells called B-cells when an immunogen is exposed. The ability to attach to specific antigens and remove them is a shared property among this diverse assortment of serum globulins, which make up the antibodies. The five main classes of immunoglobulins are immunoglobulin A (IgA) [16, 17], IgG, IgM, IgE, and IgD; each of these classes of immunoglobulins has its own unique set of biologic characteristics. Multiple methods, including ELISA and single radial immunodiffusion, can be used to measure the concentrations of IgA, IgG, and IgM in the blood. One significant criterion for differentiating viral hepatitis from other illnesses associated with jaundice was the presence of raised immunoglobulin levels in the sera of individuals with the disease. Compared to the control groups, HBV patients exhibited substantially greater levels of IgA, IgG, and IgM, according to multiple publications. When it comes to the humoral immune response, the complement system is another key component. The liver is responsible for the majority of complement protein synthesis, however tissue macrophages and fibroblasts also contribute to the complement system by producing a small number of proteins. The complement system consists of at least twenty proteins in the serum. The nine principal complement components are denoted by the initial capital letter "C" followed by an identifying number ranging from 1 to 9. The numbers represent the activation order of the components, with the exception of C4, which is activated before C2. When an antigen and an antibody react, it triggers the complement system, which in turn lyses the target or improves phagocytosis. The recruitment of highly polymorphonuclear (PMN) cells is another consequence of this activation [18, 19]. A single radial immunodiffusion assay was used to determine the concentrations of C3 and C4 in the samples. Patients with liver disease have complement system abnormalities; thus, it is reasonable to suppose that their complement components are reduced due to decreased synthesis in the liver. There was a statistically significant decrease in C3 serum levels in acute HBV, but no such decrease in C4 serum levels [20, 21]. When compared to CHB, where C3 serum levels were not drastically reduced, C4 levels were.

Immunoglobulins [IgG] Levels in patients of acute, chronic hepatitis B and control groups recorded 1466.64 ± 481.00, 1355.15 ± 373.19 and 1063.00 ± 398.11 respectively. The results showed (Mean ± S.D) that there was a significant increased $P<0.05$ of IgG levels in acute hepatitis B than those of control group.

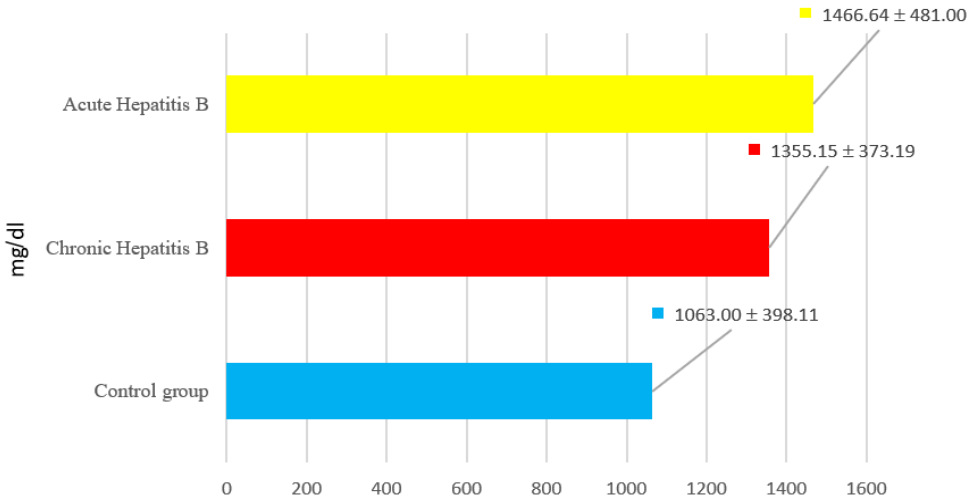

Figure 1. Immunoglobulins [IgG] Levels in patients of acute, chronic hepatitis B and control groups.

According to these results, IgG levels were normal in the CHB-HC group, which is consistent with earlier research that demonstrated a considerable rise in IgG levels in CHB patients. These results go counter to those of earlier research that found no statistically significant difference between healthy groups and HBV patients in terms of IgG levels. Patients with HBV had elevated IgG levels because their sera had been exposed to exogenous and autogenous HBV antigens for an extended period of time.

