Estimation of GM-CSF 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:

This study was designed to estimation granulocyte macrophage-colony stimulating factor (GM-CSF) in HBV infected patients and individuals vaccinated with recombinant HB vaccine.

METHODS:

Study groups were classified into patient group 31(15 acute (AH) and 16 chronic (CH)), 33 vaccinated group (20 responder (RD) and 13 Non-responder (NRD)) and 16 healthy control (HC) during May to November 2007. Blood samples were taken from patients and hospitals staffs to detection hepatitis B surface antigen(HBsAg), anti-hepatitis B core antibody(IgM)(Anti-HBc Ab(IgM), anti hepatitis B surface antibody) (Anti-HBs Ab), GM-CSF level in serum by enzyme linked immunosorbent assay (ELISA) test.

RESULTS:

A highly significant elevation ($p<0.001$) of GM-CSF level was reported in AH patients compared to the CH patients and HN controls, according to F-test, while in CH patients the elevation was not significant as compared to HN control ($p>0.05$) according to LSD- test. The level of GM-CSF also significantly higher ($p<0.05$) in RD at age ≥30 years than NRD and HN groups by F-test. Also a significant differences was recorded when compared between HN versus RD and RD versus NRD ($P<0.027$) and ($P<0.023$) respectively.

CONCLUSION:

In this study highly significant elevation of GM-CSF levels was observed in AH patients compared with CH patients and healthy control. GM-CSF level also significantly higher in RD at age ≥30 years than NRD and HN groups.

KEYWORDS: granulocyte, macrophage , hepatitis B virus, vaccine.

INTRODUCTION:

Hepatitis B virus is the prototype agent for a virus family called Hepadnaviridae [(1)]. In primary infection, HBsAg becomes detectable after 4-10 weeks of incubation period, followed by anti-HBc antibody (HBcAb) IgM isotype during early period [(1)]. Chronic HBV infection is most commonly defined as being present when a person tests positive for HBsAg for at least six months [(2)].

Vaccines consist of HBsAg produced by a recombinant DNA in yeast cells or in continuous mammalian cell lines [(3)]. The immune response to the hepatitis B vaccine in healthy subjects is extremely variable, following administration of three doses of vaccine under optimal conditions. Approximately 1 to 3% of neonates and up to 10% of healthy adults does not produce adequate levels of antibodies after three doses of the currently available vaccines, and can be considered nonresponders [(4)].

Granulocyte macrophage-colony stimulating factor is a cytokine that participates in the growth and differentiation of myeloid and monocytic lineage cells which include dendritic cells, monocytes, macrophages, and granulocyte lineage cells [(5)]. Granulocytes and macrophages during immune responses are enhanced by GM-CSF, since it plays an important role in inflammation and immune response in HBV infection [(6)]. Recombinant human GM-CSF is commercially available and is used to improve the immunological function of patients with various diseases and repair hematological disorders. However, its use in the treatment of chronic viral hepatitis is still controversial [(7)].

MATERIALS AND METHODS:

The study groups were classified into patients group with total number of 31 (21 males and 10 females) (15 acute and 16 chronic) patients and vaccinated group (33 healthy individuals) who

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were previously vaccinated against HBV through six months to one year. This group classified into responder group was positive for anti-HBs antibody (20 individuals) and Non-responder was negative for anti-HBs antibody (13 individuals) and healthy control group (16 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 GMCSF level (Marseille Cedex 9 /France) By ELISA test intended for the qualitative detection.

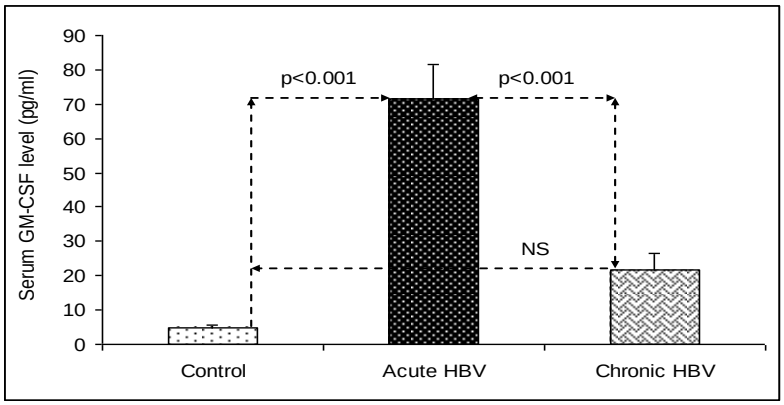
Analysis of data was performed by using statistical package for social science (SPSS) version 11.5. Results are expressed as mean ± S.E. Statistical differences were determined by LSD test for multiple comparisons after ANOVA. P value < 0.05 was considered statistically significant. The present study was done to estimation of G–MCSF in HB virus infected patients and individuals vaccinated with recombinant HB vaccine.

