The Possibilities of Chronic Renal Failure Patients Contracting Occult Hepatitis B Virus Infection, Sudan

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Abstract

Introduction: Chronic kidney disease (CKD) is a major cause of death in sub-Saharan Africa. The effects of the CKD on the host and the continuous therapeutic measures increase the hypothesis of blood-borne diseases transmission. Objective: This study aimed to find the frequency of occult hepatitis B virus (OBI) in patients of chronic renal failure (CRF) and to study the possibilities of infection acquisition. Methods: During 2017 and 2019, two hundred CRF patients under regular haemodialysis and attending Gezira Hospital for Renal Diseases and Surgery were recruited. Plasma specimens were collected and used for detection of hepatitis B surface antigens (HbsAg), total hepatitis B core antibodies (anti-HBc) and hepatitis B virus DNA isolation. Nested PCR reaction was followed to identify HBV. Socio-clinical data for each participant was obtained. Results: Male patients represented 64% (128/200), most frequent age group was from 41 to 60 years with percentage of 56.5% (113/200), 86% (172/200) of CRF patients were received blood while 42% (84/200) get HBV vaccination. Hepatitis B core antibodies were found in 54% (108/200) of studied cases, and 22% (42/188) of tested DNA were positively amplified for target gene. Conclusion: This study found high frequency of OBI in CRF patients, to reduce the transmission of the disease, possible hypotheses should be studied, including blood transfusion, haemodialysis process and HBV vacc-
1. Introduction

The steady increase in cases of chronic kidney failure (CKD) in developing countries has a tremendous impact on health and economic output [1]. Where it can be said that the continuous medical intervention for patients with CKD, such as hemodialysis, has proven to have many side effects, such as the hypothesis of transmission of blood-stream infections [2]. Cases of people undergoing hemodialysis are increasing worldwide due to the spread of underlying diseases such as diabetes mellitus, hypertension and environmental pollutants [3] [4]. In Sudan, chronic glomerulonephritis, obstructive nephropathy and autosomal dominant poly-cystic kidney diseases are also observed as risk factors among CKD patients [5]. Several reasons have an impact on the emergence of the problem of kidney failure, including the loss of interaction due to the spread of infectious diseases [6] and changes in lifestyle such as rapid urbanization [7]. We also cannot ignore the findings of some studies that determined that race lack of an organized chronic disease management program may affect the epidemiology of kidney failure [8].

Hepatitis B virus (HBV) infection is a global health dilemma with approximately 296 million chronically infected individuals by 2019 [9], and during the past five decades, developing countries remained the most endemic regions [10]. Sero-positivity as an indicator of HBV spread was found to be high in Sudan especially in Gezira State [11]. Generally, HBV infection is diagnosed by the presence of HbsAg in the blood; which is an outer protein expressed in excess when the virus replicates in the liver. However, existence of HBV genome in human even without HbsAg known as occult HBV infection (OBI) [12] [13]. Cases of OBI may have the presence or absence of hepatitis B virus antibodies; anti-hepatitis B core antibody (anti-HBc) and anti-hepatitis B surface antibody (anti-HBs) [14].

Conventionally, HBV is diagnosed by serological techniques to detect antigens or antibodies. The HBSAg is often used for routine diagnosis since it is considered as the hallmark of infection. During acute infection, anti-HBc, initially both IgM and IgG, appear 1 - 2 weeks after the appearance of HBSAg, while IgG persists during chronic infection. In the formulation of immunological concepts the presence of anti-HBs represents response to HBV infection [15]. In the advent of molecular diagnostics, it has been shown that a number of individuals may harbor DNA of HBV at very low levels in their liver and/or serum despite the absence of detectable HBSAg by currently available assays [16]. In line, a study said that infection with the virus is limited in the case of presence of DNA in people.
who carry anti-hepatitis B core antibodies, but the transmission hypothesis re-
 mains, as in the case of blood transfusion [2]. Methods for screening of OBI by 
DNA detection are essential when HBsAg is absent, thus, its consider as golden 
standard for diagnosis. Commonly used techniques are, real-time PCR, trans-
scription based mediated amplification (TMA) and nested-PCR [17].