Immunoglobulins [IgM] Levels in patients of acute, chronic hepatitis B and control groups recorded 139.94 ± 15.09, 109.20 ± 14.00, and 111.6 ± 9.54 respectively. IgM levels (Mean ± S.D) illustrated in Figures 2, showed that (Mean ± S.D) was no significant difference (P>0.05) in IgM levels between patients' groups and control group.

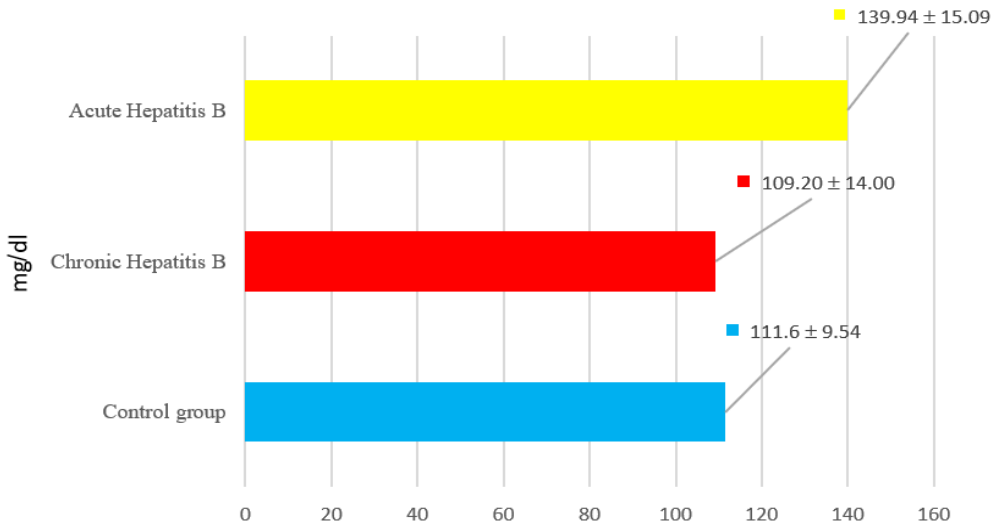

Figure 2. Immunoglobulins [IgM] Levels in patients of acute, chronic hepatitis B and control groups.

Consistent with other research, this study found no statistically significant difference between the IgM levels of the healthy group and CHB-HC patients. Additionally, the results ran counter to other research that found considerably higher IgM levels in patients with AHB and CHB. A main antigenic challenge or persistent antigen, linked to the presence of a selective deficiency in B-cell responsiveness, causes the rise of IgM levels in HBV infection. These findings showed a more significantly increase in IgA Levels in chronic hepatitis B (405.73 ± 25.09) groups than those

of control group (202.48 ± 14.93). There was no significant difference between acute hepatitis B group (190.17 ± 11.00) and control group (P>0.05).

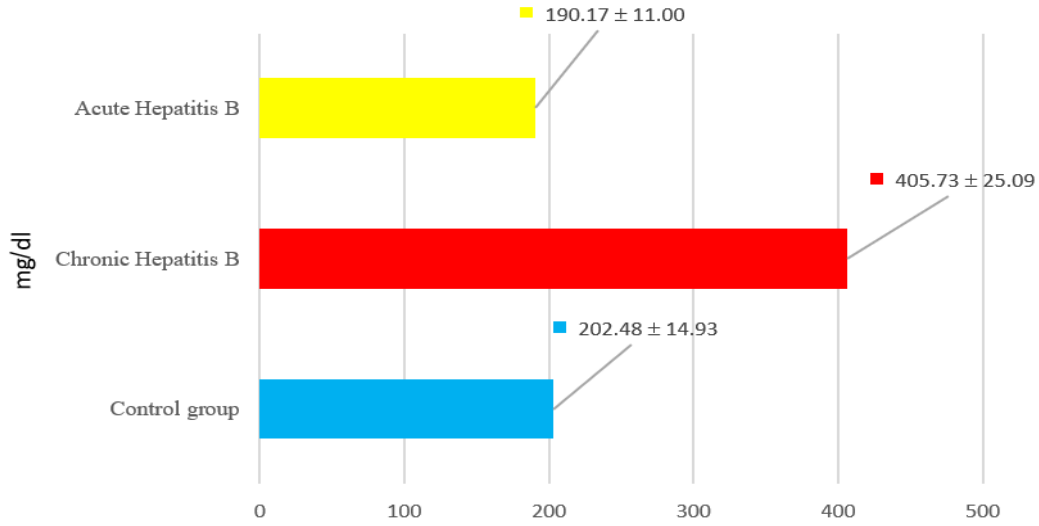

Figure 3. Immunoglobulins [IgA] Levels in patients of acute, chronic hepatitis B and control groups.

