

Estimation of Immunoglobulin (IgG and IgM) in Hepatitis B Virus Infected Patients and Individuals Vaccinated with Recombinant HB Vaccine

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ABSTRACT:

BACKGROUND:

Hepatitis B virus (HBV) infection is a major public health problem. Successful clearance of the virus depends on a complex interaction between the virus and the host immune response.

OBJECTIVE:

The purpose of this study was to assessment of some immunoglobulins (IgG and IgM) in hepatitis patients and vaccinated groups compared with healthy control.

METHODS:

Study groups were classified into patient group 35 (15 acute (AC) and 20 chronic (CH)) and 35 vaccinated group (20 responder (RD) and 15 Non-responder (NRD)) and 18 healthy non vaccinated control group (HN). This study extended through a period from May to November 2007. Blood samples were taken from patients and hospitals staffs in Nanakaly, Erbil and Rizgary Teaching Hospital/ Erbil/ Iraq to detection of IgG and IgM level in serum by immunoturbidimetric test.

RESULTS:

The mean serum IgG level significantly increased in CH patients ($p < 0.05$) compared to AH and HN control groups, in contrast IgM level increased high significant ($p < 0.001$) in AH than CH and HN groups. No significantly differences in serum immunoglobulins (IgG and IgM) between vaccinated individuals and HN group ($p > 0.05$).

CONCLUSION:

Present study indicate that serum IgG level was higher in CH patients whereas IgM level increased in AH. No significant differences in their levels were observed between vaccinated individuals and HN group.

KEYWORDS: hepatitis, immunoglobulin, chronic, acute, vaccine

INTRODUCTION:

Antibodies or immunoglobulins are formed by B lymphocytes, each individual has a large pool of different B lymphocytes that have a life span of days or weeks⁽¹⁾. B cells display immunoglobulin molecules on their surface, these immunoglobulins serve as receptors for a specific antigen⁽¹⁾.

Hepatitis is an inflammation of the liver, characterized histologically by hepatocellular necrosis and infiltration of the liver by inflammatory cells⁽²⁾. The term of viral hepatitis is reserved to the infection of the liver caused by a small group of viruses having a particular affinity to the liver^(3, 4). Hepatitis B virus constitutes a major economic and public health problem throughout the world, due to the high rate of morbidity, mortality and the development of chronic carrier state which may progress to chronic hepatitis, liver cirrhosis and hepatocellular

carcinoma⁽⁵⁾. Furthermore, the other factor for hepatitis is the immunological factor that causes immunological abnormalities such as autoimmune hepatitis, which is an autoimmune disease response to the liver tissues by autoantibodies⁽⁶⁾.

Control of HBV infection requires the combined action of both the innate immune response and the humoral and cellular arms of the adaptive immune response⁽⁷⁾. The humoral response is also critical for long-term clearance of HBV and protection from infection with HBV. B-cell antibody production neutralizes free viral particles and can prevent reinfection⁽⁸⁾. In patients who recover from acute HBV infection, activated T-helper cell type 2 (Th2) CD4⁺ T-cells induce B-cell production of HBsAb, HBcAb and HbeAb⁽⁹⁾. HBsAb is important in providing protective immunity against subsequent HBV infections and is the bases of protection in vaccinated individuals. Antibody against HBcAg constitutes the first markers of acute HBV infection. HBeAb is an early sign of recovery from acute self limited hepatitis. Seroconversion to anti-HBe is associated with less severe liver disease

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(4,8). Indeed, the detection of IgM anti-HBc is diagnostic of acute HBV infection⁽¹⁰⁾.

MATERIALS AND METHODS:

The study groups were classified into patients group with total number of 35 (25 males and 10 females) (15 acute and 20 chronic) patients and vaccinated group (35 healthy individuals) who were previously vaccinated against HBV through six months to one year. This group classified into responder group were positive for anti-HBs antibody (20 individuals), Non-responder were negative for anti-HBs antibody (15 individuals) and control group (18 apparently healthy non vaccinated individuals). They were chosen to match the age and sex of the study groups to serve as negative control, during the period between May to November 2007.

Blood samples were taken from patients and hospitals staffs and employees in Erbil Teaching Hospital, Nanakaly Hospital for Blood Diseases and Rizgary Teaching Hospital or from blood donors, who voluntarily came to Blood Bank Units. The study protocol includes viral assay for grouping study groups into acute, chronic, responder, non responder and healthy control which achieved by detection of HBsAg (Biokit,

3000-1130, Spain), and Anti-HBc Ab(IgM) in serum (Murex anti-HBc IgM, Murex Biotech Limited, C08GE18GB, UK). Hepatitis B vaccine assay for detection of Anti-HBs antibodies in serum (WB-2396, China) by using ELISA test. Immunological assay for estimation of serum IgG and IgM levels in above groups (Immunoturbidimetric test is intended for quantification of IgG and IgM in the serum. Parsazmun, Iran).

Analysis of data was performed by using statistical package for social science (SPSS) version 11.5. Results are expressed as mean \pm S.E. Statistical differences were determined by LSD test for multiple comparisons after ANOVA. P value < 0.05 was considered statistically significant⁽¹¹⁾.

