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Underlying diseases, nutritional assessment, viral incidence and HBV immunity status in patients undergoing hemodialysis

Maryam Musavi[1], Aida Rasoolzadeh[2], Mohammad Salehi[3,4*], Hadi Fazel[2]

Abstract

Introduction: Underlying kidney diseases and their progression cease the function of kidney and raise the need for regular maintenance dialysis. Furthermore, nutritional requirements and blood born viral infections affect their quality of life.

Objectives: This study aims to investigate viral incidence, underlying kidney diseases, nutritional status; and hepatitis B virus (HBV) immunity status in hemodialysis (HD) patients.

Patients and Methods: A total of 330 end-stage renal disease (ESRD) patients were assessed for human immunodeficiency virus (HIV), HBV, hepatitis C virus (HCV) viruses using Enzyme-linked immunosorbent assay (ELISA) assay. The underlying kidney diseases were confirmed by a physician. Eighty-two subjects with diabetic nephropathy are referred to dietitian for nutritional assessments and body mass index (BMI) measurement. Hepatitis B surface antibody (Anti-HBs) tests were done at two different times of year for 94 qualified subjects.

Results: Out of 330 patients 82 (24.8%) had diabetic mellitus (DM), 5 (1.5%) myocardial infarction (MI), 3 (0.9%) systemic lupus erythematosus (SLE), 55 (16.7%) hypertensive nephrosclerosis (HN), 3 (0.9%) obstructive nephropathy, 5 (1.5%) autosomal dominant polycystic kidney disease (ADPKD), and 4 (1.2%) glomerulonephritis. 45 cases (13.6%) had DM and HTN simultaneously. Eleven percent of diabetic nephropathy (DN) patients had severe malnutrition. Only five men (1.51%) were positive for HBV. No incidence of HCV and HIV was seen. Findings showed a dramatic change for anti-HBs after 6 months.

Conclusion: Despite the advances in medicine, malnutrition and viral diseases still threaten dialysis patients. DM was the most common underlying disease. The safety of dialysis patients for HBV is compromised after regular dialysis, underscoring the importance of strict adherence to vaccination program.

Keywords: Kidney failure, Diabetic nephropathy, Nutritional assessment, Virus diseases

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Introduction

The influence of underlying kidney diseases and their progression to end-stage renal disease (ESRD) have been demonstrated in several reports (1). The stage of ESRD is a condition in which glomerular filtration rate (GFR) does not exceed 15 mL/min for 1.73 m^2 (2). Diabetes is the causal agent of diabetic nephropathy (DN) and the deterioration of kidney function, which is a common complication in both type 1 and type 2 diabetes. According to the last update on DN, nearly 30% to 40% of patients with diabetes develop DN (3). In patients with DN, the tiny blood vessels (glomeruli) are damaged in kidney. To compare patients with diabetes undergoing maintenance hemodialysis (HD) and those without diabetes undergoing HD, the prognosis of the former is worse (4).

Detection of malnutrition is critical to prevent further nutritional depletion and poor patient outcomes by implementing preventive measures and interventions (5). As a critical measure of health care systems, nutritional assessment provides better quality of life. Patients with kidney malfunction need to be screened at regular intervals for identification of nutritional disorders. Dietary management of patients with poor nutritional awareness is an essential and modifiable factor that may influence chronic kidney diseases (CKD) processes and finally may lead to ESRD. It is important to note that both diet overload and malnutrition need to be balanced in DN patients. Nutritional management has a key role in preventing the progression of nutrition-associated alternations especially in DN patients (6).

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[1]Healthy Ageing Research Center, Neyshabur University of Medical Sciences, Neyshabur, Iran. [2]Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran. [3]Blood Borne Infections Research Center, Academic Center for Education, Culture and Research (ACECR), Razavi Khorasan Branch, Mashhad, Iran. [4]Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran.

***Corresponding Author:** Mohammad Salehi, Email: mohammadsalehi73@mail.um.ac.ir, mohammadsalehi73@gmail.com

Implication for health policy/practice/research/ medical education

In a study on hemodialysis patients (Neyshabur, Iran), we found that DM is a major underlying disease for kidney injury. The percentage of other potential underlying diseases reported as well. About one tenth of the patients undergoing dialysis are malnourished. There is still concern about contracting viral diseases and transmitting them to others through the dialysis settings. The process of dialysis can lead to a decrease in people's immunity to HBV.