Although he found no statistically significant difference in IgA levels between AHB patients and normal controls, his findings that CHB-HC patients had elevated levels of IgA are consistent with this observation. In contrast, CHB patients' IgA levels were significantly higher than normal controls. While they discovered a slight increase in IgA levels in CHB patients, they discovered a substantial increase in thalassemic individuals with CHB. When compared to healthy groups, the CHB-HC and CHB groups did not show significantly raised IgA levels, according to another study. The epithelial cells of mucosal surfaces, such as the bile duct and gall bladder lining, create IgA. As a result, individuals with liver illness typically have higher serum IgA levels, which are linked to extra- and entra-hepatic biliary blockage.

As part of the humoral immune response, B cells that are unique to the infection undergo development into plasma cells that generate antibodies. In order to destroy and engulf HBV-infected cells, HBsAb can attach to HBsAg, inducing antibody-dependent cytotoxicity and phagocytosis. In cases of chronic hepatitis B virus infection, B cell-mediated humoral immunity is frequently disregarded. Rituximab and other B cell-depleting drugs can restart HBV replication, which in turn increases liver inflammation and can be deadly, even in patients whose infections have cleared [24]. Since HBcAg triggers B cell maturation independently of helper T cells, it is the principal target of the humoral immune response. HBcAg-specific B cells are linked to the natural history of CHB and are more prevalent than HBsAg-specific B cells. During antiviral therapy, the B cell responses elicited by HBcAg are greatly reduced. In individuals with CHB infection who stopped therapy, clinical recurrence can be predicted by HBcAb levels at the end of treatment. In vitro, B cells specific for HBcAg develop into plasma cells with an IgG+ memory B cell phenotype, but B cells specific for HBsAg do not undergo plasma cell maturation and exhibit a dominant IgM+ phenotype. Despite these functional and morphological distinctions, innate immunity and genes for cross-presenting DC recruitment are strongly expressed by HBcAg-specific and HBsAg-specific B cells. Another possible strategy for immunotherapy is the production of B lymphocytes specific for HBsAg. During the immune active and inactive carrier stages of chronic HBV infection, B cell activation is high as evidenced by upregulation of genes encoding the activation markers CD69 and CD83 and downregulation of genes encoding IRs, which is in line with HBeAg seroconversion. At different stages of the infection, B cell functions vary. There is strong evidence in the scientific literature linking the body's immune activity, particularly the T lymphocytes dominated cellular immunological response, to the occurrence and progression of hepatitis B virus following infection [25]. Nevertheless, research on how hepatitis B virus affects T cells and subsets in various ALT levels is scarce. This is where the investigation and study of this paper begins. Since the majority of infected individuals are already carrying the hepatitis B virus, the data show that the normal ALT group does not differ significantly in how different loads of the virus affect T cells and their subsets. The majority of the body has achieved immunological tolerance, meaning that the hepatitis B virus has

successfully balanced out with the immune system. Virus loads do not seem to correlate with immune cell counts because a robust immunological response is not elicited at any viral load level [26]. At this point, the viral load and the number of immune effector cells are irrelevant because the body's immune system may not have fully recognized the hepatitis B virus. Consequently, the immune response may not have been elicited, and the body's load may not have been significant. Thus, antiviral treatment is not utilized at this stage of hepatitis B infection; rather, vigilant surveillance is the primary clinical practice.

Complement C3 Levels in patients of acute, chronic hepatitis B and control groups recorded (140.3 ± 10.09), (97.84 ± 8.64) and (108.82 ± 9.12) respectively. The results of C3 levels shown in Figures 4, illustrate that there (Mean ± S.D) was no significant difference (P>0.05) in C3 levels between patients groups and control group.

