IMMUNOLOGICAL AND BIOCHEMICAL PARAMETERS OF CHRONIC HEPATITIS B INFECTION AMONG PATIENTS IN DUHOK CITY – KURDISTAN REGION / IRAQ

AWAZ A. SAADI, BSC, MSC*
AHMED M. SALIH, MSC, PHD, **
MUAYED A. MERZA, MBCHB, MSC, PHD, FID***

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ABSTRACT

Background: Hepatitis B virus infection (HBV) is regarded as serious public health problem and approximately two billion people are infected with the virus. The presence of HBsAg with the clinical and biochemical features of hepatitis B infection reflects onset of the infection. It is sometimes very difficult to distinguish between inactive HBV carrier and patients with active HBeAg negative chronic hepatitis B. The current study aimed to measure the immunological and biochemical markers other than HBsAg in patients with chronic hepatitis B virus (HBV) attended the infectious diseases unit at Azadi Teaching Hospital in Duhok.

Subjects and Methods: Eighty nine chronic hepatitis B patients were included in this study from June 2015 through December 2015. Serum samples were collected from each patient and measured for HBsAg, anti HBs Ab, HBeAg, anti HBe Ab, and total Anti HB core Ab by using a rapid test (PLASMATEC One Step Multi –HBV Test Device). The HBsAg level was further tested by ELISA (HBsAg ELISA Test Kit, PLASMATEC Laboratory Products). The HBeAg & HBeAb were confirmed by immune-chemiluminescent assay. Levels of Alanine Aminotransferase (ALT), Aspartate Transaminase (AST), and Alkaline Phosphatase (ALP) in each serum sample were also measured.

Results: Out of the 89 HBs Ag positive patients 51(57.3%) were males and 38 (42.7%) were females by using ELISA test, 21(23.6%) were positive for HBeAg, and 76 (85.4%) were positive for anti-HBe antibodies by using Chemiluminescent Immunoassay (CLIA) techniques. There were 68 cases of HBe Ag negative and anti HBe Ab positive, 13 patients were HBe Ag positive and anti HBeAb negative and only 8 cases were both HBe Ag and anti HBeAb positive. Among the male patients, 10(47.61%) and 44(57.9%) were positive for HBeAg and anti-HBeAb respectively, whereas for the females, 11(52.40%) and 32(42.10%) patients were positive for HBeAg and anti-HBeAb respectively. Regarding the ALT, 11(12.35%) were upper than normal range in which 7 (7.9%) were elevated (40-80 IU/mL) and 4 (4.5%) were twice upper than normal limit (>80). For AST, 12 (13.48%) patients were upper than normal range in which 11(12.35%) were elevated and only 1 (1.12%) was twice upper than normal range. In ALP, only 7(7.9%) of the patients were upper than reference range.

Conclusion: From the current study, it has been concluded that HBeAg and anti Hbe Ab are dependable indicators for the active stage of HBV replication in carrier patients when both of these variables are linked with elevated levels of serum ALT. Furthermore, most increased levels of HBsAg quantitatively were found among the early and young adults who were in the age range of (24- 44) years.

Keywords: HBsAg, anti HBs Ab, HBeAg, anti HBeAb, Total HB core Ab, ALT, AST, ALP

Infection with Hepatitis B virus (HBV) is regarded as a global threat to public health.
health that causes significant morbidity and mortality in the liver. Approximately two billion people are infected with HBV worldwide. It is estimated that 44% of cases of cirrhotic diseases and 47% of hepatocellular carcinoma (HCC) are localised in Africa. Recently, it has been considered that Iraq has the intermediate endemiﬁcity of Hepatitis B infections reﬂected by 3% sero-prevalence of hepatitis surface antigen (HBsAg) in normal population.

The infection of HBV consequences leads to a broad spectrum of liver disease starting from chronic hepatitis, cirrhosis, and HCC. Treatment of HBV infections with antiviral agents is based on the precise and accurate interpretation of pathophysiologic parameters. Conforming to World Health Organization report, the distribution of HBV infection in the South Asian region estimates to be 2/8(4%). In 2001, the WHO grouped Bangladesh in the moderate to high risk group of countries for HBV infection. The existence of HBsAg with the clinical and biochemical features of acute hepatitis reflects the onset of acute infection in patients from low endemic areas but not in patients from high or intermediate endemic regions in which acute and chronic infection may be misdiagnosed if the patient’s carrier status is unknown. To monitor vaccine-induced immunity and to distinguish acute, self-limited infections from chronic HBV infections, usually serological tests are used.

