<u>Original Article</u> Contribution of Hepatitis B Contribution to Anemia in Dialysis Patients with Chronic Renal Failure, Iraq

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Abstract

Chronic renal failure are caused by impaired kidney function; this organ is essential in the metabolism, filtration, and excretion of compounds. Human hepatitis B virus is common in dialysis patients with chronic renal failure, and chronic kidney disease (CKD) is also associated with anemia in dialysis patients. In this study, 50 (36 men and 14 women) dialysis patients from Imamian Al-Khademian city, with ages between 30 and 77 years, and a healthy group (control group) with ages ranging between 30 and 62 years, were evaluated. Detection of hepatitis B virus by a molecular technique of real-time PCR and the concentration of erythropoietin hormone detected by the ELISA technique. The results showed that the prevalence of dialysis patients aged 41–50 and 60–51 was 20% and 18%, respectively. The detection of Hepatitis B from the serum of dialysis patients' samples showed that HBV was seen in 15 (30%) of the 50 serum samples. The concentration of the erythropoietin hormone in dialysis patients' samples was lower than in the healthy groups (a control group). Also, the concentration of erythropoietin hormone was significantly lower in dialysis patients' immunity. Also, people with hepatitis B have a lower level of the erythropoietin hormone, which is a major cause of anemia in dialysis patients. **Keywords:** Dialysis patients, Kidney failure, Hepatitis B virus, Erythropoietin hormone

1. Introduction

Chronic renal failure causes many side effects on health and usually causes an immune system response. Renal failure happens when the kidneys don't work as well as they should. This is made worse by different things and can lead to diabetes, high blood pressure, cancer, and other diseases (1). The dialysis patients' immunity becomes weak, so they cannot prevent infections with viruses such as hepatitis B (2). Chronic kidney failure happens over time and leads to kidney failure, which makes the kidneys work less and less until they stop working altogether in the last stages of the disease (3, 4). Anemia is a complication that happens during chronic renal failure, though most common in people with 3-5 stages; it is due to many symptoms, including renal failure, anemia, and anorexia. The pathogenesis of anemia in patients and the reduction of erythropoietin with chronic kidney disease (dialysis patients) is very important (5).

Hepatitis B is one of the viral infections related to kidney failure (6). However, hepatitis B infection can cause severe CKD (7). Hepatitis B is one of the most important causes of renal failure and an etiologic factor in secondary glomerular diseases (8). The goal of this study was to find out how hepatitis B affects renal failure caused by anemia in people with long-term diseases who get dialysis.

2. Materials and Methods

2.1. Sampling

This study evaluated renal failure dialysis patients from Imamian Al-Khademian city during March– June 2021, including 36 men and 14 women aged between 30 and 77 years, and 20 healthy individuals (control group). Blood samples of about 5 mL from dialysis patients and the healthy group were obtained, then left at room temperature for about 30 minutes. Separate the serum from the blood of dialysis patients and healthy individuals in the control group by centrifuging for 5 minutes and transferring it into tubes. Blood samples of dialysis patients (serum) are kept in tubes and then frozen at -20° C until used.

2.2. Molecular Detection of HBV

The DNA was extracted using viral DNA extraction kits (intron biotechnology, Korea) following manufacturer instructions. For hepatitis virus diagnosis, the samples from each patient were tested for HBV DNA using Nested PCR techniques, as described by Kaneko, Feinstone (9). The commercial Real-Time PCR kit (COBAS Taqman HBV test; detection cut: 6 IU/mL) was used to test each sample that had HBV DNA found by nested PCR.

2.3. Measurement of Erythropoietin Concentration

Erythropoietin levels were determined using the Enzyme-linked immunosorbent assay (ELISA) (EPO ab Kit). This assay has high sensitivity and excellent specificity for the detection of EPO-Ab.

2.4. Statistical Analysis

The values were presented as mean \pm SEM using SPSS, the variables were analyzed with t-tests, and the data were investigated with Pearson's $\chi 2$ tests. (*P*>0.05) was considered to be statistically significant.

3. Results

The dialysis patients from chronic renal dialysis were 50, taken from the central dialysis at Imamian Al-Khademian medical city in the age range of 30 to 77 years, also classified according to gender into 36 (64%) men's dialysis patients, 14 (28%) and women's dialysis patients (Table 1).

 Table 1. The distribution of the dialysis patients with renal failure according to gender

Gender	Dialysis N (%)	Control N%
Men	36 (72)	14 (70)
Women	14 (28)	6 (30)
Total	50 (100)	20 (100)

In this study, dialysis patients' age range between 30 and 77 years represented 30% of the study sample, and the age group > 77 years represented an increased percentage of 40% (Table 2). The results showed that 20 and 18% of patients aged 41–50 and 51–60 years were infected, respectively (Table 2).

