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STUDY THE CORRELATION OF NEOPTERIN WITH INTERLEUKIN-6 IN HEPATITIS B PATIENTS IN AL-RAMADI CITY

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ABSTRACT

Background/Purpose: Hepatitis B is a potentially life-threatening hepatitis B virus (HBV) liver disease. It is one of the major health issues in the world. It may result in chronic infection and puts populations at high risks of death because of cirrhosis of the liver and liver malignancy. The objective of the present study was to measure two parameters in chronic viral hepatitis patients (Neopterin, Interleukin-6). These analytes were examined in chronic hepatitis B(CHB) patients to compare the association between these markers with healthy controls & study. **Methodology:** A 50 CHB patients and 28 healthy controls (HC) have been involved in the current study, matched in age and sex. Based on commercially available ELISA kit, serum concentration of Neopterin and IL-6 are estimated. **Results:** Serum concentrations of Neopterin & IL-6 have been significantly greater in the CHB group in comparison with the control group. There has been a positive relationship between serum neopterin and IL-6 levels in CHB. Several biochemical tests were performed for patients and healthy subjects, such as ALT, AST, ALP, GGT, T.S.B. The results showed that the patients group had a considerable increase in the level of ALT, AST, ALP, and GGT compared to the control group. There has not been any considerable relation between sex and age with serum Neo & IL-6 (both $p > 0.05$) in the control and patient groups. In conclusion, this study clarified the important role of Neopterin and interleukin-6 in the prediction, cause, development and treatment in early stages of CHB. **Conclusion:** This study clarified the important role of Neopterin and interleukin-6 in the prediction, cause, development and treatment in the early stages of CHB.

Key words: Hepatitis B, Neopterin, Interleukin-6.

Abbreviations: Chronic hepatitis B (CHB), Neopterin (Neo)**,** Interleukin-6 (IL-6)**,** Hepatitis B virus (HBV), Interferon-gama (INF- γ).

I. INTRODUCTION

Viral hepatitis is a chronic infection, including the main viral replication cycle site of the liver [1]. Some viruses, such as A, B, C, D, E can cause hepatitis [2]. HBV infections remains an extensive common health issue which may result in chronic and acute hepatitis, hepatocellular carcinoma, and hepatic cirrhosis [3]. It is estimated that 2 billion people are infected with the HBV, and that includes about 350 million chronically infected; 0.50-1.20 million deaths result from the HBV-related complications, each year [4].

Neopterin, a pyrazino- [2, 3-d]-pyrimidine derivative, is a cyclic guanosine monophosphate metabolite produced by activated T lymphocytes and macrophages following interferon induction (IFN-γ) [5]. While IFN- γ is the most effective inducer of neopterin release, several other pro-inflammatory soluble mediators, such as TNF-α and bacterial components as lipopolysaccharides, can also work as cofactors to promote its release. Neopterin in blood, urine, and stool can be assessed. The release of neopterin begins about three days before T-cells hit the full proliferative. Increased biosynthesis of neopterin usually occurs a week before particular antibodies show in the

blood, so it is suggested that neopterin be the early predictor for the inflammatory reaction [6]. Neopterin is increased in some of the diseases associated with acellular immune reaction such as autoimmune diseases, viral infections, HIV disease, inflammatory diseases, cellular living bacterial or parasites, organ donation rejection events, and some cancerous illnesses [7]. It was noted that the levels of Neo are generally high in many other pathogens where the cellular immune system is active. Neopterin levels can therefore be calculated to measure the degree of immune system activation which can be beneficial as a simple and accurate diagnosis in the laboratories [8].

IL-6 is a cytokine with a wide variety of biological activities. It is a mediator for replacing the immunoglobulin class and controlling the response of the acute phase. It is also an example of inflammation in the body. IL-6 can be used as an investigation indicator [9]. High levels of IL-6 are correlated with many inflammatory and malignant illnesses; it was thought to be a combination of inflammatory reactions and an inflammatory marker [10]. Recent study detected high serum levels of IL-17 and IL-37, but low serum levels of IL-38 in Iraqi Men with CHB than in healthy controls [11]. Thus, the objective of the study is evaluating the serum Neo & IL-6 concentrations as well as their correlations with each other in CHB disease.