The other two important markers of hepatitis B infection are Serum HBV DNA and hepatitis B e antigen (HBeAg) that is reflective for active viral replication and the risk of infection transmission. Estimation of serum HBV DNA level is one of great importance for liver disease activity assessment and differentiating various aetiologies of hepatitis activity in HBV carriers that play as hepatocellular carcinoma development also it helps in decision making for antiviral therapy. Data from the clinical and experimental studies suggest that serum HBeAg may play as an immunoregulatory role in natural infection and is considered as immuno tolerant agent. Some HBeAg negative chronic hepatitis B patients may follow sero-conversion from HBeAg to anti-HBe antibodies or may develop after years or decades of the inactive carrier state. Furthermore, it represents a later immune reactive phase in the natural history of infection and characterized by periodic reactivation with a pattern of ﬂuctuating levels of HBV DNA and ALT/AST and active hepatitis. It is sometimes very difﬁcult to distinguish true inactive HBV carrier and patients with active HBeAg negative chronic hepatitis B in whom phases of spontaneous reduction of infection may occur.

However, it is a crucial consideration particularly during defects and shortages of laboratory techniques that measure the HBV DNA viral load which affects the approach of initiating and justiﬁcation of the antiviral therapy. In consequences, the alternative serological and biochemical markers may be helpful in determining the explicative activity of the HBV. The present study was aiming at evaluating the serological and biochemical markers during the course of HBV chronic infection and making the correlation between them and with the status of the disease that might help in ﬁnding some
alternative parameters that interpret the status of the HBV infection in the areas with defects of advanced lab facilities.

PATIENTS AND METHODS
This study was performed in Duhok Medical Research Centre (DMRC) at the Medical College - Duhok University, between June 2015 and December 2015. The research has been approved by the ethical committee of the health directorate of Duhok governorate and the consent has been provided by all participating patients. Eighty nine (38 females and 51 males) consecutive serum samples of patients with HBV infection were taken from central laboratory in Azadi teaching hospital in Duhok city. Patients’ data have been recorded including age, sex, residency and the date of sample collection.

All the cases were confirmed having HBV infection by rapid test for immunological markers; HBs Ag, anti HBsAb, HBeAg, anti HBeAb, and total HB coreAb using (PLASMATEC One Step Multi –HBV Test Device). HBsAg was measured quantitatively (HBsAg ELISA Test Kit, PLASMATEC Laboratory Products).

Biochemical Tests
The serum sample of each patient has been subjected to biochemical to estimate the levels of alanine aminotransferase (ALT) at references range from (5-40 IU/L), aspartate aminotransferase (AST) at reference range (up to 40 IU/L). Alkaline phosphatase (ALP) is measured within reference range of (up to 100 IU/L). ALP was measured using BIOLABO colorimetric Kit for quantitative determination. Both ALT and AST were estimated using [Boeki Prestige 24i - Bolis 24i kit].

Immunological Tests
For total HB core Ab and Anti HBs measuring was estimated by the rapid test (PLASMATEC Laboratory Products) using (ONE STEP Multi-HBV TEST DEVICE). HBsAg was measured quantitatively by (HBsAg ELISA Test Kit, PLASMATEC Laboratory Products), HBeAg and anti HBeAb titre was estimated in biochemistry section in Heave Paediatric Teaching Hospital in Duhok city by using Chemiluminescent Immunoassay (CLIA) techniques (DiaSorin LIAISON® HBeAg and LIAISON® Anti-HBe Kit).

STATISTICAL ANALYSES
Results were analysed by SPSS version (p value < than 0.05 was considered significant).