Table 2. Dialysis patients distribution

Groups	age	No of patients % group	No of Control % group
group A	30-40	15 (30)	6 (30)
group B	41-50	10 (20)	4 (20)
group C	51-60	9 (18)	4 (20)
group D	>77	16(32)	6(30)
Total		50 (100)	20 (100

The detection of Hepatitis B from the serum of dialysis patients' samples showed that HBV was seen in 15 (30%) of the 50 serum samples (Table 3). The HBV genome, detection by Real-time PCR for the presence of virus in serum dialysis patients (Figure 1) showed that the viral loads of hepatitis B were between 30×10^2 - 260×10^2 IU/ML.

Table 3. Percentage of hepatitis B in dialysis patients

Result	Dialysis patients (N)	%
Positive group	15	30%
Negative group	35	70%
Total results	50	100%

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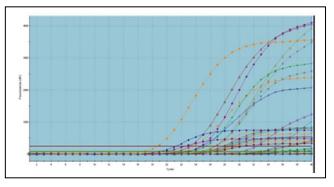


Figure 1. Result of Real-time PCR test detection of hepatitis B

This study showed a reduction in the standard level of the erythropoietin concentration for dialysis patients compared with healthy groups. The concentration of hormone in dialysis patients' men (positive and negative for hepatitis B) decreased from (3.899 pg/ml to 7.533 pg/ml, respectively, compared with men in the healthy group (control) (13.71pg/ml), while the concentration of erythropoietin in women dialysis patients group was positive (2.975 pg/ml and negative-8.546 pg/ml) compared to the healthy women (control group) (15.101pg /ml) (Figure 2).

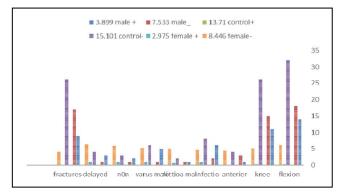


Figure 2. The concentration of Erythropiotein according to the gender

4. Discussion

The hepatitis B virus (HBV) is a known infectious risk for chronic kidney disease. Factors contributing to infection in hemodialysis patients include exposure to blood products, standard hemodialysis equipment, and immunodeficiency. This study showed that in men, in dialysis patients (72%), chronic renal failure was more common than in women (28%). This result was consistent with the results of Besisik, Karaca (10). Cooreman, Leroux-Roels (11) showed that men are more likely to reach renal failure sooner than women due to the daily effort of men compared to women, and also because the creatinine in the muscles increases the mass in men compared to women, which leads to a high ratio of creatinine in the muscles of men, which leads to kidney damage. It is seen in dialysis patients, so men are more affected than women in dialysis patients (12). The result of this study showed that in dialysis patients, 10 samples were positive for HBV. Cabrerizo, Bartolomé (13) and Oesterreicher, Hammer (14) showed that infection of HBV in dialysis patients was higher compared to the control group. In this study, the diagnosis (hepatitis B) in dialysis patients with renal failure in men and women was made using the real-time PCR technique. A study on hepatitis B virus infection in a North American adult hemodialysis patient population showed that 15 (30%) of dialysis patients had a positive viral genome, and the results of the study agree with Minuk and Sun (15), who showed that 17 (34%) of the infectious with virus tested gave positive results for viral nucleic acid. The study on occult hepatitis B virus infection prevalence in hemodialysis patients from Egypt showed a significant decrease in the erythropoietin hormone for men and women. Also, erythropoietin is responsible for the production of red blood cells (RBC) and causes anemia in dialysis patients who are infected with HBV (16); this result was in agreement with Yakaryilmaz, Alp Gurbuz (17). The results of this study showed that the low levels of erythropoietin in dialysis patients with hepatitis B were decreased more than in dialysis patients negative for hepatitis B, and the study was similar to the results of a study conducted by Cengiz, Turhan (18), who mentioned that hepatitis (B) targets the renal glomerular, which activates erythropoietin and causes hypoxia, which leads to lower production of erythropoietin and causes anemia, for dialysis patients with end-stage of kidney failure (19, 20).

Hepatitis (B) is associated with chronic renal failure in dialysis patients, which causes low immunity in patients and also leads to a reduction in the concentration of erythropoietin, which causes anemia in dialysis patients.

Authors' Contribution

Study concept and design: M. H. J.

Acquisition of data: S. H. R.

Analysis and interpretation of data: S. G. A.

Drafting of the manuscript: E. J. M.

Critical revision of the manuscript for important intellectual content: S. H. R.

Statistical analysis: M. H. J.

Administrative, technical, and material support: S. G. A.

Ethics

The Ethical Committee of Ibn Sina University of Medical and Pharmaceutical Science, Iraq approved the present study protocol.

Conflict of Interest

The authors declare that they have no conflict of interest.

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