Hepatitis B and C are the most important liver problems in end stage renal 
failure patients undergoing hemodialysis and with varying prevalence between 
countries and even between different dialysis units in the same country [18]. In a 
North American study, the prevalence of occult hepatitis B virus in adults un-
dergoing dialysis is four to five times higher than the results of positive HBsAg 
results [19]. Patients undergoing haemodialysis have a potential increase risk of 
both HBV and OBI; impaired host immune response, multiple transfusion re-
quirements, shared dialysis equipments, invasive procedures that they undergo 
and low response rates to HBV vaccination are the main predictors of OBI 
transmission in such situations [4]. In spite of that, haemodialysis units in Sudan 
trying to follow the globally acclaimed renal authorities for management of pa-
tients including control of hepatitis viruses, a significant deviation in perfor-
ance had been observed [20]. Some of the followed procedures include regular 
screening and allocating dialysis units for HBV patients, but it can be said that 
the viral screening protocol does not include OBI, thus the current study aimed 
to find out, in Gezira Hospital for Renal Disease and Surgery, the frequency of 
OBI and HBV DNA using serological and molecular methods, and to highlight 
existing risk factors.

2. Methods

2.1. Ethical Considerations

Ethical approval was obtained from the Ministry of Health in Gezira State and 
Faculty of Medical Laboratory Sciences, University of Gezira, Sudan. All study 
participants informed by the study objectives and written consents were taken. 
The confidentiality of result was secured by use of correspondence codes rather 
than written participant’s name. The results were referred to a clinician for ap-
propriate intervention.

2.2. Study Population and Settings

This is a cross-sectional laboratory based study, conducted in Gezira Hospital 
for Renal Diseases and Surgery in Wad Madani city which is the capital of the 
Gezira State; it lies in the east-central region of the Sudan and it’s a well-populated 
area with a fertile land suitable for agriculture. The period of study was from 
2017 to 2019. Participants were 200 patients with chronic renal failure under-
going regular haemodialysis medication and previously recorded as negative for 
HBV by rapid immunochromatography test (ICT) at the time of study. A val-
dated questionnaire was used to collect demographic and clinical data. The col-
lected data were analyzed using Statistical Package for Social Science (SPSS) ver-
sion 16, Chi square of <0.05 considered significant. Patients with acute renal failure admitted for dialysis were excluded from study subject.

2.3. Sampling

Five ml of venous blood were collected from each participant using sterile syringe and drawn into two EDTA containers. After complete blood clotting plasma samples were separated and kept at −20°C until used.

2.4. Serological Detection of HBsAg and Anti-HBc

Test for HBsAg done in Faculty of Medical Laboratory Sciences, University of Gezira using commercial kits, sandwich ELISA (Fortress diagnostics). The absorbance was read using ELISA micro-plate reader (Awareness Technology, Model: 303 PLUS, USA) at wavelength 450 nm, and reference filter at wavelength 630 nm. The cut-off value was calculated to evaluate the results. The electrochemiluminescence immunoassay (ECLIAS) was used to detect total hepatitis B core antibodies (anti-HBc) which qualitatively detect IgG and IgM using full automated cobase 411 machine (Hitachi High-Technology Corporation, Japan) and commercial kits.

2.5. Viral DNA Isolation

Phenol chloroform isoamyl alcohol (25:24:1) method was followed for DNA isolation [21]. Five hundred µL of 5% sodium dodecyl sulphate (SDS) 20 µL of proteinase K were added to 250 µL of plasma sample. Mixture was incubated at 56°C for one hour and at 95°C for 10 minutes. Then, 500 µL of phenol chloroform isoamyl alcohol (25:24:1) were added. After centrifugation, the upper layer of mixture was transferred into a new eppendorf tube. In order to obtain sufficient upper layer the last three steps were repeated extra two times. An amount of 20 µL of NaCl 9% and 1000 µL of cold absolute ethanol were added. The mixture was incubated at −20°C overnight. In the next day, supernatants were discharged after centrifugation to obtain DNA pellet. Tubes were left to dry and washed with 200 µL of 70% ethanol. The tubes were shaken until the pellet disappeared. Final centrifuge was made and the supernatants were discharged. Tubes were inverted open for 2 hours and re-suspend in 50 µL of sterile distilled water. The DNA was preserved at −20°C.