RESULTS
The total number of patients included in the study was 89. The mean age of the patients was (32.10± 13.398) years. Fifty one cases (57.3%) were males and thirty eight (42.7%) were females. Baseline characteristics of the study parameters are demonstrated in (Table 1).

| Parameters | Mean ± SD | No[%] |
|------------|-----------|-------|
| Age [yrs.] | 32.10 ± 13.398 | 89     |
| Gender     |           |       |
| Male       |           | 51 [57.3] |
| Female     |           | 38 [42.7] |
| HBsAg      | 16238.14± |       |
| IU/mL      | 2488.675  |       |
| HBeAg      | 97762.54± | =Mean±(|SD| |
| IU/ml      | 291656.512|       |
| Anti HBeAb | 75445.06930|±2  |
| IU/ml      | 12324.584914|     |
| Anti HBs   | 2.00 ± 0.00 |       |
| IU/mL      |           |       |
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Total Anti HBc Ab U/ml 1.00 ±0.00
ALT IU/L 29.20± 41.259
AST IU/L 28.2262± 18.88560
ALP IU/L 51.001± 42.034

Moreover, 38 (50.0%) of positive anti HBeAb were among age group in young adults.

Regarding the frequency of chronic HBV infections among age groups, it has been found that the highest frequency was within the age group (25-44) years (47%) followed by age group (15-24) years (27%) as in table 2.

Other immunological parameters like HBeAg and anti HBe Ab were analysed and correlated with age and with the biochemical variables.

Table 2: Frequency of Chronic HBV Patients among Age Groups.

| Age group | Frequency [ % ] |
|-----------|----------------|
| 1-14      | 5 [ 5.6% ]     |
| 15-24     | 24 [ 27.0% ]   |
| 25-44     | 42 [ 47.2% ]   |
| 45-64     | 15 [ 16.9% ]   |
| 65-More   | 3 [ 3.4% ]     |
| Total     | 89 [ 100.0% ]  |

Table 3: Gender of Patients Regarding Hbs Ag

| Gender | Frequency [ % ] |
|--------|----------------|
| Female | 38 [ 42.7% ]   |
| Male   | 51 [ 57.3% ]   |
| Total  | 89 [ 100.0% ]  |

Serological parameters:
All parameters of serological analysis HBsAg, HBeAg and anti HBeAb are shown in table (4&5).

Table 4: Hbeag & Anti Hbeab Frequencies and Percentages

| Parameter | Frequency [ % ] |
|-----------|----------------|
| HBeAg     | Positive 21/89 [ 23.6% ] |
|           | Negative 68/89 [ 76.4% ] |
| Anti HBeAb| Positive 76/89 [ 85.4% ] |
|           | Negative 13/89 [ 14.6% ] |

Table 5: Correlations of Age Category with all Immunological Parameters: Hbsag, Hbeag and Anti Hbeab

| Age category | HBs Ag [ % ] | HBe Ag [ % ] | Anti HBe Ab [ % ] | Frequency, % Total |
|--------------|-------------|-------------|-------------------|-------------------|
| 1-14         | 5 [ 5.6% ]  | 3 [ 14.3% ] | 2 [ 40.0% ]       | 5                 |
| 15-24        | 24 [ 27.0% ]| 7 [ 33.3% ] | 19 [ 79.2% ]      | 42                |
| 25-44        | 42 [ 47.2% ]| 10 [ 47.6% ]| 38 [ 50.0% ]      | 42                |
| 45-64        | 15 [ 16.9% ]| 1 [ 4.8% ]  | 14 [ 18.4% ]      | 15                |
| > 65         | 3 [ 3.4% ]  | 0 [ 0.0% ]  | 3 [ 3.9% ]        | 3                 |

One of the main aim of this study to analyze the serological parameters as data of both HBeAg and HBeAb are the most crucial categories to investigate the patient’s progression of the disease in HBV infection. The parameters were
grouped into three categories, when HBeAg positive and anti HBeAb negative, HBeAg negative with anti HbeAb positive, and both the parameters of HbeAg and anti HBeAb are positive. This has been revealed in correlation with age variable in table 6.

| Age | HbeAg –ve/ HBeAb+ve | HbeAg+ve/ HBeAb-ve | HbeAg+ve/ HBeAb+ve |
|-----|---------------------|---------------------|---------------------|
| 1-14 | 2/68 [2.94%]         | 3/13                | 0                   |
| 15-24 | 17/68 [25%]         | 6/13                | 3/8 [37.5%]         |
|     |                     |                     | [4.15%]             |
| 25-44 | 32/68 [47.05%]      | 3/13                | 5/8 [62.5%]         |
|     |                     |                     | [23.07%]            |
| 45-64 | 14/68 [20.60%]      | 1/13                | 0                   |
|     |                     |                     | [7.69%]             |
| ≥ 65 | 3/68 [4.41%]        | 0                   | 0                   |
| Total | 68                  | 13                  | 8                   |

Rapid test for HBs Ag and Anti-HBs Ab.: All the patients were HBs Ag positive and anti HBs Ab negative. Among HBeAg positive 10/21 (47.61%) were male and 11/21 (52.4%) were female. In anti HBe Ab positive, 44/76 (57.9%) were male while 32/76 (42.1%) were female.

