Numbers of PFCanti-HBc in patients with Chronic Hepatitis B

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Date of acceptance 16/1/2006

ABSTRACT

Plaque forming assay was used in enumerate the number of plaque forming cells of anti-HBc (PFC anti-HBc) in eight patients with Chronic Hepatitis B (CHB) and eight health humans as a control group . We found statistical increase in number of PFC anti-HBc in patients with CHB when compared their results with control group. The percentage of patients that have IgG anti-HBc was 100 % and this percentage was 87.5 %. From this study we can suggest there is a spontaneous ability in these patients to production of anti-HBc.

INTRODUCTION

Many of studies had assured the chronic infection with hepatitis B virus (HBV)may develops to autoimmune diseases for example Systemic Lupus Erythematosus (SLE) (1,2,3) and a lot of diseases that associate with immune complex because the high presence of antigen and antibodies in patients sera for example Glomerulonephritis and Polyarteritis nodosa (4). Hepatitis B core antigen (HBc Ag)is strong antigen and has high ability to stimulate immune system to produce specific antibody(anti-HBc) this happens because the high ability of HBc Ag to stimulate B-Lymphocytes to release antibodies (anti-HBc) after bound this antigen with specific receptor on B-Lymphocyte. This binding cause the most important signal that stimulates B-Lymphocytes independently of T-Lymphocytes(5,6). Anti-HBc divided into two classes:1-IgM anti-HBc which appears (in serum)with rise of hepatitis B surface antigen (HBs Ag) (in serum) 2 - IgG anti-HBc that appears(in serum)during acute phase and still for many years(in chronic state)(7).High titer of IgM anti-HBc indicates to rising into liver cells injury and increace in viral activity(8)thus this antibody(anti-HBc) plays an important role in HBV pathogenicity and its classes are determining the stages of disease (Chronic hepatitis B (CHB)) therefore a study of antibodies productive cells (to anti-HBc) gives a good prospect about the pathogenecity of CHB(9).There is a type of cells can produce of antibodies this cell is B – Lymphocyte thus it takes responsibility of cellular and humeral immune response (4) . Plaque forming assay had been used at the first time in 1963 by Jerny (10) and it had been developed by many investigator (11).It used to enumerate antibodies productive cells spontaneously of many autoimmune diseases such as Rheumatoid arthritis (11, 12).We used it to enumerate anti-HBc productive cells spontaneously (Plaque forming cells anti-HBc (PFC anti-HBc) because this type of antibody play an important role in immunopathogenicity of CHB (4).

MATERIALS AND METHODS:

1- Patients and control group: Eight patients with chronic hepatitis B (CHB) according to clinical examination, serological tests and biochemical tests. And eight healthy human as control group(from Central public health laboratory) . Clinical & laboratory features of patients and control group show in table -1- .Heparinized blood and serum were collected from all patients and control group. All these cases have not any marker for other viruses except HBV markers(HIV , anti-HCV, anti-HAV) and nobody had received any immunosuppressive or antiviral drug for treatment. To detect any viral markers ELISA tests were used and Signal radial immunodiffusion method was

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used to determinate the concentration of Immunoglobulin types (IgG, IgM, and IgA)

2- Cells isolation: Lymphocytes were isolated from heparinized peripheral blood by density gradient centrifugation by used Lymphoprepe (Flow Lab.)(13). and washed three times with phosphate buffer slain (PBS). Number of viable cells was determined by trypan blue exclusion test (14).The viable cells adjust to 10^6 cells/ml by PBS.

3- Plaque forming assay of anti-HBc: we used Jerny method that modified by Zgair (15) to count plaque forming cells anti-HBc(PFC anti-HBc) that is meaning We counted B-Lymphocytes which produce anti-HBc spontaneously.

4- Detection of anti-HBc :Enzyme linked immunosorbant assay (ELISA)were used to detect IgM anti-HBc & IgG anti –HBc (Hepanostica organon).

Table-1- Some clinical, serological and Biochemical features of patients with CHB and control group.

