

**Antibacterial effect of date palm (*Phoenix dactylifera* L.) pit aqueous
extract on some bacteria cause urinary tract infection.**

Najla'a Nabhan Yassein

Study complement activity and humoral immune response in chronic hepatitis B patients

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Abstract

Fifty (50) serum samples were collected from patients with chronic hepatitis B (CHB) who were admitted to Hepatology and Gastroenterology Teaching Hospital in Baghdad, aged from 20-65 years during the period from February / 2008 and the mid of February / 2009, also serum samples were collected from Fifty (50) healthy HBs Ag carrier was discovered accidentally through attending blood bank for donation of blood, aged from 18-52 years and served as a control group. In this study, the serum levels of immunoglobulins (Igs) including IgG ,IgM , IgA are evaluated in both groups . The results review that the median level of IgG and IgA is higher significantly in chronic patients than carrier group, whereas the median level of IgM is slightly increase in chronic patients than carrier group, on other hand, the serum complement components (C3 and C4) were evaluated in CHB patients in comparison to carrier group. It was found that there was a significant decrease in the levels of C3 and C4 among chronic group compared to carrier group. The results of this study indicate that serum complement and immunoglobulins activities was impaired in chronic hepatitis B patients

Key words: chronic hepatitis B,C3 , C4 , IgA ,IgG .

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**دراسة فعالية المتمم والاستجابة المناعية الخلطية في مرضى التهاب الكبد الفيروسي البائي
المزمن**

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الخلاصة

تم جمع 50 نموذج مصلي من مرضى مصابين بالتهاب الكبد الفيروسي المزمن نوع (ب) و(50) فردا من الحاملين لأصحاء للمستضد السطحي لفايروس التهاب الكبد نمط (ب) من المراجعين للمستشفى التعليمي لأمراض الجهاز الهضمي والكبد في بغداد ومصرف الدم المركزي في بغداد للفترة من الاول من شباط 2008 ولغاية منتصف شباط 2009، وكانت أعمار المرضى المصابين بالتهاب الكبد الفيروسي المزمن نوع (ب) تتراوح ما بين 20-65 سنة و أعمار الحاملين لأصحاء للمستضد السطحي لفايروس التهاب الكبد نمط (ب) تتراوح ما بين 18-52 سنة. تضمنت هذه الدراسة تحديد المستويات المصلية للغلوبولينات المناعية (غلوبولين المناعي G و الغلوبولين المناعي A و الغلوبولين المناعي M) ومكوني المتمم (C3 و C4) في المرضى المزمنين والحاملين لأصحاء، حيث أظهرت النتائج ارتفاعا معنويا في مستوى الغلوبولينات المناعية (A , G) لدى المرضى المزمنين مقارنة مع الحاملين لأصحاء و ارتفاعا غير معنوي في مستوى الغلوبولين المناعي (M) لدى المرضى المزمنين مقارنة مع الحاملين لأصحاء ، و من جهة اخرى كان المستوى المصلي لمكوني العامل المتمم منخفضا في الاشخاص المزمنين بالمقارنة مع الحاملين لأصحاء . لذلك فأن نتائج هذه الدراسة أكدت بان فعالية المتمم و الكلوبولينات المناعية كانت غير طبيعية في المرضى المصابين بالتهاب الكبد الفيروسي البائي المزمن

الكلمات المفتاحية: التهاب الكبد الفيروسي البائي المزمن ، IgA , IgG

Introduction

Hepatitis B virus (HBV) infection is a major public health problem and its outcome depends on the kinetics of the virus host interaction and in particular on the strength of the innate and adaptive humoral and cellular immune response (1).

The humoral immunity is mediated by specific antibodies that recognize and react to a challenge, therefore the humoral immunity or antibodies – mediated arm of the immune system as well as the humoral part mediated by complement components, thus the serial

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estimation of serum immunoglobulin and complement may provide as useful marker for disease progression and therapeutic monitoring (1). The adaptive cellular immune response plays an important role in the hosts defense against viruses such as HBV because it is specifically recognizes HBV –infected cells, also induces maintains protective HBV –specific memory (2). Specific antibody patterns have been associated with different phases and outcomes of HBV infection and are widely used as diagnostic tools antibodies against HBs Ag constitute the first markers of acute HBV infection. Antibodies against HB Ag are an early sign of recovery from acute self limited HB. Seroconversion to anti-HBe is associated with less severe liver disease (3). Antibodies against the glycoproteins of the S, pre S1, and pre S2 region are neutralizing and appear when HBs Ag is cleared during recovery from HBV infection. Their production is a T-cell depended process (4), therefore the aim of the study was undertaken to evaluate complement activity and humoral immunity in chronic hepatitis B patients.

Subjects and Methods

Subject groups include the following:

chronic hepatitis B patients groups

A total of (50) patients with CHB who were admitted to Hepatology and Gastroenterology Teaching Hospital in Baghdad, aged from 20-65 years with male to female ratio of 2.2:1. The patients were suffering from different clinical symptoms with previous risk factors for transmission of HBV infection.

Control groups

A total of 50 healthy HBs Ag carriers were discovered accidentally through attending blood bank for donation of blood, aged from 18-52 years with male to female ratio of 2 :1

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**Quantitative Estimation of Serum Immunoglobulins (IgG, IgM, and IgA) and
Complement Components (C3, C4) by Using Single Radial Immunodiffusion
(SRID) test**

Principle of the test

The concentration of Igs, complement component C₃, C₄ and alpha 2 macroglobulin were measured by a single radial immunodiffusion SRID method in which equal volumes of reference sera and test samples were added to wells in agarose containing monospecific antisera.