Another thing that threatens the life quality of HD patients is viral diseases persisting even with modern methods of dialysis treatment. As a global public health issue, they are at increased risk of catching viral infections as they mostly receive maintenance dialysis (7). The underlying causes of this issue are broad including the impaired cellular immunity and some parenteral route such as blood transfusion, and frequent hospitalizations. Moreover, prolonged exposure to infectious materials through the process of extracorporeal circulation for a long period could aggravate the situation (7).

As the management of the nutritional aspects represents a number of advantages, and the outbreaks of viral diseases continue to occur, repeated assessments to improve nutritional status and reduce the prevalence of viral diseases in high-risk groups of all patients undergoing maintenance dialysis seems to be crucial. Despite all the efforts and advancements in the medical sciences, HD patients are still considered to be high risk group for viral diseases. Previous epidemiological studies in second populous city of Razavi-Khorasan province (North-east of Iran), Neyshabur, suggested that some viral diseases are prevalent. Dietary management of DN patients is a challenging issue as well.

Objectives

We arranged this study to report nutritional status in a chunk of HD patients with DN and viral disease assessment of almost all HD patients in this region. Additionally, the underlying kidney diseases and HBV immunity status in these patients were investigated.

Patients and Methods

Cases and measurements

This cross-sectional study was performed at academic center for education, culture and research (ACECR) on blood sample of chronic HD patients which are outsourced by the only main well-equipped HD center of Neyshabur, Salamat dialysis center (January 2021-March 2022) catering daily up to 150 individuals with ESRD. Out of 48 HD machines, one was dedicated to positive viral cases to avoid cross-contamination. After informed consent, all 330 patients were screened for DN to be assessed for nutritional assessment, the body mass index (BMI) as an anthropometric measurement and biochemical tests. Nutritional assessments were evaluated by dietitian using efficient method of subjective global assessment (SGA) and complete medical history was done by center's physician. The website of https://www.calculator.net/bmi-calculator.html was applied to calculate BMI. World Health Organization's (WHO's) recommendation was used to interpret BMI. Over screening, we found some other potential kidney underlying disease, and then preferred to report them. To show that immunity for HBV may impaired over maintenance dialysis in ESRD patients, we analyzed hepatitis B surface antibody (anti-HBs) over a period of 6 months.

All 330 HD patients underwent for viral tests of human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). All viral tests were performed immediately using commercial ELISA kits (Pishtaz Teb Zaman, Iran). The cut-off values for seropositivity were considered according to kits instructions. Some serum biochemical have been measured using full automatic analyzer of Mindray BS-800 (China, Shenzhen).

Inclusion and exclusion criteria

Only physician-confirmed DN patients underwent nutritional assessment, BMI measurement and biochemical tests. As exclusion criteria, we exclude those who receive HBV vaccine over the period of study; people with zero immunity for HBV were excluded to figure out whether dialysis setting could weaken HBV immunity. Those who were unable to cooperate or complete relevant assessments (e.g., due to mental disorders) were excluded.

Statistical analysis

Linear regression model was performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA, USA). The statistical significance level was considered 0.05. All descriptive values including percentage, standard deviation, mean indices were prepared using SPSS software version 21. To depict figures, we utilized GraphPad Prism and Microsoft Excel 2010.

Results

Viral incidence in patients with ESRD

In 330 patients with a mean age of 52.2 ± 15.5 years (range, 12-85 years); there were 201 males (60.97%) and 129 females (39.9%). Patients with ESRD showed not any incidence for HCV and HIV in both genders. There were only three cases of men with HBV infection (1.51%) however, women were not infected with HBV (Figure 1).

Immunity status for HBV after six-month undergoing dialysis

Out of 330 patients, only 94 cases have the exact condition to assess the immunity status for HBV after 6 months dialysis. A linear regression model showed a significant difference ($P < 0.0001$, r=0.79) between patients at the beginning of study and the same subjects after 6 months

(Figure 2). On average, the mean of antibody for HBV changes from 309 ± 82 to 69.2 ± 76 over mentioned period. In some cases, these variations were drastic so that the antibody declined 33.4 times from 6130 mg/dL to 183 mg/dL. Other striking results indicate a dramatic reduction ranging from 17.5 times to 6.29 times (Figure 3).