2.6. Nested PCR for Detection of Hepatitis B Virus

Nested PCR was done to detect viral DNA of hepatitis B virus using primer pairs (Macrogen, Seoul Korea) as shown in (Table 1). PCR master mix for one reaction mixture using iNTRON’sMaximePCRPreMix (INTRON biotechnology Soul, Korea) contained 5 µL of Taq DNA polymerase, 10XPCR buffer, 10 mMdNTPs and MgCl then 2 µL of DNA, 1 µL of each 10 P mol/ml forward and reverse primer, the volume was completed to 20 µL by deionized sterile water. Using PCR system 9700 thermocycler (Singapore) the program initiated with a first denaturation step 95°C for 5 min; followed by 30 cycles at 95°C for 1 min, 56°C
Table 1. Sequences of primers used and expected PCR product sizes [22].

| Primer direction | Sequence 5’-3’ | Tm (˚C) | size   |
|------------------|----------------|---------|--------|
| Nested forward   | GTTGCCCGTTTGCTCTAA | 58.4    | 250 bp |
| Nested reverse   | AAGCCCTACGAACCCTGAA | 58.4    |        |

for 1 min, 72˚C for 2 min, and a final extension at 72˚C for 5 min. revealed amplicons were visualized in 1.5% agarose using Cleaver Scientific Ltd. gel documentation system (Model: OMNIDOC). Length of target region showed approximately 250 bp.

3. Results

3.1. Demographics of Study Subjects

In total, 200 haemodialysis patients were enrolled. Male to female ratio equal 1.8:1. Most frequent age group was from 41 to 60 years. Most of studied patients 86% (172/200) had a history of blood transfusion and only 42% (84/200) were HBV vaccinated. A proportion of cases had a history of surgical intervention and jaundice was 38% and 28% respectively (Table 2).

3.2. Sero-Detection of HBsAg and Hepatitis B Core Antibodies

All participants were previously screened for HBsAg using ICT rapid test and recorded as negative. ELISA method detected HBsAg in 6% (12/200) of studied subject while hepatitis B core antibodies using ECLIAS resulted in 54% (108/200) as positive.

3.3. Molecular Identification of HBV DNA

Nested PCR was carried out to detect hepatitis B virus DNA among specimens that revealed negative HBsAg. Out of 188 DNA specimens, 22% (42/188) were successfully amplified using nested primers pairs and yielded approximately band size of 250 bp (Figure 1).

3.4. Risk Factors Analysis

Hepatitis B core antibodies was significantly associated with marital status (Chi-square 0.03) and with lack of vaccination (Chi-square 0.00) in the studied population, while no significant association was found with other observed risk factors (Table 3). Presence of virus DNA has been associated with non-vaccination cases (Chi-square 0.03) among study patients (Table 4).

4. Discussion

The most affected by chronic kidney diseases are those who live in sub-Saharan Africa, where most countries with low-income and middle-income are located [23]. Moreover, in the past three decades, the increasing problem of chronic kidney disease and the number of deaths resulting from it has led to its being
Table 2. Baseline data of CKD participants. No 200.

| Socio-clinical demographic | Yes/No | Frequency | Percent |
|----------------------------|--------|-----------|---------|
| Sex                        | Male   | 128       | 64      |
|                            | Female | 72        | 36      |
| Age group                  | 1 - 20 | 5         | 2.5     |
|                            | 21 - 40| 56        | 28      |
|                            | 41 - 60| 113       | 56.5    |
|                            | 61 - 80| 26        | 13      |
| Marital status             | Married| 34        | 17      |
|                            | Single | 166       | 83      |
| History of blood transfusion| Yes   | 171       | 85.5    |
|                            | No     | 29        | 14.5    |
| Alcohol intake             | Yes    | 24        | 12      |
|                            | No     | 176       | 88      |
| HBV vaccination            | Yes    | 84        | 42      |
|                            | No     | 116       | 58      |
| History of surgical operation| Yes | 76        | 38      |
|                            | No     | 124       | 62      |
| History of jaundice        | Yes    | 56        | 28      |
|                            | No     | 144       | 72      |
| HCV                        | Yes    | 0         | 0       |
|                            | No     | 200       | 100     |
| HIV                        | Yes    | 0         | 0       |
|                            | No     | 200       | 100     |

