



Screening of Anti-HBc in HCV Positive Hemodialysis Patients

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ABSTRACT

This study aims to determine the prevalence of antibodies to hepatitis B core antigen (anti-HBc) in hemodialysis patients, and evaluate its role as an indicator for the diagnosis of Occult hepatitis B (OHB) in patients with hepatitis C. Study sample included 122 patients from four hospitals in Syria, all patients had undergone 3-4 hours hemodialysis sessions twice weekly, and received a hepatitis B vaccine, and they are negative for hepatitis B surface antigen (HBs Ag). Patients ranged in age between 21 -79 years (median age 54.03 years old), and the method used to study the prevalence of anti-HBc in hemodialysis patients is enzyme-linked immune sorbent assay (ELISA) and Polymerase Chain Reaction-real time(RT-PCR). The results showed that 14 patients (29%) of the control group and 34 patients (47%) of hepatitis C positive patients are Anti-HBc positive. In the next step, HBV DNA was detected using RT-PCR technique for 34 positive anti-HBc, hepatitis C infected patients. Results showed that 4 patients (12%) have Occult hepatitis B, this rate is considered high compared with the prevalence of hepatitis B in Syria, which is estimated at 5%. The results of this study confirm the importance of a screening test for anti-HBc in addition to screening for HBs Ag, as positivity of anti-HBc may be a possible indicator of Occult hepatitis B infection in patients with hepatitis C subjected to hemodialysis devices.

Keywords: Co-infection B-C, Anti-HBc , haemodialysis.

INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus are considered as the most infections responsible for morbidity and mortality in haemodialysis patients in artificial kidney sections¹. Coexistence and co-infection of these two viruses is a frequent accident in the same patient especially hemodialyse done². B-hepatitis – C-hepatitis association confers on B-hepatitis evolution some traits: delay in HBs-Ag appearance and its existing period is shorter compared to isolated B-hepatitis^[3]. C-virus depresses B-virus replication resulting in HBs-Ag levels immeasurable by ordinary methods and leading, by consequence, to occult hepatitis B conception which is defined as: negative HBs-Ag, positive HBV-DNA in blood or liver and sometimes positivity of certain serum HBV markers such as anti-HBc antibodies. Occult hepatitis B is common in C-hepatitis patients. Anti-HBc antibodies are produced since the onset of acute hepatic injury and so through chronic infection. Anti-HBc antibodies are the most important markers of HBV infection due to:

1. They may be isolated in serum window period, which may lasts for several weeks and separates HBs-Ag -necessary for B-hepatitis diagnosis-absence from anti-HBs antibodies appearance, and be the only indicator of B-hepatitis injury in this period.
2. They persist life-long
3. They don't appear in vaccinated subjects.

Figure (1) illustrates serum window period and the importance of anti-HBc antibodies screening which are incited by HBc-Ag.

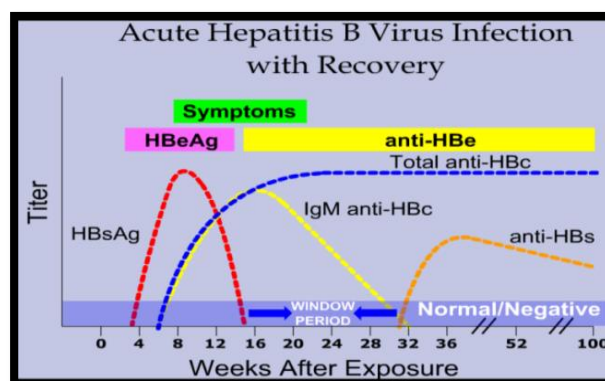


Figure 1: HBV serum markers

Occult B-hepatitis diffusion percentage reaches 58% in haemodialysis patients^{7,8}. It's associated with:

1. Increased hepatic cirrhosis occurrence
2. Increased hepato cellular carcinoma
3. Increased interferon treatment failure⁹

Occult B-hepatitis (OBH) prevalence is higher in hemodialysed patients than in subjects with normal renal function because of immunity depression and HBV high risk exposition by way of injection and medical utensils used with hemodialysed patients¹⁰ where HBV may remain contagious extra-corporeally till 7 days¹¹. Studies point to OBH is more diffuse among positive anti-HBc-Abs patients and this positivity might be a sign of active B-

hepatitis¹². Other studies confirmed that negative HBs-Ag in hemodialysed patients is not sufficient to exclude B-hepatitis¹³. So, all hemodialysed patients should be screened for HBV-DNA because it later persists in all stages of B-hepatitis.