Total Anti HbcAb:
Among the 89 studied cases, all patients were positive 89 (100%) in anti Hbc Ab marker as indicating the chronic infection of Hepatitis B. There were only 13 (14.6%) patients positive for HBeAg and negative for anti HbeAb and the other 68 (76.4%) patients were HbeAg negative and anti HBeAb and 8 (8.9%) of the patients were positive for both HBe Ag and anti HBe Ab.

Biochemical markers:The measured levels of the biochemical markers in the sera of the chronic hepatitis B patients are shown (table 7 & 8).

Table 7: Correlations of ALT Levels, with HbsAg (By ELISA) in Chronic Hepatitis B Patients

| Frequency of patients with normal levels of HbSAg* | 78 | (87.6%) | Mean of HBsAg titre |
|----------------------------------------------------|----|---------|---------------------|
| Frequency of patients with elevated ALT**          | 7  | (7.9%)  | (16238.14 IU/mL)    |
| Frequency of patients with twice upper than normal range of ALT*** | 4  | (4.5%)  | P= 0.584            |
| Total                                              | 89 | (100%)  |                     |

[In correlation of HBsAg titre with the frequency of ALT levels of patients, p value was 0.584 which was not significant \( p = \) or <0.05]

* ALT/AST Normal = < 40 IU/mL according to central Lab Reference Range, ** Range between of 40-80 IU/mL, *** Twice upper than 80 and more
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Diagram 2: Distribution of AST Marker as Normal, Elevated and Twice Upper than Normal Range

Table 9: Frequency of all Biochemical Marker Levels Upper than the Normal Limits in Regard to HbeAg/Anti HbeAb Levels

| Parameter                  | Total | ALT > 40 IU/mL | AST > 40 IU/mL | ALP >100 IU/mL |
|---------------------------|-------|----------------|----------------|----------------|
|                           |       | Elevated       | Twice[ULN]     | Elevated       | Twice[ULN]     |
| HBeAg —ve                 | 68    | 5 [7.35%]      | 2 [2.94%]      | 7 [10.29%]     | 0              |
| Anti-HBeAb+ve             |       | 2 [5.88%]      |                |                |                |
| HBeAg +ve                 | 13    | 2 [15.38%]     | 0              | 3 [23.0%]      | 0              |
| Anti HBeAb-ve             |       | 3 [23.07%]     |                |                |                |
| HBeAg +ve                 | 8     | 1 [12.5%]      | 1 [12.5%]      | 1 [12.5%]      | 1 [12.5%]      |
| Anti HBeAb+ve             |       |                |                | 0              |                |
| Total                     | 89    | 11 [12.35%]    | 12 [13.48%]    | 7 [7.86%]      |

DISCUSSION

In the current study, the biological and immunological profile has been evaluated and correlated with the prognosis of HBV infection in Duhok city that may help in finding some alternative parameters to interpret the status of the HBV infection and link them with the risk factors in the areas with defects of advanced lab facilities. The population age in this study ranged from [5-75 years] category with mean of about (32.1) years. According to the correlation between age and the presence of each of HBs Ag and HBe Ag and anti HBeAb parameters, this study revealed that approximately near to half of the cases (47.2%) were in the range (25-44) years group while the lowest one was 3/89 (3.4%) among >65 years age group. This finding is in consistent with those obtained by another study that has been done in Bangladesh14, they showed that the highest frequency was found to be within
the (21 - 40) years of age which is close to the age range that showed in this study. Moreover, it has been shown that slightly above half of cases in this study were among male patients and this might be due to the more the frequency of exposure of males to hepatitis B virus than females, however, there was limited number of chronic carrier cases thus this finding needs further studies.

In the current study results show no significant correlations among age groups with regard to HBsAg yet the highest titer (47.2%) was found among the age group (25-44) years. This has been also proved by a study which is done in Bangladesh (Asadur R.& et al.2013)14 which revealed that maximum of patients were among the age group between (21-40) which it is similar to our findings. Furthermore, all of the patients (100%) recruited in this study were negative for anti HBsAb and positive for total HBcAb which is an indicator for the chronicity of the infection15.