Underlying diseases in maintenance HD patients

The underlying diseases were diabetic mellitus (DM), coronary artery disease (CAD), systemic lupus erythematosus (SLE), hypertension (HTN), obstructive nephropathy, autosomal dominant polycystic kidney disease (ADPKD) and glomerulonephritis. As Table 1 shows the survey of 330 patients suggests that DM was the most common complication in ESRD patients. The coincidence of DM and HTN occurred in 13.6% of ESRD patients. There was no cut-clear etiology for 38.4% of ESRD patients to figure out which underlying disease is responsible for ESRD (Table 1).

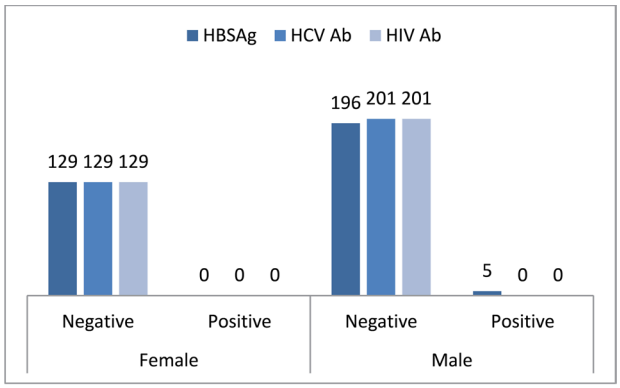

Figure 1. Prevalence of viral disease in patients with ESRD (N=330).

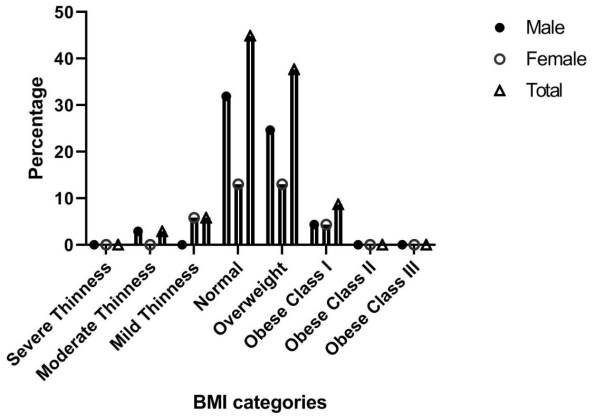

Figure 2. BMI of diabetic nephropathy patients with ESRD (N=82).

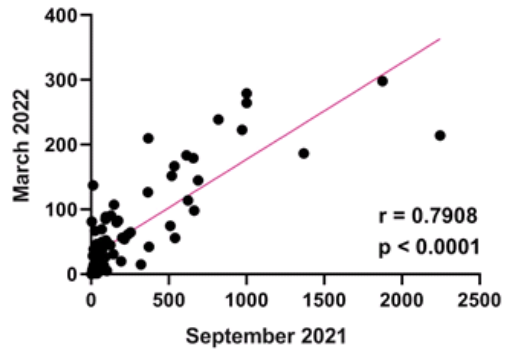

Figure 3. The anti-HBs antibody changes among ESRD patients during six months (N=94).

Nutritional assessment and laboratory findings in DN patients

Table 2 summarizes the findings from questionnaires filling out by dietitians for 82 DN patients. Eighty-two patients with an average age of 40.9 ± 16.2 years (range, 12 –81 years); there were 47 men (57.31%) and 35 women (42.68%). Eighty-five percent live in city, 94% were married, and 28% were illiterate or acquired elementary grade degree. One (1.21%) out of 82 with ESRD underwent the renal transplantation (RT). Among 82 participants, two cases (2.4%) showed an acute hypersensitivity reaction to intravenous (IV) iron. Eleven percent had severe malnutrition. Moreover, concomitant symptoms such as wasting, anorexia, nausea and constipation were the most common complications in DN sufferers. The average daily energy intake was 2974 kcal/d (Table 2, Figure 4).