RESULTS:

By reading the serum level of IgG in all study groups using F-test, there were significant differences among three studied groups ($p < 0.05$), as shown in Table (1) however, the comparison of CH with acute and control using LSD also revealed significant differences in serum IgG levels ($p < 0.05$).

Table 1: Difference in Mean Serum Level of IgG (mg/ml) Between Study Groups

Study groups	No.	IgG	P value (F-test)
		Mean \pm SE	
Acute HBV patients	15	13.494 \pm 1.162	P<0.05
Chronic HBV	20	23.53 \pm 2.852	
Healthy control	18	16.959 \pm 1.362	
HC versus AH	LSD	NS	
HC versus CH		P<0.02	
AH versus CH		P<0.001	
HC: Healthy control, CH: Chronic hepatitis, AH: Acute hepatitis.			
P<0.05: Significant , NS: Non significant			

On the other hand, acute hepatitis group insignificant decrease as compared to the control group ($p > 0.05$). Results in Table (2) showed significant high levels of IgM recorded in sera of

AH patients as compared to control and CH groups. While there was a slightly non significant increased in chronic group when compared to the healthy control.

Table 2: Difference in Mean Serum Level of IgM (mg/ml) Between Study Groups

Study groups	No.	IgM	P value (F-test)
		Mean \pm SE	
Acute HBV	15	17.492 \pm 2.709	P<0.001
Chronic HBV	20	3.889 \pm 1.081	
Healthy control	18	2.0236 \pm 0.387	
HC versus AH	LSD	P<0.001	
HC versus CH		NS	
AH versus CH		P<0.001	
HC: Healthy control, CH: Chronic hepatitis, AH: Acute hepatitis.			
P<0.001: Highly significant , NS: Non significant			

The data in Tables (3 and 4) showed that there were no significant changes in total immunoglobulins (IgG and IgM) levels in the sera of vaccinated groups compared to the HN control group ($p>0.05$).

Table 3: Mean Serum IgG in (mg/ml) Between Vaccinated Study Groups and Healthy Control According to Age Distribution

Study groups	Serum IgG (Mean \pm SE)				
	No.	Individuals age \geq 30 years	P value (F-test)	Individuals age <30 years	P value (F-test)
Responder	20	19.312 \pm 1.329	P>0.05	17.864 \pm 0.607	P>0.05
Non-responder	15	17.685 \pm 1.475		20.273 \pm 1.088	
Healthy Non-vaccinated	18	15.955 \pm 1.917		17.682 \pm 1.919	
HN versus RD	LSD	NS		NS	
HN versus NRD		NS		NS	
RD versus NRD		NS		NS	
RD: Responder, NRD: Non-responder, HN: Healthy non-vaccinated control					
NS: Non significant					

Table 4: Mean Serum IgM in (mg/ml) Between Vaccinated Study Groups and Healthy Control According to Age Distribution

Study groups	Serum IgM (Mean ±SE)				
	No.	Individuals age ≥30 years	P value (F-test)	Individuals age years	P value (F-test)
Responder	20	1.46±0.033	P>0.05	2.252±0.252	P>0.05
Non-responder	15	1.935±0.299		1.783±0.126	
Healthy Non-vaccinated control	18	2.084±0.122		1.973±0.114	
HN versus RD	LSD	NS		NS	
HN versus NRD		NS		NS	
RD versus NRD		NS		NS	
RD: Responder, NRD: Non-responder, HN: Healthy non-vaccinated control					
NS: Non significant					

DISCUSSION:

By reading the serum level of IgG in all study groups using F-test, there were significant differences among three studied groups ($p<0.05$), however, the comparison of CH with acute and control using LSD also revealed significant differences in serum IgG levels ($p<0.05$). On the other hand, acute hepatitis group insignificant decrease as compared to the control group ($p>0.05$).

Significant high levels of IgM recorded in sera of AH patients as compared to control and CH groups. While there was a slightly non significant increased in chronic group when compared to the healthy control. The serum levels of Igs (IgG and IgM) in patients with HBV infection have been investigated by various workers but the results were contradictory. Significant higher levels IgG and IgM found in chronic and acute HBV compared to control group⁽¹⁰⁻¹³⁾.

Moreover, significant elevation of total IgG in chronic HBV sera was found compared to healthy groups, this agree with earlier report of Thomas *et al.*,⁽¹⁴⁾ .It may indicate a persistent insult to immune response with relative high level of viral activity. However the results of the present study disagree with Eyigun *et al.*,⁽¹⁵⁾ who found that patients with acute HBV had greater IgG serum levels than other clinical groups.

The data in tables (4.15 and 4.16) showed that there were no significant changes in total immunoglobulins (IgG and IgM) levels in the sera of vaccinated groups compared to the HN control group ($p>0.05$).

Generally the levels of immunoglobulins were higher in the sera of HBV infected patients than RD, this be may this is attributed to the complexity of the HBV antigens, or high dose of HBV or due to the continues replication of HBV and shedding their antigens into the circulatory system which could be encounter by the immune system, however the vaccine is less complex, and their dose decreased by a period of time and part of them can be phagocyted by macrophage.

CONCLUSION:

Present study indicate that serum IgG level was higher in chronic hepatitis patients whereas IgM level increased in acute hepatitis patients. No significantly differences in serum (IgG and IgM) were observed between vaccinated individuals and HN group.

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