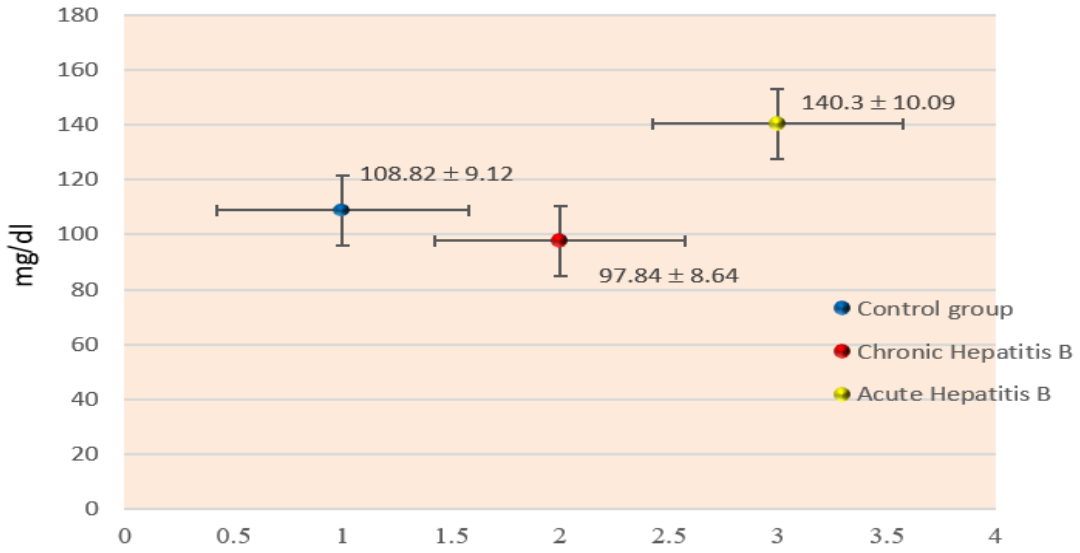

Figure 4. Complement C3 Levels in patients of acute, chronic hepatitis B and control groups.

Consistent with other research, our findings show that C3 serum levels in CHB patients did not drop considerably. Previous research found that C3 levels in HBV patients' sera were significantly lower than normal, presumably because their livers were unable to produce as much C3. These results contradicted that conclusion. The circulating immunological complex detected in the sera of AHB patients is responsible for the observed decrease of C3 levels. The decreased protein synthesis of C3 levels was associated with low C3 levels in CHB patients, which may be due to an increase in C3 degradation as well as an increase in the extensive involvement of the liver parenchymal. Complement C4 Levels in patients of acute, chronic hepatitis B and control groups recorded (25.37 ± 3.81), (27.65 ± 5.83) and (36.22 ± 7.19) respectively. C4 levels illustrated in Figure 5, show a significant decrease in C4 levels of AHB and CHB groups, when compared with and control group.

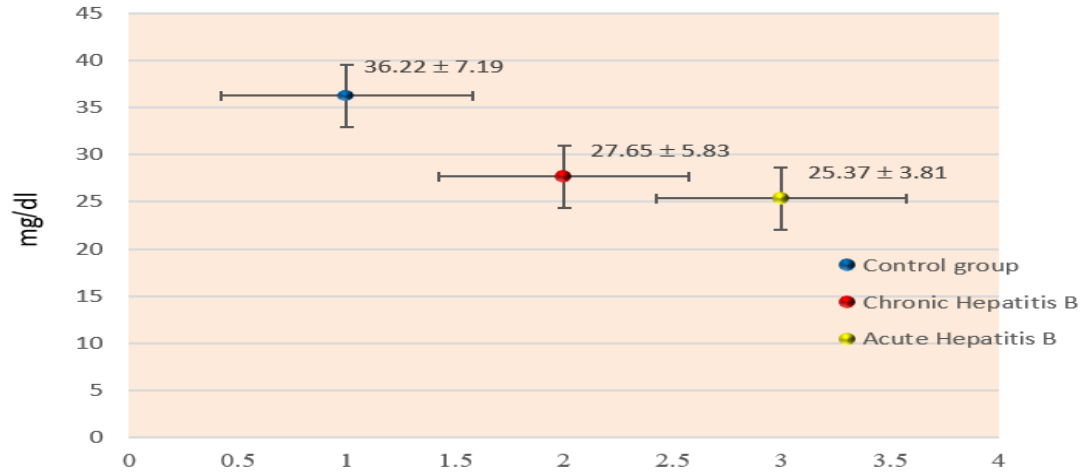

Figure 5. Complement C4 Levels in patients of acute, chronic hepatitis B and control groups.