In HBeAg and anti HBeAb markers there were only 13 patients (14.60%) found to be HBeAg positive but with only 2(15.38%) of elevated ALT that was most likely to be within the ‘active stage’ of infection but none of the cases were twice upper the normal range of ALT. On the other hand, in the current study the high rate of patients who were positive for anti-HBeAb was found among the age group between (25-44) years while the lowest rate was within (1-14) years. This is suggesting that most likely the chronicity of infection was established among the patients during neonatal period of their life or could be transmitted vertically with HBV through perinatal time, this is also supported by other studies when they stated that the a chance of developing chronic infection among neonates infected with HBV through perinatal transmission14.

In the present study, when patients categorised based on the ALT levels into [normal, elevated and twice upper the normal] and compared with the HBsAg titre, it was found that HBsAg did not correlate significantly with the level of ALT among the whole studied patients [p 0.584]. This means that HBsAg titre on its own could not be relied on for the evaluation of the liver function and enzymes level.

Also, it has been found that none of the liver biochemical markers (ALT and AST) were significantly associated with the immunological markers (HBsAg, anti HBeAb and anti HBeAb), but only (7.35%) of the HBe Ag negative and anti HBeAb positive had elevated ALT and only two patients with HBeAg positive and anti HBeAb negative had elevated ALT. It seems that sero-conversion of HBeAg to anti HBeAb in the patient’s serum is linked with the decrease or normalization of the level of serum ALT. This has been supported by another study which revealed that elevated ALT of chronic hepatitis B were correlated significantly with detectable HBsAg, HBeAg and HBeAb levels14, this means that raised ALT level is reflecting more vigorous response to the replicating HBV and therefore more hepatocyte damage4.

A study by (Hussain AB, and et al 2004) revealed that out of 50 patients, 48 positive samples of HBeAg HBV DNA PCR hepatitis B virus carriers had elevated serum ALT level. They concluded that detectable HBeAg should be considered as

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a surrogate marker for HBV DNA in hepatitis\textsuperscript{16}. Also this has been also proved in another study which showed that most children who are HBeAg positive have elevated ALT levels and sero-conversion to anti-HBeAb is common near or shortly after the onset of puberty\textsuperscript{13}. Moreover, in developed countries, most probably HBV infection is usually acquired during adulthood through sexual transmission and injecting drugs\textsuperscript{9,17}.

Regarding ALP results, there were few cases of upper than normal range among groups with both HBe Ag and anti HBe Ab positives. This might indicate the occurrence of liver damage however, further studies in larger scale are needed and other investigations are considered to be done.

It has been suggested that those who are HBeAg positive are better tolerating the antiviral therapy\textsuperscript{18}, and consequently, the sero-conversion from HBeAg-positive status to an anti-HBe Ab positive status has been used to indicate antiviral response and results in a reduced morbidity and mortality\textsuperscript{9,19}.

In the present study, as most cases of HBV were HBeAg negative and anti HBeAb positive, they have lost HBeAg and seroconverted to anti-HBeAb, accordingly the majority have gone into an ‘inactive phase ‘of chronic HBV infection\textsuperscript{20}. However, some patients’ after HBeAg seroconversion develop HBeAg-negative immune active disease, whereas others who have the inactive phase of HBV may experience a reversion to HBeAg positive immune active disease or develop HBeAg-negative immune active hepatitis\textsuperscript{21}.

Furthermore, during the natural course of HBV chronic infection, the loss of HBeAg appearance and the expression of antibodies directed against it, (anti-HBeAb) often represent the end of viral replication\textsuperscript{20}. In this regard, HBeAg remain the most crucial immunological and biomarker as an indicator for the stage of the disease in a place where there are inadequate diagnosis facilities like molecular analysis\textsuperscript{21}.

In conclusion, this study determined the main objectives as the outcome of our findings indicates that most of the cases showed either in ‘Immune tolerance phase’ or ‘active phase. Determinants such as HBsAg HBeAg, and anti HBeAb remain the most important markers for the diagnosis, of the infection specially if these markers are associated with age category to specify the period of transmission of the disease. However, it is recommended for further investigations such as molecular analysis like (PCR) to be done to determine the level of viral load (HBV DNA) in relation to other markers, to understand exactly the phase of infection in order to assess the treatment of the disease.