Laboratory findings for biochemical tests of DN patients with ESRD have been outlined in Table 3 as well. Fasting blood sugar (FBS) mean of DN patients was 164 ± 81 mg/dL. Hemoglobin A1c (HbA1c) average, another important test indicating diabetes, was 8.6 ± 1.3% (range: 7.1-12%). The mean for creatinine (Cr) and urea were 7 ± 2.4 mg/dL and 115 ± 31 mg/dL respectively (Table 3). Figure 3 shows the relationship between BMI and gender. In the present study, the average BMI was 24.4 ± 4.47 kg/m^2 (Min: 16.1, Max: 34.6). Forty percent of DN patients with ESRD were overweighed and 10 % had obesity class I.

Table 1. Underlying diseases and common complications in patients with ESRD (N=330)

Underlying disease, No. (%)	No. (%)
DM	82 (24.8)
SLE	3 (0.9)
HTN	55 (16.7)
Obstructive uropathy	3 (0.9)
ADPKD	5 (1.5)
Glomerulonephritis	4 (1.2)
DM+HTN	45 (13.6)
Other complications	**No. (%)**
DR	80 (24.2)
MI	5 (1.5)
Unknown etiology	127 (38.4)

DM: Diabetic mellitus, SLE: Systemic lupus erythematosus, HN: hypertensive nephrosclerosis, ADPKD: Autosomal dominant polycystic kidney disease, DR: Diabetic retinopathy, MI: myocardial infarction.

Table 2. General, clinical status and nutritional assessments in ESRD patients with DN (N=82)

Parameters	Value
Number of dialysis (per week, hour)	3, 4 h
Years on dialysis (y), Mean ± SD	4 ± 2.9
Body weight (kg, range)	67, 37-98
Smoking or drug abuse, No. (%)	5 (6.1)
General status, No. (%)	
Consciousness disorder	1 (1.2)
Visual impairment	0 (0)
Motor impairment	1 (1.2)
Speech impairment	1 (1.2)
Hearing impairment	1 (1.2)
History, No. (%)	
Iron allergy	2 (2.4)
Biopsy	3 (3.6)
Peritoneal dialysis	2 (2.4)
Family history of dialysis	3 (3.6)
Renal transplant	1 (1.2)
Hepatitis B vaccination	61 (74.3)
Blood receiving	4 (4.9)
Food allergy	1 (1.2)
History of COVID-19	70 (85.3)
Gastrointestinal/digestive disorder, No. (%)	
Nausea	9 (11)
Vomiting	2 (2.4)
Diarrhea	0
Reflux	2 (2.4)
Anorexia	14 (17)
Constipation	22 (26.8)
Dysphasia and chewing problems	1 (1.2)
Denture problems	5 (6.1)
Clinical status, No. (%)	
Wasting	10 (12.2)
Subcutaneous dystrophy	2 (2.4)
Edema	1 (1.2)
Ascites	1 (1.2)
Skin, hair, nail changes	1 (1.2)
Weakness/tremors	4 (4.9)
Recent physical activity level, No. (%)	
Sedentary	29 (35.3)
Mild	43 (52.4)
Moderate	7 (8.5)
Extreme	3 (3.6)
Medicine or supplement intake history, No. (%)	
Nephro-Vite ®	40 (48.8)
Folic acid	44 (53.6)
CaCo3	48 (58.5)
L-carnitine	63 (76.8)
Vitamin C	73 (89)
B complex vitamin	32 (39)
Vitamin B1	17 (20.7)
Omega-3 fatty acids	35 (42.7)
Vitamin E	32 (39)
Vitamin D3	68 (83)
Nutritional parameters	
Fluid (cc)	1340±488
Protein (g/d)	73±12
Energy (kcal/d)	2074±313
Low phosphorus and potassium	56 (68.3)
Low fat	7 (8.5)
Low protein	13 (15.8)
Eating way, No. (%)	
Oral nutrition	82 (100)
Nil per os (Nothing by mouth)	0 (0)
Total parenteral nutrition	0 (0)
Enteral nutrition	0 (0)
Kind of vascular access, No. (%)	
Temporary catheter	7 (8.5)
Permanent catheter	3 (3.6)
Fistula	60 (83.3)
Graft	2 (2.4)
Current status of pregnancy/lactation	
Pregnant & Nursing	0

ESRD, end-stage renal disease; DN, diabetic nephropathy.