The findings corroborate those of earlier research that found C4 levels in the sera of AHB patients to be much lower. Serum C4 levels were substantially lower in CHB patients, as shown in numerous prior investigations. Finally, studies have shown that decreased serum complement components are associated with liver illness through at least two different mechanisms: (1) In AHB, immune complex activation occurs in some patients. (2) In advanced hepatocellular illness, the rate of complement component production is low.

Lymphocyte Transformation Assay: The results were expressed as an assay. The transformed cells in patients of acute, chronic hepatitis B and control groups recorded (33.92 ± 5.68), (31.07 ± 3.99) and (53.07 ± 9.80) respectively. The transformed cells (Mean ± S.D) were significant less $P<0.05$ in acute and chronic hepatitis than those of control group. In vitro lymphocyte transformation was performed by using culture of separated lymphocyte or whole blood. Radioactive thymidine incorporation rate measurements of DNA synthesis are the gold standard for evaluating the response to PHA. Examining the morphological alterations of lymphocytes under the oil immersion light microscope is another morphological way to measure lymphocyte transformation. Polyclonal activators, like PHA, can stimulate cell DNA production, which is a measure of proper T-lymphocyte responsiveness. Symptoms of hepatic disorders, such as acute and chronic hepatitis B, include a decrease in PHA responsiveness; this decrease occurs in the early stages of the disease and then progressively returns to normal. Patients with CHB have chronically decreased PHA reactivity, but healthy carriers show no abnormalities. Some researchers have drawn the conclusion that the functional integrity of T-lymphocytes in infected individuals determines the eventual fate of HBV infection based on these observations. Multiple researchers have found that CHB patients have impaired cell-mediated immunity, as assessed by in vitro studies of lymphocyte proliferation in reaction to mitogens like PHA or conA.

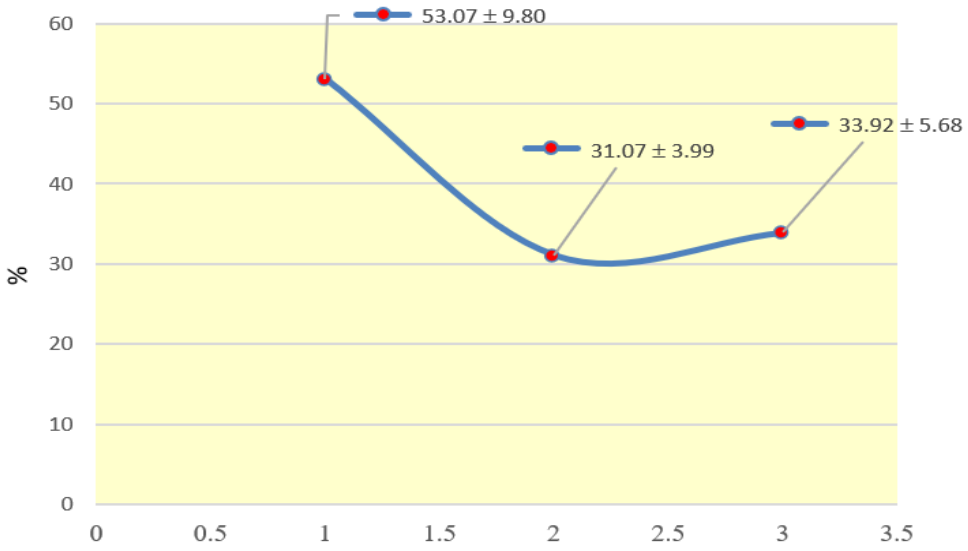

Figure 6. The transformed cells in patients of acute, chronic hepatitis and control groups.

Consistent with earlier research, this study found that acute, healthy carriers, and those with chronic hepatitis B had a reduced lymphocyte transformation capacity in response to PHA compared to the control group. Changes in plasma lipoproteins are a prevalent cause of the poor T-cell response to mitogens as PHA in chronic liver disease, according to some writers. Because there are fewer lymphocytes in the peripheral blood of patients with AHB and CHB, the Tlymphocyte response to PHA is diminished. Immunosuppressive factors, linked to high viral replication levels, were responsible for the decreased lymphocyte PHA capacity in AHB and CHB; these findings demonstrated the function of these factors in the immunological pathogenic processes. Studies have shown that healthy carriers have a normal lymphocyte response to PHA, which means that there is no major liver damage and no overall impairment of the immune system's cellular response, with the exception of the persistence of HBsAge. The proliferation response with improved T-cell activation techniques, with the use of flow cytometry for a more precise assessment of the surface expression of several activation antigens on lymphocytes. This includes IL-2 receptors as well as the proliferation

response following T-cell activation in vitro via CD3 and CD2 molecules. Their theory postulated that CHB patients' immunological responses were due to either an insufficient number of activated T-lymphocytes in the body or a selective deficiency in lymphokine synthesis.