This study is recent in Syria. It aimed to determine anti-HBc antibodies prevalence in hemodialysed patients injured by C-hepatitis, to screen HBV-DNA by real-time PCR thereafter, to evaluate the role played by anti-HBc-Abs in detecting OBH, and, finally, to present a group of recommendations could be an active contribution to specialists in this field.

MATERIALS AND METHODS

This study involved 122 patients distributed into two groups:

- 1) First group: hemodialysed patients free of C-hepatitis: 50 patients, all from Al-Assad university hospital – Latakia
- 2) Second group: hemodialysed patients harmed by C-hepatitis, 72 patients from: Latakia National Hospital (28 patients), Latakia Military Hospital (17 patients), Damascus Kidney Surgical Hospital (27 patients).

It's worthy of mention that all patients were HBs negative and HBV vaccinated; and HBs-Ag was screened periodically once a three months in hemodialysis centres.

This study was released in the period extending from 2010 to 2012; males 66/122; females 56/122; and the mean age was 54.03 ± 11 years.

Patients characteristics (age, sex, hemodialysis period, renal failure aetiology, and blood transfusion history) were defined and registered.

The following tests were performed:

1. Anti-HBc-Abs screening by enzyme linked immunosorbent assay method, using ELISA device, presented in laboratory department in Al-Assad university hospital – Latakia, and commercial kits belong to Biolisa[®] Company. The enzymatic reaction was done on a plastic micro plate in order to detect total serum anti-HBc-Abs directed against HBc-Ag. In this reaction, serum anti-HBc-Abs (if exist) compete with peroxidase enzyme labelled antibodies (presented in the reagent) to bind HBc-Ag fastened on the solid phase. By adding the substrate, the results are interpreted as following:
 - 1) Slight colour or its absence means that sample contains anti-HBc-Abs
 - 2) Dark colour means that sample contains none.
2. HBV-DNA screening by RT-PCR method, using PCR equipment presented in Tishreen military hospital – Damascus, and HBV QPCR manual-48T[®] kit

originating by Chinese Bioer[®] association was used.

A definite site was amplified by Taq-DNA polymerase enzyme and two labelled primers. The device directly quantitates the amplified DNA at the end of each reaction round. During transcription, tincture molecules bind to recently synthesized DNA and released in the next hot round when the two chains DNA separated. So, fluorescence and its intensity increasing are measured according to DNA amplification exponential equation. Across fluorescence values projection on amplification rounds number, relative DNA concentration is outlined according to logarithmic measure and the results are compared to serial standards with known DNA concentration.

RESULTS AND DISCUSSION

Chi square test and T-student test were used to relate each of the separated variables (hospital, sex, renal failure aetiology, hemodialysis period, transfusion and age) to anti-HBc-Abs and RT-PCR conclusions. The results were statistically significant when the differences at design threshold are (P value ≤ 0.005).

Anti-HBc-Abs prevalence study

14 out of 50 (28%) hemodialysed patients free of C-hepatitis were anti-HBc-Abs positive; whereas 34 out of 72 (47%) patients infected by C-hepatitis were positive to these antibodies. This illustrates that anti-HBc-antibodies are more diffuse in C-hepatitis hemodialysed patients. Our conclusion was in agreement with Moroccan Lahsouna, M's study conducted in 2009[15].

Anti-HBc-Abs prevalence study in C-hepatitis infected patients

- **Relation with sex**
Out of 72 C-hepatitis hemodialysed patients, 34 were males (67.6%), and 11 were females (32.3%). We didn't observe any statistical significant relation with sex (P value = 0.085)
- **Relation with age**
Patients ages ranged from 21 to 79 years; mean age in anti-HBc-Abs positive patients (34/72) was 54.5 ± 10 years, and in anti-HBc-Abs negative patients (38/72) was 53.6 ± 12 years. There weren't any significant statistical differences (P value = 0.742)
- **Relation with renal failure aetiology**
Renal failure aetiologies distributed into: Hypertension 24/72: positive patients were 14 (29.4%). Diabetes mellitus 16/72: positive patients were 7 (20.5%) Other diseases 32/72: positive patients were 17 (50%) After statistical study, we didn't observe any relation statistically significant between the test result and renal failure aetiology (P value = 0, 66).