Discussion

DN, a chronic condition of DM, leads to the loss of kidney function. According to the CDC, diabetes is the leading cause of ESRD and is associated with increased morbidity and mortality in maintenance HD patients. Findings of the present study showed DM accounts for 24.8% of ESRD. In the United States, diabetes accounts for 30% to 50% of end-stage renal failure representing a significant public health concern in both types of absolute insulin insufficiency and relative insulin deficiency (3).

To have better quality of life in HD patients particularly in those with metabolic disturbance of T2D, nutritional status should not be ignored in HD settings. In other words, dietary adherence can determine outcomes in DN sufferers. In addition, the detection of malnutrition is important to prevent further nutritional depletion in patients with hyperglycemia. Implementing preventive measures such as nutritional counseling and psychosocial interventions could be a highly effective approach in DN conditions (8). Thereby, some simple methodologies including SGA have been developed to manage nutritional quality of DN sufferers and lessen the effects

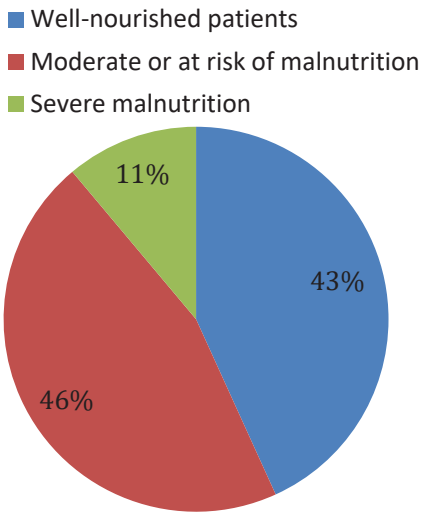

Figure 4. Nutritional status of the DN patients with ESRD (N=82).

Table 3. Plasma laboratory findings of DN patients with ESRD (N = 82)

Test item	Result	Unit	Normal range
Calcium	8.2±0.8	mg/dL	8.6-10.5
Cr	7±2.4	mg/dL	0.6-1.2
FBS	164±81	mg/dL	70-115
Ferritin	576±456	µg/L	18.2-341
PTH	382±305	Pg/mL	14.5-87.1
Potassium	5.3±0.9	mEq/L	3.5-5.5
Phosphorus	5.1±1.2	mg/dL	2.6-4.5
Sodium	141±3.8	mEq/L	133-148
HbA1c	8.6±1.3	%	3-6
Urea	115±31	mg/dL	13-43
Urea (After dialysis)	41±16	mg/dL	13-43
ALB	3.6±0.29	mg/dL	3.5-5.2

of malnutrition.

The result of malnutrition assessment in the present study using efficient method of SGA showed that severe malnutrition is present in nearly 11% of the incident cases of DN patients undergoing maintenance HD. This result was lower than that reported in some other research projects conducted in China (19%), Belgium (15.5%), Japan (38%), Pakistan (21.3%) and India (23%) (9-13). On the other hand, some research performed in Palestine on severely malnourished diabetic individuals suggests lower prevalence of 9.4% in comparison with our study (14). In a very recent study, malnutrition assessment showed that the prevalence of malnutrition in CKD patients (stage 4 and 5) with diabetes was higher than that in the uncombined group (9).

Supplementation as an important means of treating malnutrition could ameliorate the outcomes of DN (15). Profound impact of nutritional interventions has been suggested in several studies. In one study, findings suggest that oral supplementation of vitamin D3 was associated with decrease in the complications of DN (16). Additionally, the lipid profile and glutathione levels of DN sufferers have ameliorated following a high-dose of vitamin E supplementation. Alpha-lipoic acid administration also showed to be helpful in reducing neuropathic symptoms and quality of life. Several dietary antioxidants including resveratrol, curcumin, selenium, soy, catechins, coenzyme Q10, omega-3 fatty acids, zinc and vitamin C have been proven to affect oxidative stress and the capacity for antioxidant response as well (17). Participants of the current study with DN were frequently nourished by appropriate supplementations of L-carnitine (76.8%), vitamin C (89%), vitamin D3 (83%), and CaCo3 (58.5%).