II. PATIENTS AND METHODS:

A: The design of the study and population

A 50 CHB patients (25 males and 25 females), age ranged between 11 and 64 years, have been enrolled in this research. This study was conducted during the time from December 2020 to March 2021.

All patients selected, have no clinical history of recent. The patients have been evaluated prior to initiating any treatment. They are healthy without symptoms of jaundice or chronic liver disease. Patients were compared to 28 (12 males, 16 females) healthy control subjects age ranged from 11-64 years.

B: Sample collection

Five ml of fresh blood was drawn by vein puncture with a plastic syringe from each patient and healthy human. The blood specimen was shifted to a plain tube, after which the tube was centrifuged for 10 mins at (3000 xg). Serum was then aliquoted into several eppendorf tubes and frozen at -20 ° C until used.

C: Evaluation of biochemical

The tests of the liver functions (serum alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and (T.S.B) have been made as well in all subjects with the use of an auto-analyzer.

D: Serological part of the study: -

The serological parameters used in this study were firstly checked by serum Neo and IL6 were measured by Enzyme-Linked Immunosorbent Assay (ELISA). Those kits are provided by (Neopterin My-bio-source company, USA) and (Interleukin-6 Cusabio company, China).

E: Statistical analysis:

Statistical analysis has been carried out with the use of the Statistical Package for Social Sciences (SPSS) software (SPSS Inc., Chicago, U.S.) version 25.0 with descriptive statistical analysis, all study parameters were demonstrated as mean ± standard deviation (SD). One-way ANOVA test determined the significance of differences between individuals. Spearman has confirmed the relationship between parameter relationship analysis. P-value less than 0.05 were considered to be significant.

III. RESULTS AND DISCUSSION:

A 50 CHB patients (25 males and 25 females) and 28 (12 males, 16 females) healthy control, have been enrolled in this research.

Biochemical investigations (liver enzymes)

Table (1), lists the results that have been obtained in the present study from which we can see there was the concentration of ALT, AST, ALP, GGT were increased in patients compared to healthy control subject (**37.74 ±**

13.51), (36.80 ± 15.78), (166.84 ± 40.52) and **(18.38 ± 5.678)** respectively**.** The statistical analysis (Table 1), it was found that the level of T.S.B among (CHB) patients group has been higher compared to the healthy control with the mean was **(0.88 ± 0.65 IU/L)**.

There has not been a considerable correlation between sex and age with serum ALT, AST, ALP, GGT, T.S.B rates (both $p > 0.05$) within any of the patients group.

As non-significant differences of age between the group of patients and healthy control subject.

Table 1: (ALP, ALT, AST, GGT and T.S.B levels for all Control and Patients groups).

Parameters			Mean ± S. D	p-value
ALP	U/L	**cases**	**166.84 ± 40.52**	**0.052**
		controls	**115.57± 11.39**	
ALT	U/L	**cases**	**37.74 ± 13.51**	**0.002**
		controls	**20.86 ± 8.08**	
AST	U/L	**cases**	**36.80 ± 15.78**	**0.001**
		controls	**25.64± 7.30**	
GGT	U/L	**cases**	**18.38 ± 5.678**	**0.000**
		controls	**8.64± 3.79**	
T.S. B	mg/dL	**cases**	**0.88 ± 0.65**	**0.070**
		controls	**0.65± 0.17**	

Serological markers

Table (2), lists the results that have been obtained in the present study from which we can see there was the concentration of Neo & IL-6 concentration was increased with a significant difference in CHB cases compared to a healthy control subject. The serum level of the neopterin in CHB group has been **18.5±6.649** nmol/L (range 7.18-32.4 nmol/L), while it has been **6.80± 2.59**nmol/L (range 1.58-9.81 nmol/L) in the control group, and this increase has been discovered to be statistically significant ($p < 0.05$) (Fig1). The serum level of Interleukin-6 in CHB group was **389.53 ± 189.2** pg/mL (range 858.92-115.52 pg/mL), whereas it was **138.52 ± 84.46** pg/mL (range 336.49-109.74 pg/mL) in the control group. The average level of Interleukin-6 of patients with CHB increased in comparison with the control group, such increase has been discovered to have statistical significance ($p < 0.05$) (Fig. 2).

Table 2: (Neo and IL-6 levels for all Control and Patients groups).