Relation with blood transfusion times:

Table (1) shows the relation of this kind.

Table 1: the relationship between anti-HBc-Abs positivity/negativity and blood transfusion times.

| Blood Transfusion times | Patients No. | Anti-HBc (+) | Anti-HBc(-) |
|-------------------------|--------------|--------------|-------------|
| No transfusion | 43 | 20 (58.8%) | 23 (60.5%) |
| Less than 10 times | 21 | 9 (26.4%) | 12 (31.5%) |
| More than 10 times | 8 | 5 (14.7%) | 3 (7.8%) |

Applying Chi square test to the previous table reveals no significant statistical relationship between the test result and blood transfusion times.

After Erythroboitin injections had been used to treat anaemia secondary to renal failure, the number of patients requiring blood transfusion reduced. So, exposition probability of viral hepatic diseases transmitted through contaminated blood decreased as well, and this procedure has really contributed in prevention.

In our random sample, we didn't observe any significant statistical relation between studied variables (sex, age, hemodialysis period, and blood transfusion times) and anti-HBc-Abs positivity. This conclusion is in accordance with Aghakhani, A's study conclusions which fulfilled in 2010.¹⁶

HBV-DNA prevalence study in positive anti-HBc-Abs with C-hepatitis patients

All 34 positive anti-HBc-Abs samples –originally negative HBs-Ag- were submitted to RT-PCR in view to screen HBV genome and so occult B-hepatitis detection. Positive HBV-DNA was found in 4 out of 34 samples (about 12%). Helmy, A studied 169 C-hepatitis patients in Saudi Arabia in 2006 and found positive anti-HBc-Abs in 50% of patients and HBV-DNA presented in 15.8%^[17]. This conclusion is near to ours, and illustrates the importance of anti-HBc-Abs screening in C-hepatitis where negative HBs-Ag doesn't rule out B-hepatitis. Doaa, A's study conducted in 2009^[18] is concordant with that. The existence of 30 negative HBV-DNA patients out of 34 positive anti-HBc-Abs ones points to one of two possibilities:

- 1) The anti- bodies that bound to HBc-Ag in wells were non-specific and false in nature.
- 2) Anterior HBV exposition and posterior resolution; the anti-HBc-Abs induced during anterior exposition last life-long.

Military Hospital positive anti-HBc-Abs amount to 4 out of 34 (11.76%); furthermore; there was no HBV-DNA positive patient.

This percentage (11.76%) in comparison to anti-HBc-Abs prevalence percentage –both in National Hospital (41.18%) and Kidney Surgical Hospital (47.06%)- is a reduced one. This fact is explained by: existing of enough nurses controlling patients during renal washing session, the small number of patients in artificial kidney

department which play a role in reducing contagion transmission, and the existence of enough hemodialysis devices permitting time to sterilization both by chemical and thermal means.

Kidney Surgical Hospital had –in addition to two HBV-DNA positive patients- the highest percentage (47.06%) of positive anti-HBc-Abs; this state is ascribed to the great number of patients exceeding capacity power of the centre; where the total number of patients reached (260) allowing no sufficient time to sterilize washing device and the number of nurses is small, so contamination transmission via unchanged gloves is potential when moving between patients.

CONCLUSION

Our study has revealed that anti-HBc-Abs can be an indicator to B-hepatitis in C-hepatitis patients in whom negative HBs-Ag is insufficient to rule out the diagnosis.

In hemodialysis units, before connecting the patient to washing device, C-hepatitis patients should be examined in order to detect occult B-hepatitis. To this end; anti-HBc-Abs should be screened routinely besides HBs-Ag to rule out OBH precisely.

The importance of perfect and correct sterilization of hemodialysis washing devices and medical equipment, such as: scissors, barometers apparatus and stethoscopes. It's mandatory to clean and disinfect external surfaces of hemodialysis washing devices after each use and to condense awareness and control programs in hemodialysis centres.

Proposal of RT-PCR – HBV-DNA screening technique availability in hemodialysis centres; this test could be reserved for positive anti-HBc-Abs patients in order to ascertain infection occurrence in this well-known high risk group.

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