Role of Innate Immunity in Patients with HBV Infection

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Abstract:

Background: The hepatitis B virus [HBV] is an infectious disease and its one of the great public health problem, its outcome depends on the kinetics of the virus-host interaction and specifically on the strength of the innate and adaptive, humoral and cellular immune response. Thus interactions between the virus and the components of immune systems plays an important role in the pathogenesis of the disease. This study aimed to evaluate innate immune response in different clinical courses of HBV infection by estimation serum levels of granulocytemacrophagecolony stimulating factor (GM-CSF), Interleukin-1 (IL-1), Interleukin-8 (IL-8) , immunoglobulin A (IgA), IgM, IgG and Complement component (C3and C4) levels.

Aim of study: to evaluate role of immune response in clinical course of hepatitis B infection

Patients and Method: This study based on 78 patients with HBV infection attending the Public Health Laboratories. Their ages ranged from (14-65) years, (58 males and 20 females) compared with 20 healthy subjects (12 males and 8 females) as control group. This study extended from first of January 2015 to first of January 2017.

The patients were classified to three groups on the bases of serologic markers (HBsAg, HBe Ag, HBc Ab IgM) according to WHO department of communicable disease surveillances and response, liver function tests which are used as supportive indicator for the liver injury, history of the illness, and full clinical assessment. The first group includes twenty-eight patients with acute HBV infection, second group include twenty cases identified as chronic healthy HBsAg carriers group and the last third group include thirty cases of chronic HBV group

Results: By using enzyme immuno assay [ELISA] technique, serum levels of interleukin-1alpha [IL-1α], interleukin-8 [IL-8], and granulocyte-macrophage-colony stimulating factor [GM-CSF] were measured in all patients compared to that of healthy control group. The mean levels of IL-1α and IL-8 showed a significant increase in serum of patients with newly infected (acute) HBV and chronic HBV compared with studied control group [P=0.04 and P=0.001 respectivel]. However, the mean serum levels for IL-1α and IL-8 recorded a non significant increase in patients with chronic healthy HBsAg carriers [P=0.17 and P=0.4 respectively]. While the mean serum levels of GM-CSF showed a significant rise in acute HBV only [P=0.01].

Moreover, serum immunoglobulins [IgG, IgM, IgA] and complement component [C_3, C_4] levels also evaluated by using single radial immunodiffusion test (SRID). The mean serum IgA, IgG and IgM levels showed a significant increase in chronic HBV group as compared to that of control group [P=0.000 ,P=0.000 and P=0.001 respectively].. The mean serum C_3 and C_4 levels showed a significant lower serum level in all groups compared to control studied group [P=0.000].

Conclusion: this data demonstrate that immunological differences do exist between different clinical groups with HBV infection and may reflect the role of the innate immune system in host defense and disease.

Key word: HBV, Innate immune, Complement component, Immunoglobulins.

Introduction:

Despite, the availability of effective vaccine HBV remains a great public health problem with 2 billion people infected worldwide and the 10th leading cause of death, between 500,000 and 780,000 patients die annually from HBV associated liver disease and most of these deaths occur in developing countries [1]. HBV infection is a dynamic condition, in which the relation between virus and patients host immune response influences the progression of the disease [2], and its infection may associate with a large spectrum of disease (ranging from very mild and asymptomatic illness to the most severe liver diseases including: fulminant hepatitis, cirrhosis and hepatocellularcarcinoma). [3]

Hepatitis B virus is a non cytopathic virus and trauma to liver is mainly caused by the host immune response. Cytokines has the major role in the defense against infection cause by virus, both indirectly, through designing pattern of host immune response and directly, through stopjng viral replication and involved in the non-cytolytic clearance of HBV during acute infection and during T cell-mediated virus control. Their effects against HBV are approved in a different of experimental studies models [4, 5]. However, in the context of an inflammatory response against a virus, cytokines may lead to liver injury by signaling substance in the immune response against pathogens, it play a key role in the pathogenesis of hepatitis/, cirrhosis and HCC [6].