Phagocytic cells activities (Phagocytosis of *Staph. aureus* by phagocytic cell) in patients of acute, chronic hepatitis and control groups recorded (60.35 ± 8.96), (54.83 ± 7.13) and (75.91 ± 10.00) respectively. phagocytic cells activities (Figure 7) were a highly significant decreased P<0.05 in acute and chronic hepatitis than those of control group. The term "phagocytosis" refers to the process by which phagocytic cells consume and kill particle material like germs. Macrophages, monocytes, and neutrophils are the three most important types of phagocytic cells. To take action, phagocytic cells must first detect foreign substances, then move to attach to them, then ingest them, and finally destroy them inside the cell using a variety of antimicrobial methods. The presence of HBV in the blood was identified using DNA hybridization and cell separation techniques. Patients infected with herpes simplex virus (HSV) had high levels of free-monomer length HBV in their polymorphonuclear leukocyte cell fraction. The reason these cells contain viral DNA is unclear, but it could be associated with viral replication or phagocytosis. The importance of the monocyte-macrophage cell line in host defense against viruses is being more and more acknowledged. The phagocytosing macrophages lyse virus-infected cells, impose cellular cytotoxicity that is dependent on antibodies, and display a range of antiviral actions that are intrinsic and extrinsic. In addition to their roles as lymphocyte support and antigen presenters, monocytes play a significant role in the immune response to viruses. The primary criterion for assessing phagocytosis in patients is the detection of peripheral blood leukocytes, which include the quantity of neutrophils and monocytes. Blood tests can help explain it and quantify the phagocytic cells' function using methods including chemotaxis, bactericidal activity, reducing nitroblue tetrazolium (NBT), and superoxide anion (O2-) generation.

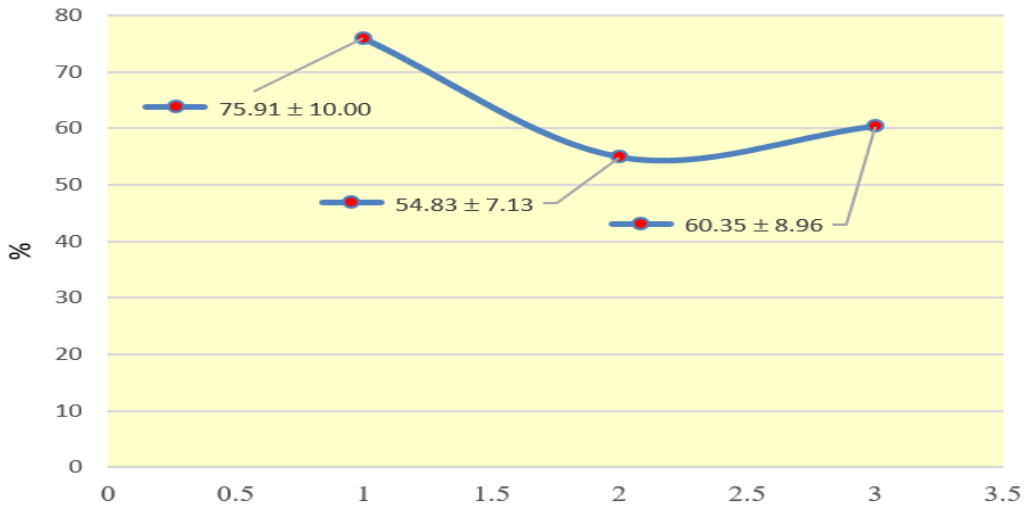

Figure 7. Phagocytic cells activities in patients of acute, chronic hepatitis and control groups.