Parameters			Mean ± S. D	p-value
Neo	nmol/L	**cases**	**18.5±6.649**	**0.000**
		controls	**6.80± 2.59**	
IL-6	pg/mL	**cases**	**389.53 ± 189.2**	**0.004**
		controls	**138.52 ± 84.46**	

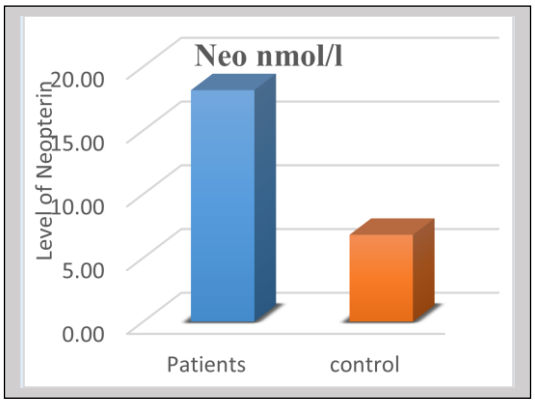

Figure 1: Neopterin Serum levels of the groups

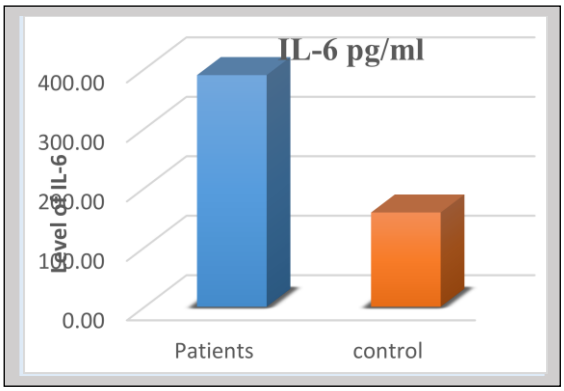

Figure 2: IL-6 Serum levels of the two two groups

The liver disease pathogenesis which results from HBV is mainly based on the mechanisms which are mediated by the immune system. None-the-less, it can seldom result from the direct hepatotoxic damages. The main immune system-mediated hepatotoxicity mechanism is destroying infected hepatocytes via the cytotoxic T cells [12]. In this study, we tend to documented that Neo & IL-6 were expressively elevated within the serum of CHB patients in comparison to the control group. In the infection of the hepatitis B, IFNγ, which is free from cytotoxic T cells, stimulates the macrophages within the liver, which stimulates the discharge of neopterin from those macrophages [13]. IFNγ generation will increase as a result of activating T lymphocytes with varied specific antigens, which are primarily microorganism antigens [14].

Neopterin can be considered as an early important marker to monitor the activity of the infectious diseases [15]. The levels of the serum neopterin have not only been associated with the disease activity, they are as well informative and useful for the early distinguishing between the infectious patients and the non-infectious ones [16]. It was proposed that the levels of the serum neopterin can be a significant differential diagnosis indicator of the non-infectious and viral hepatitis types [17]. High levels of the neopterin have been discovered in the hepatitis B e anti-gen positive (HbeAg +) chronic hepatitis B patients. It can be concluded that there is an association between the increased levels of the neopterin and HbeAg-positivity [18]. The medical specialty research results advised that Neo levels are often utilized as associate degree inflammatory markers in kids with Hepatitis-B-related chronic disease [19]. An increase in Neo levels within the HBsAg carriers compared to the healthy people [20]. The results of our study show an increase in Neo level in the serum of CHB patients. These results are consistent with the previous studies [18,20]. We have a tendency to found a high increase in Neo levels within the CHB because the stimulation of the cellular system in comparison with HCs.

The levels of the Neopterin are decreased in the case of suppressing the immune system, because of the infectious illnesses like the CHB. In contrast, in the case of treating the CHB by an IFN treatment, the concentration levels of the neopterin are increased because of stimulating the immune system [21]. The increased levels of the neopterin in the fluids of the body may be discovered immediately prior to the incidence of clinical signs at the incubation period's end, and it has shown a considerable increase with the appearance of the clinical signs. High Neo concentration levels are articulated progressively in the diseases throughout the entire activities of the macrophage and monocyte are intense. The release of the Neopterin starts 3 days prior to the proliferation of the T lymphocyte reaching the peak and the neopterin may be utilized as an early inflammation indicator due to observing an increase in the production of the neopterin nearly 1 week prior to the point where specific anti-bodies become positive. Following the appearance of the neutralizing anti-bodies in the period of the convalescence, none-the-less, the secretion of the neopterin is decreased to the normal levels [22].