Although BMI does not differentiate between muscle and fat mass, greater survival is associated with higher BMI. That is to say, lower mortality risk has been seen in obese DN patients with ESRD. Although it could be considered as a survival advantage in this population, targeting higher BMI levels is not necessarily recommended to get better clinical outcome. For dialysis patients, unintentional weight loss may lead to poorer clinical outcomes but intervention measures to lose weight intentionally is linked to better outcome (18). Approximately half of subjects in this study were suffering from obesity or being overweight and excessive body weight whether overweighed or obese was found in 50% of diabetic individuals with DN.

A sufficient energy intake is mandatory to avoid protein energy wasting (PEW) in ESRD patients. Recent studies have demonstrated that, upregulated sodium-glucose cotransport and glomerular hyperfiltration enhance energy required in tubular cells of DN patients' kidney (19). Protein intake appeared to be major determinants of nutritional status in HD patients. A safe amount of protein intake to have weight-stable conditions is suggested to be about 0.8 to 1.3 g/kg body weight (20). We observed that the average daily energy intake and protein intake of DN patients with ESRD were 2974 kcal/d and 73 g/d respectively.

Diabetes and HTN are the two most common causes of kidney failure overloading the body with toxins. In addition, other disorder including SLE, obstructive nephropathy, ADPKD and glomerulonephritis could hurt kidney. Other complications like DR, myocardial infarction (MI), and CAD can be seen in people with renal failure. The present study showed 24.8% DN, 0.9% SLE, 24.2% DR, 16.7% HTN, 0.9% obstructive uropathy, 1.5% ADPKD, 1.2% glomerulonephritis, 1.5% MI, 38.4% unknown etiology and 13.6% coexistence of DM plus HTN in all 330 participants. Al Saran et al in Riyadh reported that the cause of chronic renal failure included the following: DN in 82 patients (41%), hypertension in 40 patients (20%), chronic glomerulonephritis in 12 patients (6%), hypoplastic kidney in 4%, lupus nephritis

in 3%, unknown etiology in 22%, obstructive uropathy in 2%, and tubulo-interstitial nephritis and contrast-induced nephropathy in 1% each (21). In addition, the main causes of CKD in very recent report were DN (28.4%), followed by hypertensive nephrosclerosis (HN) (19.3%), glomerulonephritis (9.2%), ADPKD (3.7%) and 19.7% undetermined cause (22). Glomerulonephritis (31.7%), HN (26.7%), and DM (16.7%) were reported as the main underlying diseases in the HD group of Rodrigues et al study (23).

Biological indicators can be routinely monitored by measuring serum biomarkers in HD patients. Examination of blood biomarkers in DN patients undergoing HD in this study indicates the following outcomes; hypocalcemia, high P levels, increased synthesis of PTH, high level of FBS and HbA1C, high amount of urea and creatinine. In brief, the synthesis of calcitriol is compromised in ESRD and then the intestinal absorption of Ca is reduced. The body increases the synthesis of PTH to compensate Ca level, which may gradually lead to systemic mineral and bone disorders. Failure to excrete biological toxins could raise the serum level of P, urea and creatinine and high-circulating level of these metabolites is associated with elevated mortality in dialysis patients (23).

The prevalence of blood-borne viruses in HD patients varies considerably throughout the world. It was hypothesized that viral disease in HD patients may be associated with poor clinical outcomes and despite following the guidelines on universal precaution and regular virology tests, viral infections are still considered a big concern in the HD settings (24). HD units are mostly infected by hepatotropic viruses of HBV, HCV indicating not perfect adherence to hygiene standards is the most striking cause of viral diseases transmission among patients in dialysis units (25). Through phylogenetic analysis, a systematic review of 31 reports on HCV outbreaks confirmed nosocomial transmission which highlights the potential of health care system as a causative agent of outbreaks (26).