The sample diffuses radially through this gel and the substance being assayed form a precipitin ring with the monospecific antisera. Ring diameters were measured and a reference curve is constructed on graph paper. Unknown concentration was determined from the references standard curve (5).

Procedure

Before starting procedure, the plate were opened and left for 5 minutes at room temperature for evaporation of any water (if present in wells) due to storage of 4°C.

1. Five µl of each serum sample was dispensed by Hamnilton syringe in to one well of each plate (containing 16 wells) for three classes of Igs, two types of complement component and alpha 2 macroglobulin.
2. The plate were left opened for (10–20) minutes, then covered and left at room temperature (20–25) °C for (3–4) days for precipitin ring to be formes.
3. The diameter at each immune precipitating using formed around each well was measured in mm by immune viewer and the concentration of each class of Igs and complement level was calculated from standard curve.

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Statistical analysis

The usual statistical methods were used in order to assess and analyze our results and included:

Descriptive statistics: including

- a. Mean (M).
- b. Standard deviation (SD).
- c. Statistical tables.

Inferential statistics: Data have been analyzed statistically using SPSS program version 11.

Analysis of quantitative data was done using t-test and ANOVA (analysis of variance).

Acceptable level of significance was considered to be below 0.05.

Results and discussion

The humoral immunity is provoked by specific antibodies that distinguish and react to a challenge, for that reason the humoral immunity or antibodies – mediated arm of the immune system as well as the humoral part mediated by complement components, as a result the serialized assessment of serum immunoglobulin and complement may make available marker as useful for disease progression and therapeutic monitoring (6).

In this study, the serum levels of immunoglobulins (Igs) including IgG ,IgM , IgA are evaluated in both groups . The results review that the median level of IgG and IgA is higher in chronic patients than carrier group, whereas the median level of IgM is slightly increase in chronic patients than carrier group(table and figure 1).

These results are in concurrence with study done by González-Quintela *et al.* (7) who has recorded an increased level of IgA and IgG among CHB patients as compared to carrier group, also a study done by Joshi *et al.* (8) in India who has demonstrated an increased levels of IgG ,IgA and IgM in patients with CHB.

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serum levels of immunoglobulins (Igs) including IgG, IgM and IgA are synthesized in the lymphoid system and cleared by the reticuloendothelial system, including kupffer cells of liver, polyclonal increase in serum immunoglobulins are normal response to infections (9).

The raised immunoglobulins level in liver disease may be due to in part of inability of kupffer cells to sequester exogenous antigen from the gastrointestinal tract (10), behind that the filtering function of the liver is impaired as a result of distortion of hepatic architecture and microcirculation, so virus may therefore enter the systemic circulation and stimulate Ab production by the reticuloendothelial system (11).

The other important explanation may be the over production of Igs through HBV infection mediated immunoreaction and there is a much evidence that CHB infection is associated with the accelerated host immunoresponse, So HBV infection is often associated with a variety of systemic immunologic reactions or abnormalities such as circulating immune complexes, autoantibodies, and autoimmune manifestations (12).

Concerning the serum complement components (C3 and C4) were evaluated in CHB patients in comparison to carrier group. It was found that there was a significant decrease in the levels of C3 and C4 among chronic group compared to carrier group (table and figure 1), these results resembled those reported by other investigators (13,14), so complement activation is one of the earliest responses to infection including viral hepatitis and its level has been shown to be reduced in those disease (6). Other studies were reported a significant reduction in serum C3 and C4 levels in some patients with viral hepatitis during different phases of the disease (15,16) and the decrease of complement levels in liver diseases has been assumed to be the result of failure of components synthesis in the liver (6). More over the low levels of complement in such patients were caused by complement fixing immune complexes composed of hepatitis associated antigen (HAA) and antibody to HAA, so it was more important explanation for decrease of complement levels in chronic HBV patients as compared to carrier group was due to impaired hepatic synthesis of complement components

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(14). In conclusion, the altered levels of serum complement and immunoglobulins might be responsible for abnormal immune response in patients with in chronic hepatitis B patients.

Table1:-The differences in the mean of immunoglobulin and complement components (mg / dl) of chronic patients and carrier group. Unit for each parameter = mg / dl.

Humoral Parameters	Number	Mean	Std. Devia	t-test P-vale	Signaificant
C4 healthy carrier HBV	50	41.16	12.30		
chronic HBV	50	27.00	15.18	.000	HS
Total	100				
C3 healthy carrier HBV	50	133.40	31.52		
chronic HBV	50	101.60	30.58	.000	HS
Total	100				
IgA healthy carrier HBV	50	273.98	132.04		
chronic HBV	50	349.58	132.76	.005	HS
Total	100				
IgM healthy carrier HBV	50	196.18	84.02		
chronic HBV	50	205.80	111.56	.627	NS
Total	100				
IgG healthy carrier HBV	50	1496.80	413.04		
chronic HBV	50	2119.68	719.49	.000	HS
Total	100				

Note :-C3 and C4 : Complement type 3 and 4.

IgA,IgMand IgG: Immunoglobulins type A,M and G .

NS: Non significant. HS: highly significant

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