IL-1 is a pro-inflammatory cytokine that play a role in diseases caused by infection by HIV, avian influenza, human papilloma and hepatitis B and C viruses is secreted by T cells and NK cells, and has antiviral, immunoregulatory, and anti-tumor properties [7], and IL-8 is a chemokine and an important mediator in the innate immune response with well-defined immunomodulatory effects on T-cell function and inflammatory response, some evidence has suggested that this chemokine may play an important role in the immunopathogenesis of HBV infecion [8]. Rise in serum level of IL-8 have been seen in Chronic HBV cases with active chronic hepatic inflammation as well as in cases with acute HBV [9]. Also, in other study found that serum IL-8 level increase during the episodes of HBV reactivation, the rise of viremia levels is paralleled by an increase of serum IL-8 level and that these events precede the onset of hepatic flare [10]. Also (GM-CSF) is a cytokine produced by macrophages; NK cells; T cells; fibroblasts, and endothelial cells. It can rise neutrophil number and enhance the differentiation and maturation of myeloid dendritic cells to improve the functions of antigen-presenting cells, as well as induce the development and perpetuation of Th1 responses given its immune modulatory functions, there for (GM-CSF) has been used as an adjuvant in different studies as an immunomodulting treatment for the cancers and HIV infection. In addition, some recent studies suggest that the use of GM-CSF as a vaccine adjuvant may induce suppressive immune responses in some cases of HBV infection Xianzheng Wang [11].

Subjects, Materials and Methods:

Our study based on 78 patients infected with HBV infection, all patients were currently attending the Public Health

Laboratory in Kirkuk city. Patients were selected after full clinical assessment, and all patients were subjected for questionnaire on the disease manifestation and their medical history. Our patients were classified to three groups on the bases of serologic markers [hepatitis B surface antigen "HBsAg": HBe Ag: hepatitis B core IgM antibody "HBc Ab IgM]) according to WHO department of communicable disease surveillances and response, liver function tests, and full clinical assessment. The first group includes twenty-eight patients with acute HBV infection, they had a recent onset of jaundice, elevated liver enzymes and were HBs Ag +ve, HBeAg +ve, and HBcAb (IgM) +ve. Twenty cases identified as chronic healthy HBsAg carriers group, were symptom free, had hepatic enzyme values within the normal levels with HBeAg –ve. Thirty cases of chronic HBV group, had more than 6 months of recognized HBsAg +ve, associated with elevated liver enzymes.

The mean value with the standard deviation (SD) for each value was determined. The statistical significance of difference in mean of a certain continuous outcome (dependent) variable between more than 2 groups (independent variable) was assessed by ANOVA test. P value lower than the 0.05 level of significance was considered statistically significant. The statistical significance between 2 groups was analyzed by chi-square test as indicated.

Results:

By using ELISA technique, serum levels of IL-1α: IL-8 and GM-CSF were measured in all patients compared to that of control studied group. The mean serum levels of IL-1α and IL-8 showed a significant increase in patients with acute HBV and chronic HBV compared with control group [P=0.04 and P=0.001 respectively] tables (2)&(3). However, the mean serum levels of IL-1α and IL-8 recorded a non significant increase in patients with chronic healthy HBsAg carriers [P=0.17 and P=0.4 respectively] tables (2)&(3). While the mean serum levels of GM-CSF showed a significant increase in cases with acute HBV only (P=0.01) table (4).

Moreover, serum [IgG, IgM, IgA] and complement component [C_3, C_4] serum levels also evaluated by using single radial immunodiffusion test (SRID). The mean serum IgA, IgG and IgM levels showed a significant increase in chronic HBV group as compared to that of control studied group [P=0.000 , P=0.000 and P=0.001 respectively]tables (5) (6)&(7). The mean serum C_3 and C_4 reported a significant low levels in all groups compared to control group (P=0.000) tables (8) & (9).

Table (1): Age and gender distribution of (HBV) infected groups.

Groups		Healthy control N = 20 (%)	Acute HBV N = 28 (%)	Chronic Healthy HBsAg carrier N = 20 (%)	Chronic HBV N = 30 (%)
Age (years) Range Mean± SD		(20-52) 31.6 ± 11.2	(14-54) 34.26 ±10.8	(19-54) 35.9 ±10.9	(18-65) 40.6 ± 15.3
Gend	Male	12	21	16	21
	Female	8	7	4	9

P= 0.53 non significant for gender between groups.
P = 0.153 non significant for age between groups.
P=0.0001 significant between male and female.