Figure 1. PCR gel presentation of HBV DNA. Lane M 100bp DNA ladder. Lanes 2, 3, 4 and 5 positive cases showing typical band size of 250 bp.
Table 3. Risk factors associated with hepatitis B core antibodies in study subjects. No 108.

| Risk factor               | Yes/No | Frequency (%) | Chi-Square |
|---------------------------|--------|---------------|------------|
| Sex                       | Male   | 71 (65.7)     | 0.658      |
|                          | Female | 37 (34.3)     |            |
| Age group                 |        |               |            |
| 1 - 20                    | Yes    | 3 (2.8)       | 0.05       |
|                          | No     | 41 (38)       |            |
| 21 - 40                   | Yes    | 41 (38)       |            |
|                          | No     | 54 (50)       |            |
| 41 - 60                   | Yes    | 10 (9)        |            |
|                          | No     | 54 (50)       |            |
| 61 - 80                   | Yes    | 10 (9)        |            |
|                          | No     | 54 (50)       |            |
| Marital status            |        |               |            |
| Married                   | Yes    | 24 (22.2)     | 0.03       |
|                          | No     | 84 (77.8)     |            |
| History of blood transfusion | Yes | 90 (83.3)     | 0.422      |
|                          | No     | 18 (16.7)     |            |
| Alcohol intake            |        |               |            |
| Yes                       | Yes    | 10 (9.3)      | 0.275      |
|                          | No     | 98 (90.7)     |            |
| No vaccination            |        |               |            |
| Yes                       | Yes    | 69 (63.9)     | 0.00       |
|                          | No     | 39 (36.1)     |            |
| History of surgical operation | Yes | 35 (32.4)     | 0.082      |
|                          | No     | 73 (67.6)     |            |
| History of jaundice       |        |               |            |
| Yes                       | Yes    | 32 (29.6)     | 0.637      |
|                          | No     | 76 (70.4)     |            |

Table 4. Risk factors associated with HBV DNA detection in study subjects. No 42.

| Risk factor               | Yes/No | Frequency (%) | Chi-Square |
|---------------------------|--------|---------------|------------|
| Sex                       | Male   | 31 (73.8)     | 0.201      |
|                          | Female | 11 (26.2)     |            |
| Age group                 |        |               |            |
| 1 - 20                    | Yes    | 1 (2.4)       | 0.588      |
|                          | No     | 11 (26.2)     |            |
| 21 - 40                   | Yes    | 22 (52.4)     |            |
|                          | No     | 8 (19)        |            |
| 41 - 60                   | Yes    | 22 (52.4)     |            |
|                          | No     | 8 (19)        |            |
| 61 - 80                   | Yes    | 11 (26.2)     |            |
|                          | No     | 22 (52.4)     |            |
| Marital status            |        |               |            |
| Married                   | Yes    | 9 (21.4)      | 0.503      |
|                          | No     | 33 (78.6)     |            |
| History of blood transfusion | Yes | 32 (76.2)     | 0.084      |
|                          | No     | 10 (23.8)     |            |
| Alcohol intake            |        |               |            |
| Yes                       | Yes    | 5 (11.9)      | 1.000      |
|                          | No     | 37 (88.1)     |            |
| No vaccination            |        |               |            |
| Yes                       | Yes    | 12 (28.6)     | 0.035      |
|                          | No     | 30 (71.4)     |            |
| History of surgical operation | Yes | 18 (42.9)     | 0.473      |
|                          | No     | 24 (57.1)     |            |
| History of jaundice       |        |               |            |
| Yes                       | Yes    | 14 (33.3)     | 0.322      |
|                          | No     | 28 (66.7)     |            |
added to the list of the leading causes of death in sub-Saharan Africa along with malaria, tuberculosis and AIDS [24] [25].