Interleukin-6 (IL-6) is firmly connected to the event of HBV contamination, that is among tumorigenesis and immune disorders, we tend to argue that the blockade of the IL6 might boost the effectuality of the treatment in the patients who have diseases associated with the HBV. Accordingly, similar clinical preliminaries have been desperately required in order to explain the pathologic process of IL6 perform and make sure the role of IL6 within the development of HBV-associated diseases [23]. Initiated monocytes are the most supply of IL6 in the blood, once inflammation happens, macrophages and monocytes are the primary receptive cells which

manufacture the IL6 [24]. Which is why, IL-6 has been at the first idea to market the enlargement and stimulation of the lymphocyte population, promote lymphocyte separation, and control the response of the acute-phase [25].

The previous study had demonstrated that IL-6 level in liquid body substance is enhanced in HBV-contaminated patients and are considerably greater in the patients with the severe case [26]. HBV will infect the monocytes of the peripheral blood, and might effectively imitate in those cells [27]. Interestingly, our present result in concurrence with this finding, we tend to found a high increase in the levels of the serum IL6 within the CHB. Because of the chronic active liver disease, the decrease within the process of the antigens by the Kupffer cells and also the poly-clonal initiation of internal organ antigens enhanced liquid body substance the levels of IL6. In the chronic severe hepatitis (CSH), Kupffer cell phagocytosis loss, massive hepatocytes necrosis, as well as the endotoxemia, which results from decreasing the function of the intestinal mucosa and defaults in the elimination of the endotoxin as a result of the liver injuries, resulted in stimulating the mono-nuclear phagocyte system in producing higher levels of the IL6 [23]. Additionally, due to the fact that the liver is that major organ which is answerable for the disposal of IL6, severe liver damage can weaken evacuation of IL6, resulting in a rise in plasma levels of IL6, at the same time, elevated IL-6 levels will instigate expansion and separation of cytotoxic T-cells [28]. Which will cause the inflammation of the liver in addition to destroying the immunological cells. In the CHB patients, the levels of the IL6 in the hepatitis B early anti-gen (HBeAg) (+) and HBV-DNA (+) patients have been considerably greater compared to the patients with HBV-DNA (−) and HBeAg (−). Post the interferon treatment, the levels of the IL6 serum have been considerably reduced in HBV-DNA (+) and HBeAg (+) patients, which indicates that the replication of the HBV is associated with the levels of the IL-6, which have a synergistic anti-viral impact [29]. Which is why, IL6 is a helpful marker as well to monitor the activity of the disease and the therapeutic efficacy in the hepatitis B patients, undetectable levels of the serum IL6 (i.e. less than 3pg/mL) throughout early acute exacerbation (AE) stage in the CHB patients might signify the patients with the favorable clinical results, which indicates that the IL-6 can be a beneficial clinical predictor of the prognoses [30].

There has not been any significant correlation between sex and age with serum Neo & IL-6 rates (both $p > 0.05$) within any of the patients group.

Biomarker correlations

Based on the results shown in Table 3, the level of Neo had positively correlation with IL-6 ($P < 0.01$), also positively relationship with GGT ($P< 0.05$).

There has not been any significant correlation found between the levels of the serum neopterin and AST, ALP, ALT and T.S.B levels in patients with the chronic hepatitis B.

There has not been any significant correlation discovered between serum Interleukin-6 levels and AST, GGT, ALP, ALT and T.S.B levels in CHB patients.

Table 3: (Correlation coefficient of Neo with IL-6 and GGT in CHB patients)

	Neo	IL-6	GGT
Neo	**1**	**.298****	**.262***
IL-6	**.298****	**1**	**-.012**
GGT	**.262***	**-.012**	**1**

**. Correlation is significant at the ($p<0.01$)level (2-tailed).

*. Correlation is significant at the ($p <0.05$)level (2-tailed).

IV. CONCLUSION

In conclusion, this study clarified the important role of Neopterin and interleukin-6 in the prediction, cause, development and treatment in the early stages of CHB.

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