Table (2): Mean serum IL-1α levels (pg/ml) in the study groups.

Values pg/ml IL-1α	Healthy control	Acute HBV	Chronic healthy HBsAg carrier	Chronic HBV	P value
Minimum	8.7	10.	8.7	16	0.04
Maximum	14	75	87	80.0	
Mean	11.3	26.9	23.25	28.8	
SD	2.03	13.01	19.19	16.02	

Table (3): Mean serum IL-8 levels (pg/ml) in (HBV) infected groups.

Values Pg/ml IL-8	Healthy control	Acute HBV	Chronic healthy HBsAg carrier	Chronic HBV	P value
Minimum	1.1	60	8	53	0.001
Maximum	16	135	11	350	
Mean	5	99.3	8.85	100.9	
SD	2	17	1.23	53.7	

Table (4): Mean serum GM-CSF levels (pg/ml) in (HBV) infected groups.

3Values Pg/ml GM-CSF	Healthy control	Acute HBV	Chronic healthyHBsAg carrier	Chronic HBV	P value
Minimum	0.1	0.1	0.1	0.1	0.1
Maximum	2.5	102.5	13.5	102.5	
Mean	0.92	17.42	2.58	7.28	
SD	1.13	32.32	4.49	21.26	

Table (5): Mean serum IgG levels (mg/dl) in the study groups.

Values mg/dl IgG	Healthy control	Acute HBV	Chronic healthy HBsAg carrier	Chronic HBV	P value
Minimum	774.5	448.7	529	409.9	0.000
Maximum	1348.7	2010.6	1697	3079.6	
Mean	1055.4	1082.5	1045.1	1694.1	
SD	210.9	451.7	391.9	749.	

Table (6): Mean serum IgM levels (mg/dl) in the study groups.

Values mg/dl IgM	Healthy control	Acute HBV	Chronic healthy HBsAg carrier	Chronic HBV	P value
Minimum	40.6	26.5	26.5	30.3	0.000
Maximum	137.1	214.5	149.1	338.5	
Mean	89.4	103.8	81.7	161.6	
SD	29.4	49.7	34.4	96.8	

Table (7): Mean serum IgA levels (mg/dl) in the study groups.

Values mg/dl IgA	Healthy control	Acute HBV	Chronic healthy HBsAg carrier	Chronic HBV	P value
Minimum	96.5	29.8	130.8	50.4	0.00
Maximum	296.5	284.8	423.3	647.0	
Mean	172.9	175.3	234.9	382.0	
SD	64.8	69.2	86.3	213.4	

Table (8): Mean serum complement C3 levels (mg/dl) in the study groups.

Values mg/dl C3	Healthy control	Acute HBV	Chronic healthy HBsAg carrier	Chronic HBV	P value
Minimum	88.3	26.2	46.3	11.9	0.000
Maximum	195.3	114.9	120.6	150.1	
Mean	128.4	80.3	84.14	64.9	
SD	27.47	23.16	26.58	39.57	

Table (9): Mean serum complement C4 levels (mg/dl) in the study groups.

Values mg/dl C4	Healthy control	Acute HBV	Chronic healthy HBsAg carrier	Chronic HBV	P value
Minimum	20.4	9.3	5.9	2.8	0.000
Maximum	40.9	32.6	32.6	36.6	
Mean	31.6	21.0	20.69	18.08	
SD	5.66	6.76	8.7	10.63	

Discussion and Conclusion:

In this study statistical analysis revealed no significant difference between studied groups according to age and sex table (1), also HBV infection in this study occurred mainly in adults. This is similar to the findings of other studies [12]. The finding of significant lower number of HBV infection among females patient than males may be due to the hormonal differences between them and in turn, their effects on the immune responses.