Information	CHB	Control
Number of cases	8	8
Age groups	20-48	22-40
Mean of age in years	37	33
HBs Ag	8	0
Anti-HBs	0	0
ASMA	0	0
ANA	0	0
TSB mg/dl	2.1(P< 0.05) 3.95 ±	0.9+ 0.12
ALT (2-15 IU/L)	± 31.1 (P < 0.001) 55.25	9.3+ 5.3
IgG mg/dl	59 (P < 0.05 2910±)	1397+512
IgM mg/dl	298±98 (P< 0.01)	120 + 71
IgA mg/dl	170 NS± 280	301+ 125

NS: Non Significant
ANA: Anti-nuclear antibody
ASMA: Anti-Smooth muscle antibody
ALT: Alanine aminotransferase.
TSB: Total serum bilirubine

RESULT AND DISCUSSION:

Statistical increases were found in number of plaque forming cell anti-HBc (PFC anti-HBc)that got it from peripheral blood for patients with CHB when compare these results with results of control group table -2- .The plaque (we found it) were specific for cells which produce anti-HBc and we have confirmed from that by using a negative control ,because a presence clones of B-Lymphocytes which release heterophile antibodies ,the last in charge of prozone phenomena that appear usually in hemagglutination (16).The size of plaques were various because the differences in B-Lmphocytes active which produced anti-HBc and the differences in antibodies classes that produced by B-Lymphocytes in spontaneously(PFC anti-HBc).That is proving these antibodies were polyclonal antibodies . We found percentage of patients with CHB that have IgG anti-HBc was 100 % and this percentage was 87.5 % in patients with CHB that have IgM anti-HBc(table-3-) . We and many Investigator found there is anti-HBc only In sera of patients with CHC(4 ,15) these results have affirmed the high ability of HBc Ag to stimulate immune system exactly B-Lymphocytes independently or dependently T-Lymphocytes to produce anti-HBc in patients with CHB (5 , 6). Some investigator else has showed the ability of HBc Ag to activate CD 4^+ cells (T-helper) and these cells can control B-Lymphocytes activation to produce many types of antibodies besides anti-HBc (17,18) . After all that we can say, HBc Ag can urge the immune system to produce polyclonal antibodies spontaneously.

Table-2- Numbers of PFC anti-HBc / 10^6 viable cells (Lymphocytes) that were taken from peripheral blood of patients with CHB and control group.

Number of cases	CHB(PFC anti-HBc /10^6 viable cells).	Control (PFC anti-HBc /10^6 viable cells).
1	14	1
2	16.2	1.3<
3	11.1	0.9
4	20	2
5	7.3	7
6	25	3
7	17	2
8	23	4
mean	16.7 (P < 0.001)	2.65
SD	2.9±	2.1±

PFC: Plaque forming cell.
SD : Stander deviation.

Table -3- Numbers and percentages of individuals who have IgG anti-HBc and IgM anti-HBc in patients with CHB and control group.

	CHB	Control
Number of cases	8	8
Number of individuals that have IgG anti-HBc	8	0
Percentage of individuals that have IgG anti-HBc	100 %	0 %
Number of individuals that have IgM anti-HBc	7	0
Percentage of individuals that have IgM anti-HBc	87.5 %	0 %

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اعداد الخلأيا المكونة للويحات لب الفيروس في مرضى التهاب الكبد الفيروسي المزمن نمط –ب-

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الخلاصة:

استخدمة طريقة حساب اعداد الخلأيا المكونة للويحات لحساب اعداد الخلأيا المكونة للويحات اضداد لب الفيروس (فيروس التهاب الكبد الفيروسي نمط –ب-) في ثمان مرضى يعانون من التهاب الكبد الفيروسي المزمن نمط –ب- و ثمان اشخاص اصحاء يمثلون مجموعة السيطرة ز حيث وجد ارتفاع معنوي احصائي في اعداد الخلأيا المكونة للويحات لب الفيروس لدى الأشخص المصابين بالتهاب الكبد لفيروسي المزمن عند مقارنة النتائج مع مجموعة السيطرة . وعند التحري عن اضداد لب الفيروس وجد ان نسبة المرضى الحاملين لاضداد لب الفيروس صنف غاما كانت 100% . اما نسبة المرضى الحاملين لأضداد لب الفيروس صنف ميو كانت 87.5% . من هذه الدراسة يمكن الأستنتاج على وجود قابلية ذاتية لدى هؤلاء المرضى على انتاج اضداد لب الفيروس بصورة ذاتية.