RESULTS:

The serum concentration of GMCSF in 15 AH, 16 CH and 16 healthy controls were presented in Table (1) and Figure (1). Statistical analysis reveals a highly significant elevation of serum GM-CSF in AH infection (P<0.001) as compared to CH and healthy control. However, there were no significant elevation of GM-CSF serum levels in patients with CH infection as compared to healthy subjects (p>0.05) Table (1) and Figure (1).

Table 1: Difference in mean serum level of GM-CSF (pg/ml) between study groups.

Study groups		No.	GM-CSF Mean ±SE	P value (F-test)
Acute HBV		15	71.592±9.773	P< 0.001*
Chronic HBV		16	21.765±4.662	
Healthy control		16	5.603±0.483	
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				



study groups (P<0.05) analyzed by F-test, the higher levels of serum GM-CSF was found among RD group, and lower value found among NRD group 19.109 ± 0.987 versus 4.419 ± 5.734 respectively. On the other hand serum level of GM-CSF was not significantly differ among <30 years old studied groups by LSD. However a significant differences was present when compared between HN versus RD and RD versus NRD P<0.027 and P<0.023 respectively Table 2.

Table 2: Mean serum GM-CSF in pg/ml between vaccinated study groups and healthy control according to age distribution.

Study groups	Serum GM-CSF (Mean ±SE)				
	No.	Individuals age ≥30 years	P value (F-test)	Individuals age <30 years	P value (F-test)
Responder	20	19.109±0.987	P<0.05	20.288±7.656	P>0.05
Non-responder	13	4.419±5.734		4.419±0.987	
Healthy non-vaccinated control	16	5.395±0.9104		5.765±0.536	
HN versus RD	LSD	P<0.027		NS	
HN versus NRD		NS		NS	
RD versus NRD		P<0.023		NS	
RD: Responder, NRD: Non-responder, HN: Healthy non-vaccinated control vaccinated					
P< 0.05:Significant, NS: Non significant					

DISCUSSION:

In this study, it was determined that the serum GM-CSF concentration was a highly significant elevated during acute phase as compared to CH and healthy control (P<0.001), Table (1) and Figure (1)

However, there were no significant elevation of GM-CSF serum levels in patients with CH infection as compared to healthy subjects (p>0.05).

These results were in accordance with pervious results reported by Al- Wabel *et al.*, in 1993 and Kubota *et al.*, in 1995 who found that some patients with chronic viral hepatitis showed elevation in GM-CSF level over the baseline level measured in all of the normal individuals, but this difference was not statistically significant [6- 8]. Furthermore, Kountouras *et al.*, reported in their study, increase in GM-CSF level in chronic HBV infection and suggested that it might serve to monitor viral presence and outcome of patients [9]. Serum GM-CSF is lower in patients with leukemia that initially present with chronic HBV, or readily develop it after institution of IFN therapy [10].

Table (2), showed in age ≥30 years a significant difference among study groups (P<0.05) analyzed by F-test, the higher levels of serum GM-CSF was found among RD group, and lower value found

among NRD group 19.109 ± 0.987 versus 4.419 ± 5.734 respectively.

Krishnamurthy *et al*., (2004) reported the seroconversion (high titer of anti-HBs) found in a group which administrated GM-CSF (used as adjuvant) was more than control group, after a single dose immunization with rHB vaccine [11]. They suggested that the efficacy of rHB vaccine was increased when administrated with GM-CSF, which produce local inflammation at the site of injection and enhance dendritic cell maturation and migration.

GM-CSF is a cytokine synthesized by activated T lymphocytes, macrophage and endothelial cells which leads to increase in the production of neutrophils and macrophage [7].

GM-CSF is used to improve the immunological function and hematological disorders. It has a role in changing cytokine production as significant enhancement of both TNF-α and IL-1 production also reduces serum HBV DNA levels significantly, which are associated with increased 2, 5-oligoadenylate synthetase activity in cultured mononuclear cells [6]. On the other hand serum level of GM-CSF was not significantly differ among <30 years old studied groups.

CONCLUSION:

GM-CSF levels was elevated in AH patients. It is also significantly elevated in RD at age ≥30 years than NRD and HN groups.

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