1 - Interleukin-1:

A significant high levels of IL-1α in sera of acute and chronic HBV patients as compared to control subjects seen in table (2).these results were in agreement with the other results reported by Tian Zhao-ju, and deCastillo *et al.*[7,13], they found that a high levels of IL-1 were frequently observed in acute and chronic HBV infected patients. Actually, this finding is in agreement with Hiroko Tsutsui [14] who demonstrated extensively the role of IL-1 family as cytokine-mediated molecular and cellular interaction in the progression of acute and chronic liver diseases also Kolarski *et al.,* [15] reported that IL-1 and other cytokines modulate liver metabolism in health and disease, physiological and pathological liver function and the evolution of liver inflammation and injury to hepatic fibrosis and liver cirrhosis.

2 - Inerleukine-8:

In the current study, by comparing three groups of HBV infected individuals with different disease profile (acute HBV, chronic healthy HBsAg carrier, and chronic HBV) regarding their IL-8 levels, it has been found that the patients in chronic HBV have the highest mean values for the examined variable followed by patients with acute HBV, and chronic healthy HBsAg carrier table (3). This result also supported by Teresa Pollicino [2], who demonstrated high level of interleukin -8 in the blood and liver tissue of HBV infected cases

3 - Granulocyte-macrophage colony-stimulating factor (GM-CSF):

In the present study statistical analysis revealed a significant elevation of serum GM-CSF in acute HBV infection as compared to healthy subjects. While, there were no significant elevation of GM-CSF serum levels in cases with chronic HBV, and chronic HBsAg healthy carriers as compared to healthy subjects table (4). These results were in accordance with previous results reported by Kubota *et al.,*[16] he found that some patients with chronic viral hepatitis, showed elevation in GM-CSF level over the baseline levels measured in all of the normal, but this difference was not statistically significant. Furthermore, M. H. Bahgat *et al*.,[17]reported increase in GM-CSF level in chronic HBV infection and suggested that it might serve to monitor viral activity and outcome of patients.

4- Immunoglobulins (Igs):

SerumIgs levels are frequently increased in cases associated with cirrhosis, and the increase serum level of a specific class of serum Ig is associated with a specific disease for example, elevated level of IgM is related with primary biliary cirrhosis (PBC), elevated IgG level with autoimmune hepatitis (AIH), and elevated IgA level in alcoholic liver disease (ALD)]. Therefore, elevated Ig level can aid diagnosis, there are no sufficient data examining the use of serum Igs as markers for assisting diagnosis of HBV-related disease. In study done by Sha Lin they measured serum IgA, IgG, and IgM serum levels

in cases with HBV and analyzed whether Ig serum level was associated with disease progression in cirrhotic cases. They found that serum level of IgA may serve as a biomarker indicating cirrhosis [18].

In our study, the serum levels of three important Igs including IgG, IgA, and IgM were evaluated in studied HBV groups in comparison to control healthy group tables (5; 6; 7). The comparison concluded that the patients in the chronic HBV have the highest mean values for all the examined variable (IgG, IgA, IgM) followed by acute HBV and chronic healthy HBsAg carrier, respectively. Significant higher levels of immunoglobulins in chronic HBV and acute HBV groups compared to control group, were also reported by other studies Dienstag and Isselbacher,[3]. This elevation could be related to elevated tissue antibodies. However, the major factor seems to be failure of the diseased liver to get red intestinal antigens, and decreased suppressor T lymphocyte function in chronic hepatic disease [19].

5- Complement Components (C_3 , C_4):

Complement activation is one of the earliest responses to infection including viral hepatitis, it is widely involved in the body's defense against the foreign pathogens and the regulation of immunity [20]. In this study low levels of C_3 and C_4 were recorded with the acute HBV, chronic HBV and chronic healthy HBsAg carrier groups table (8, 9). This observation were also reported by other researcher Chengliang Zhu [21],. HBV can incorporate the complement regulatory protein of the host into its outer membrane to evade the host's complement attack, it may also inhibit the expression of complement C3 and C4 through the aforementioned hypotheses. In addition, the decrease in the synthesis by liver cells and excessive complement component consumption would lead to decline serum levels of complement C3 and C4 in the patients with HBV lnfection [22].

Conclusion:

Based on these results, it could be concluded that the innate immune system make critical contributions to the progression of HBV.

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