More data on the occurrence of OBI in developing world is necessary, especially, in individuals of high risk for HBV due to the limited existent data and diagnostic methods as well as lack of precise prevalence rate. The importance of identifying OBI in patients with chronic kidney failure as a risk group is due to their impaired cellular immunity, undergoing regularly haemodialysis and multiple blood transfusion intervention [26]. Understanding of the occurrence and transmission of OBI among patients with CKD enables knowledge of prevention measures to limit the transmission and spread.

The age group, 41 - 60 years, for CKD participants was the most frequent, which is similar to what is published in sub-Saharan Africa [27]. Although this study did not focus on determining hemoglobin levels, anemia is a common dilemma among patients with chronic kidney failure [28] [29], since most of the participants in this study had undergone blood transfusions. In many reports, the global prevalence of OBI varies from 0 - 58% [30] [31], our study revealed 22% (42/188) had positive OBI; it could be because most of participants had undergone multiple blood transfusion. Furthermore, the lack of diagnostic methods to identify the OBI in the blood of donors, its transmission is possible and subsequently, increases with the frequency of blood transfusions. Similarly, high rates of OBI among haemodialysis cases were reported in Egypt [14], but low and high rates were also documented in the same country as showed in [32] [33] [34] [35]. Despite the implementation of effective vaccination programs, hepatitis B remains an important cause of morbidity and mortality worldwide.

Considering the studies that indicated vaccination against hepatitis B virus for those suffering from CKD [36] [37], the proportion of unvaccinated included CRF patients in the study is of concern. Therefore, we found significant association between the presence of hepatitis B core antibodies, HBV DNA and the absence of vaccination. It is also important to monitor the levels of protective antibodies formed as a result of vaccination, as the protection resulting from vaccination may reach only 85%, in addition to the fact that the effectiveness of vaccination has not been confirmed for life [38]. Furthermore, with some cases, protection against HBV may require booster doses [39]. From the current study, immune chromatography test was less sensitive method for detection of HBsAg comparing to ELISA which was strongly supported by [40] in India. The detection of the virus by both mentioned tests was previously reported as failed due to mutations in the S gene which may lead to structural changes in HBsAg [41]. It should be noted that, variation in the sensitivity of serological diagnostic methods is likely to indicate the extent of these mutations. These observations may justify the high prevalence of OBI due to lack of knowledge, attitudes about HBV vaccination, modes of transmission, its consequences, its preventive measures, the improvement in diagnosis and documentation of HBV infection. The discrepancy in the reported incidences of HBV from different parts of Sudan [11]
[42] [43] justifies conduction of further studies to consider other risk factors like race, social and health status and, provision of health services. Limitation of HB vaccination program in Sudan and targeting of other high risk groups such as medical personnel also may contribute to continuous increasing in HBV infection especially among CKD individuals.

5. Conclusion

The acquisition of OBI for studied patients with CKD may be due to the lack of vaccination, the continuation of the haemodialysis process and consequent procedures and, multi-blood transfusion intervention. Thus, monitoring of CKD patients by regular screening of OBI is strongly recommended.

Limitation of Study

This study did not classify patients by stages of chronic renal failure or duration of dialysis intervention. Furthermore, detected genes of HBV were not characterized by sequencing analysis.

Acknowledgements

The authors would like to thanks staff of Gezira Hospital for Renal Diseases and Surgery in Wad Madani city for help and support during study periode, and all technicians work in the Medical Laboratory of the hospital.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Data Availability

Data of this study can be obtained from the correspondent author.

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**List of Abbreviations**

CKD: Chronic kidney disease; CRF: chronic renal failure; HBV: hepatitis B virus; OBI: occult hepatitis B virus; HbsAg: hepatitis B surface antigens; anti-HBc: anti-total hepatitis B core antibodies; anti-HBc: hepatitis B core antibody; anti-HBs: anti-hepatitis B surface antibody.