Consistent with earlier research, this study discovered that zymosan stimulation decreased O2-production and that CHB patients exhibited significantly less bactericidal action. Phagocytosis activity was observed to be lower in AHB patients in other research. While CHB patients were in the replicative phase, their monocyte phagocytic activity was diminished. It is postulated that the inhibitory effects on neutrophil phagocytosis activity in AHB patients may be due in large part to serum inhibitory substances and circulating immune complexes. Because the virus and complexes containing the virus harm circulating monocytes, the percentages and functions of these cells decline in AHB patients. On the flip side, a reduction in INF-□ activity during the replication phase is responsible for the diminished monocyte phagocytosic activity observed in CHB patients.

CONCLUSIONS

Finally, this study concludes that certain levels of hepatitis B viral load influence immune function in differently. Consequently, it is useful for clinical understanding the immunological mechanism of viruses and using suitable

antiviral treatment to distinguish and appraise this point. There was a marked decline in the lymphocytes' transformation capacity, suggesting a drop in cellular immunity in the patient groups. When evaluating the immunological tolerance phenomena in these individuals, healthy carriers did not show a statistically significant decline in transformed lymphocyte capacity or phagocytic cell activities. Reduced non-specific immunity was demonstrated by a marked drop in phagocytic cell activity in AHB and CHB patients. Humoral immune response abnormalities suggest a malfunction in the function of T cells, which control the responses of B cells.

REFERENCES

1. De Pasquale, C., Campana, S., Barberi, C., Sidoti Migliore, G., Oliveri, D., Lanza, M., et al. (2021). Human hepatitis b virus negatively impacts the protective immune crosstalk between natural killer and dendritic cells. Hepatology 74, 550–565.
2. De Simone, G., Andreata, F., Bleriot, C., Fumagalli, V., Laura, C., Garcia-Manteiga, J. M., et al. (2021). Identification of a kupffer cell subset capable of reverting the T cell dysfunction induced by hepatocellular priming. Immunity 54, 2089–2100.e8.
3. Dong, Y., Li, X., Zhang, L., Zhu, Q., Chen, C., Bao, J., et al. (2019). CD4+ T cell exhaustion revealed by high PD-1 and LAG-3 expression and the loss of helper T cell function in chronic hepatitis b. BMC Immunol. 20, 27.
4. Durai, V., and Murphy, K. M. (2016). Functions of murine dendritic cells. Immunity 45, 719–736.
5. Fang, Z., Zhang, Y., Zhu, Z., Wang, C., Hu, Y., Peng, X., et al. (2022). Monocytic MDSCs homing to thymus contribute to age-related CD8+ T cell tolerance of HBV. J. Exp. Med. 219, e20211838.
6. Ferrando-Martinez, S., Huang, K., Bennett, A. S., Sterba, P., Yu, L., Suzich, J. A., et al. (2019). HBeAg seroconversion is associated with a more effective PD-L1 blockade during chronic hepatitis b infection. JHEP Rep. 1, 170–178.
7. Filho, R. M., Carmo, R. F., Catsman, C., Souza, C., Silva, A., Moura, P., et al. (2010). High frequency of variant alleles of the mannose-binding lectin 2 (MBL2) gene are associated with patients infected by hepatitis b virus. Viral Immunol. 23, 449–453.
8. Fisicaro, P., Barili, V., Montanini, B., Acerbi, G., Ferracin, M., Guerrieri, F., et al. (2017). Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis b. Nat. Med. 23, 327–336.
9. Fisicaro, P., Barili, V., Rossi, M., Montali, I., Vecchi, A., Acerbi, G., et al. (2020). Pathogenetic mechanisms of T cell dysfunction in chronic HBV infection and related therapeutic approaches. Front. Immunol. 11, 849.
10. Fisicaro, P., Rossi, M., Vecchi, A., Acerbi, G., Barili, V., Laccabue, D., et al. (2019). The good and the bad of natural killer cells in virus control: perspective for anti-HBV therapy. Int. J. Mol. Sci. 20(20), 5080.
11. Gabriel, S. S., Tsui, C., Chisanga, D., Weber, F., Llano-León, M., Gubser, P. M., et al. (2021). Transforming growth factor-b-regulated mTOR activity preserves cellular metabolism to maintain long-term T cell responses in chronic infection. Immunity 54, 1698–1714.e5.
12. Ghosh, S., Nandi, M., Pal, S., Mukhopadhyay, D., Chakraborty, B. C., Khatun, M., et al. (2016). Natural killer cells contribute to hepatic injury and help in viral persistence during progression of hepatitis b e-antigen-negative chronic hepatitis b virus infection. Clin. Microbiol. Infect. 22, 733.e9–733.e19
13. Hakim, M. S., Rahmadika, N., and Jariah, R. O. A. (2020). Expressions of inhibitory checkpoint molecules in acute and chronic HBV and HCV infections: implications for therapeutic monitoring and personalized therapy. Rev. Med. Virol. 30, e2094. doi: 10.1002/rmv.2094 Han, J. W., and Shin, E.-C. (2020). Liver-resident memory CD8+ T cells: possible roles in chronic HBV infection. Int. J. Mol. Sci. 22, 283.