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CONFLICT OF INTEREST

Nothing to declare.
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نشخیذه

نشاخصی:
نفوذ کسبونافاروسیونی هماییت ب، پسرگیری‌های تکمیلی هماییتی هماییتی برتری تکمیلی و نیز ابزارک میلیار کاس
ب فیروسیفه تیپه کرن. همواره تایپی‌سی‌های HBeAg
نسبت به‌تعداد‌های کلسی‌کی‌و بیولوژیکی‌های کلسی‌کی‌های
کلسی‌کی‌های هماییتی که HBSAg
نیشان دهنده‌ستیکا نهایت‌کسی‌پروییک
B، جاری‌نام نه پر زده‌نامه‌که کو ز جهان‌نا گاردبیلیا گاردبیلیا نهایتیا نهایتیا نهایتیا
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نارنگی:
نارنگی‌نا گی لیکولئی تایپ‌نا دیتابی‌نامی‌گیرنده HBeAg
بیولوژیکی ز بلی
نافذ‌یونه‌ب فیروزوسا
هماییت‌گریزکه کو بهداری بینش نافذ‌یونه‌B
یور نافذ‌یونه‌ب‌نفه‌کسی‌پروییک
ل نافذ‌یونه‌ب‌ارگه‌کسینی نازدیکی ل به‌که.

ددرنامجال
ژ لیکولئیه‌ک نوها، هات نه‌نامدان کو HBeAg و HBeAb
نیشان‌هنیکی پی بو من نین قوناخا چالاک نا
d د نافذ‌یونه‌ک هک اپارو و د در دما کو هر یکه ز فان هکی‌کیه
گیونه‌ب دب‌بی‌کی، پراتی نست اپارو ناف مزنگ دست‌پریک
و جوان‌نام کو ژ کوما تهما
(2019-4) سالیربراست بون و ده هات هسپادن

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الخلاصة

الخصائص المناعية والكيميائية- الأحيائية لمرضى تهاب الكبد المزمن B في مدينة دهوك - كردستان / العراق

خلفية الدراسة و الهدف: تعتبرعدوى فيروس التهاب الكبد B مشكلة خطيرة في مجال الصحة العامة، كما أن حوالي مليار يشخص مرضاً بفيروس HBV. خلاص الدراسة الحالية هو قياس الخصائص المناعية والوصلات الكيميائية الحيوية في بعض المرضى الذين يعانون من فيروس التهاب الكبد B المزمن.

الطريقة البحثية: تم ضم تسعة وثمانية من مرضى التهاب الكبد B المزمن في هذه الدراسة من يونيو 2012 لغاية ديسمبر 2012. وتم تجميع عينات المصل من كل مريض وقياس مستضد HBsAg، والاجسام المضادة لـ HBs Ab، وقياس HBeAg، والاجسام المضادة لـ HBe Ab، ومجموع Anti HB core Ab، وقياس ALT، وAST، والفوسفاتازالقلوي (ALP) في كل عينة مصل لكل مريض.

النتائج: من بين المرضى الإيجابيين لـ HBs Ag كان 51 (57.3٪) من الذكور و 38 (42.7٪) من الإناث والتي اجريت في الدراسة الحالية، و 81 (85.4٪) كانت إيجابية لـ HBeAg، و 76 (82.1٪) كانت إيجابية لـ anti HBeAb من مجموعات الفحوصات ELISA. التفتيش على طريقة الفحوصات المناعية الكيميائية (CLIA). أما في حالة HBeAg، كان هناك 68 حالة من متغيرات المناعية الكيميائية، و 50 (55.2٪) كانت إيجابية للـ anti HBeAb، و 2 (9.2٪) كانت إيجابية للـ HBeAb.

الاستنتاج: من الدراسة الحالية، تم التوصل إلى أن HBeAg و HBsAg تتغذى على آلية استجابة مستضد المناعية. وتم العثور على معظم مستويات الزيادة من ALT، والمستويات المرتفعة من HBsAg في المصل. وعلاوة على ذلك، تم العثور على مستويات مرتفعة من HBsAg في المرضى الذين يعانون من فيروس التهاب الكبد B المزمن.