The risk of co-infection is greater among chronic renal failure patients due to repeatedly exposure to blood from transfusions and extracorporeal circulation during HD (27). The coexistence of three viral infections of HBV, HCV and HIV was not observed in the present study. Patients with ESRD showed not any incidence of HCV and HIV in both genders. There were only three cases of men with HBV infection (1.51%) however, women were not infected with HBV. The prevalence of HBV infection in HD patients of Malhotra et al study was found to be 1.5% which was in agreement with our study (27). However, others reported higher prevalence; Duong et al showed seroprevalence of HBsAg and HCV were 7% and 6% respectively in 113 enrolled HD patients (28). These findings highlight the importance of regular serologic screening for blood-born viral specific antibodies, which play an essential role in preventing blood-borne viral transmission within HD settings.

As this study was conducted in pandemic times of Covid-19, we reported the history of COVID-19 in DN with ESRD as well. The history of COVID-19 has been observed in 85.3% of DN undergoing HD. Some research suggested that this period exacerbates the situation for ESRD so that they reported that the mortality of end-stage kidney disease patients admitted with COVID-19 infection was 17% (29).

Vaccinated patients are at a lower risk of infection with hepatitis B but in dialysis patients there are many modifiable factors that could affect the outcome. Over a period of 6 months, a logistic regression model was built for 94 patients to find out to what extent dialysis settings could weaken immune system in terms of anti-HBs. In some cases, variations were striking, the anti-HBs antibody declined ranging 33.4 times to 6.29 times ($P<0.0001$, r=0.79). This evidence suggests that dialysis setting could accelerate immunity lowering against HBV, highlighting the need for regular anti-HBs tests and vaccination. The lack of immune response to the HBV vaccine in dialysis patients is linked to older age, lower nutritional status, HLA-DR3 status, duration of dialysis time, diabetes mellitus, lower PTH level, lower hemoglobin, and lower dialysis adequacy. Although some of these are difficult to deal with, the HBV vaccine immune response might be improved by tackling these factors (30).

Conclusion

Despite the amazing advances in medicine, malnutrition and viral diseases still threaten dialysis patients. Regular maintenance HD could gradually deteriorate nutritional status of DN patients, therefore compromising their health condition. We strongly encourage dietary compliance of the patients because dietary adherence can determine outcomes. It is worth mentioning that dietary interventions provide new hope as an effective tool in improving quality of life and delaying disease progression in DN patients.

These results still highlight the importance of strict adherence to universal precautions, careful aseptic technique, proper and regular maintenance of HD units including cleaning and disinfection and instruction-based disposal of used material and every other standard and dialysis-specific infection control practices should be implemented in the dialysis units to decrease the risk of transmission of viral diseases. Besides, Environmental surfaces used in the dialysis setting must be disinfected. Immunization with HBV vaccine before doing any dialysis, isolation of patients with reactive results for viral diseases, regular surveillance for all transmittable viral infections helps drastically in hindering the spread of these viruses in HD units. Moreover, as 4.9% of DN sufferers in this study had blood transfusion experience, the blood used for transfusion should be screened for HBV, HCV, HIV and particularly human T-lymphotropic virus type

1 (HTLV1) in this region; results should be confirmed using state-of-the-arts techniques as well. To find out the efficacy of the strategies in order to eradicate the incidence of these viral infections and their trend among HD patients, we strongly recommend occasional local studies, which could promote better social repercussion. The results of this study proved that the safety of dialysis patients for hepatitis B is compromised after regular dialysis underscoring the importance of strict adherence to vaccination program.

Limitations of the study

The endemicity of the region for HTLV underscores the importance doing of this test among HD patients. Investigators of this research project were on a tight budget to perform HTLV test. We strongly advise it in future evaluation.

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Authors' contribution

Conceptualization: MS, AR.
Methodology: MS, AR.
Formal analysis: MS, AR.
Resources: MS, AR.
Investigation: MS
Data curation: MS
Visualization: MS
Validation: AR, HF, MM
Project administration: MS
Writing—original draft preparation: MS
Writing—Review and Editing: AR, HF, MM
Supervision: AR, HF, MM.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The institutional ethical committee at Academic Center for Education, Culture and Research (ACECR), Razavi Khorasan Branch, approved all study protocols (Ethical code#IR.ACECR.JDM.REC.1400.084). Accordingly, written informed consent was taken from all participants before any intervention. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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