14. Hensley, S. E., Zanker, D., Dolan, B. P., David, A., Hickman, H. D., Embry, A. C., et al. (2010). Unexpected role for the immunoproteasome subunit LMP2 in antiviral humoral and innate immune responses. J. Immunol. 184, 4115–4122.

15. Hoang, T. V., Toan, N. L., Song, L. H., Ouf, E. A., Bock, C.-T., Kremsner, P. G., et al. (2011). Ficolin-2 levels and FCN2 haplotypes influence hepatitis b infection outcome in Vietnamese patients. PloS One 6, e28113.

16. Jandus, C., Boligan, K. F., Chijioke, O., Liu, H., Dahlhaus, M., Démoulins, T., et al. (2014). Interactions between siglec-7/9 receptors and ligands influence NK cell-dependent tumor immunosurveillance. J. Clin. Invest. 124, 1810–1820.

17. Jiang, D., Chen, C., Yan, D., Zhang, X., Liu, X., Yan, D., et al. (2022). Exhausted phenotype of circulating CD8+ T cell subsets in hepatitis b virus carriers. BMC Immunol. 23, 18.

18. Kallies, A., Zehn, D., and Utzschneider, D. T. (2020). Precursor exhausted T cells: key to successful immunotherapy? Nat. Rev. Immunol. 20, 128–136.

19. Khan, O., Giles, J. R., McDonald, S., Manne, S., Ngiow, S. F., Patel, K. P., et al. (2019). TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion. Nature 571, 211–218.

20. Khanam, A., Chua, J. V., and Kottilil, S. (2021). Immunopathology of chronic hepatitis b infection: role of innate and adaptive immune response in disease progression. Int. J. Mol. Sci. 22 (11), 5497.

21. Koh, S., Kah, J., Tham, C. Y. L., Yang, N., Ceccarello, E., Chia, A., et al. (2018). Nonlytic lymphocytes engineered to express virus-specific T-cell receptors limit HBV infection by activating APOBEC3. Gastroenterology 155, 180–193.e6.

22. Le Bert, N., Salimzadeh, L., Gill, U. S., Dutertre, C.-A., Facchetti, F., Tan, A., et al. (2020b). Comparative characterization of b cells specific for HBV nucleocapsid and envelope proteins in patients with chronic hepatitis b. J. Hepatol. 72, 34–44.

23. Legat, A., Speiser, D. E., Pircher, H., Zehn, D., and Fuertes Marraco, S. A. (2013). Inhibitory receptor expression depends more dominantly on differentiation and activation than “Exhaustion” of human CD8 T cells. Front. Immunol. 4.

24. Leu, C.-M., Lu, Y.-C., Peng, W.-L., Chu, H.-T., and Hu, C. (2014). The hepatitis b virus e antigen suppresses the respiratory burst and mobility of human monocytes and neutrophils. Immunobiology 219, 880–887.

25. Li, Y., You, Z., Tang, R., and Ma, X. (2022). Tissue-resident memory T cells in chronic liver diseases: phenotype, development and function. Front. Immunol. 13. doi: 10.3389/fimmu.2022.967055 Li, C., Yu, T., Shi, X., and Yu, J. (2022a). Interleukin-33 reinvigorates antiviral function of viral-specific CD8+ T cells in chronic hepatitis b virus infection. Viral Immunol. 35, 41–49.

26. Liu, Y., Hu, X., Hu, X., Yu, L., Ji, H., Li, W., et al. (2022). T Follicular helper cells improve the response of patients with chronic hepatitis b to interferon by promoting HBsAb production. J. Gastroenterol. 57, 30–45.