The Impact of CD19+B-Cells and Some Important Parameters in Iraqi Hemodialysis Patients

Sahar Taha Hatif[1*], Ayat Majeed Zeadan[2], Manar Mohammed Sarhan[3]

[1]Department of Microbiology, College of Medicine, AL-Iraqia University, Baghdad, Iraq
ORCID: https://orcid.org/0000-0002-6202-0109
EM: sahar.aldaffaie90@gmail.com
[2]Department of Microbiology, College of Medicine, AL-Iraqia University, Baghdad, Iraq
[3]Baghdad Teaching Hospital (Dialysis Unit-The Iraqi Centre for the Industrial)

*Corresponding author: Sahar Taha Hatif (sahar.aldaffaie90@gmail.com)

Abstract

Objective: The present study was designed to identify the effect of CD19+ B-cells in Hemodialysis (HD) patients compared to healthy volunteers and, also investigates its relationship with essential parameters parathyroid hormone (PTH), vitamin D3 (VitD3). Methods: A total of (60) clinically diagnosed patients and (30) healthy group, were collected from /February 2022 to July 2022/. Information was collected through a questionnaire designed for the present study. The serum levels of CD19 were evaluated using ELISA and serum remains were collected, for PTH and D3. Results: The result of the present study showed that the mean serum CD19 levels was significantly decreased (P < 0.001) in patients compared to healthy volunteers. Although the level of PTH was significantly higher (P < 0.001) in HD patients, the results showed a significant decrease in levels of D3 (P < 0.001). Furthermore, there was a highly significant positive correlation between B-cells levels and vitamin D3 level as well as showed this study a highly significant negative correlation between B-cells and PTH. Conclusion: Chronic kidney disease (CKD)-associated immunodeficiency has been associated with a decrease in B cells, and a substantial proportion of morbidity and mortality with advanced conditions; therefore, we can support the immune system of HD patients before deterioration. In future studies, a monthly examination of the CD19 + correlations with laboratory parameters may improve outcomes and disease. Also verifying the primary immune source that affects the disturbance of the natural system of the kidney, more studies are needed to shed light on therapies with antibodies.

Keywords

Chronic renal failure (CRF) is the term used for kidney diseases that last three months or more; it is also known as chronic kidney disease (CKD), and it denotes the presence of structural or functional abnormalities in the kidney with or without a decline in glomerular filtration rate (1).Hemodialysis has a higher mortality rate(2). Compared to the general population, those with end-stage renal disease (ESRD) have impaired immunity (3). Therefore, CRF represents a primary worldwide concern and its prevalence continues to rise, it is also one of the most prevalent diseases (4).

In advanced countries, the prevalence is estimated to be between 10 and 13% in the adult population (5). According to social determinants of health and ethnicity, the incidence and prevalence of CKD also differ among nations, presumably due to the epigenetic effect. Many patients exhibit no symptoms or display generalized symptoms

like lethargy, itching, or anorexia. The diagnosis is frequently made as a result of fortuitous results from screening testing (blood and urine tests) or when symptoms worsen (6). Patients with CRF must have conservative treatment, but if renal failure has progressed, dialysis (hemodialysis, HD, or peritoneal dialysis, PD) is necessary. Many problems can arise during HD treatment, and bacterial infections are dangerous for ESRD patients sustaining HD (7). The mortality of patients undergoing HD remains relatively high in spite of recent technological advancements (8).

The lymphocyte subpopulations are further separated into CD3+ T-cells and CD19+ B-cell. Several studies have indicated that B lymphocytes (B cells) may have a protective role (9). B lymphocytes (CD19+) regulate several immune responses and inflammatory processes (10). The observed immunological abnormalities in CKD patients include : Reduced antibody synthesis in B lymphocytes, the mechanisms underlying these abnormalities are still poorly understood (11). The most widespread causes of CRF are diabetes and hypertension(12) ; also hormonal issues are common among patients(13). Calcium is released into the blood by the bones as a result of the parathyroid hormone (PTH). It also stops calcium excretion from kidneys and increases calcium absorption in the intestine by activating vitamin D (VitD3) (14).

HD patients exhibit an abnormal immune response with a decreased response to some pathogens and vaccines, increased susceptibility to infections brought on by intracellular pathogens, increased tolerance to cutaneous grafts, and a higher incidence of tumors (15). Hence, the aims of the study are to identify the effect of CD19+ B- cells in HD patients compared to control group and their roles in increase of the mortality of patients with renal failure, as well as investigate the relationship between some essential parameters (PTH, vitamin D3) and B lymphocytes concentrations and its effect on increasing the disease state.

Material and methods

This research is done at Baghdad Teaching Hospital (Dialysis Unit-The Iraqi center for the Industrial. Total of 60 clinically diagnosed samples were collected from patients and 30 healthy volunteers, including both genders and ages ranging from between (17 -74) years old, during the period from /February 2022 to July 2022/. Patients undergoing renal transplantation, those with severe diseases, and those with no laboratory data were all excluded, as were those with autoimmune diseases. Regarding scientific research ethics, the study was approved by the "Helsinki declaration for Human subjects" research (World Medical Association) (Association, 2013), patients consent to participate in the study was taken.

Information was collected through a questionnaire designed for the present study, including name, age, gender, the initiation stage of hemodialysis, medical history, disease complications, medications, death cases and other illnesses like diabetes and hypertension. As well as knowing the important bacteria that is associated with hemodialysis. The study groups were spliced into two groups: hemodialysis patients included (60) patients with CKD within an age range between 17-74 years; while the control included (30) healthy control volunteers of both gender.

Five ml Blood samples were collected from patients as well as healthy controls. After that, the blood is transferred with a sterile gel tube. The blood in a gel tube was centrifuged for 5 minutes at 3000 rpm. Some samples were put in a gel tube and left to clot for approximately 30 minutes. Then, it was kept at -20єC in Eppendorf tubes until used in the analysis, the serum levels of CD19 was evaluated quantitatively using ELISA technique; ELISA tests had been performed according to the manufacture directions (USA / Mybiosource) .The remains of the serum were collected for some parameters (PTH ,vitamin D3) using E411 Cobas device/Swiss. Using an ELISA reader set to 450 nm, interpolation from the standard curve was used to determine the CD19+ concentration results.

The mean and standard error (SE) were determined using IBM SPSS version 28.0 (16), The likelihood was also investigated using the student T-test. The probability for non-parametric data was calculated using Pearson's chi-square test. Pearson's correlation was used to determine the

relationship between the studied parameters.

Results

The present work included 60 patients with CKD on hemodialysis and 30 healthy individuals. The mean age among patients was 49.57 ± 1.89 years, whereas the mean age of control group was 45.57 ± 2.66 years (p > 0.05), as shown in the figure1. According to the gender (60%) of them were males, and (40%) were female; the males were more frequent than females, but not significantly, as shown in figure 2.
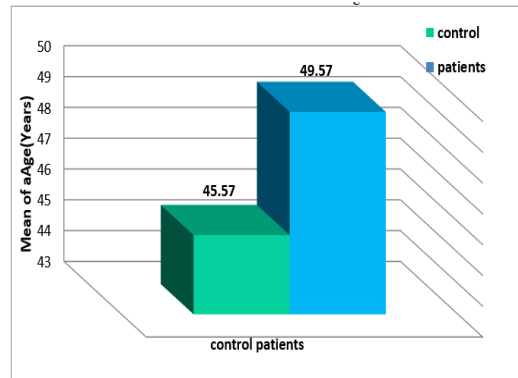
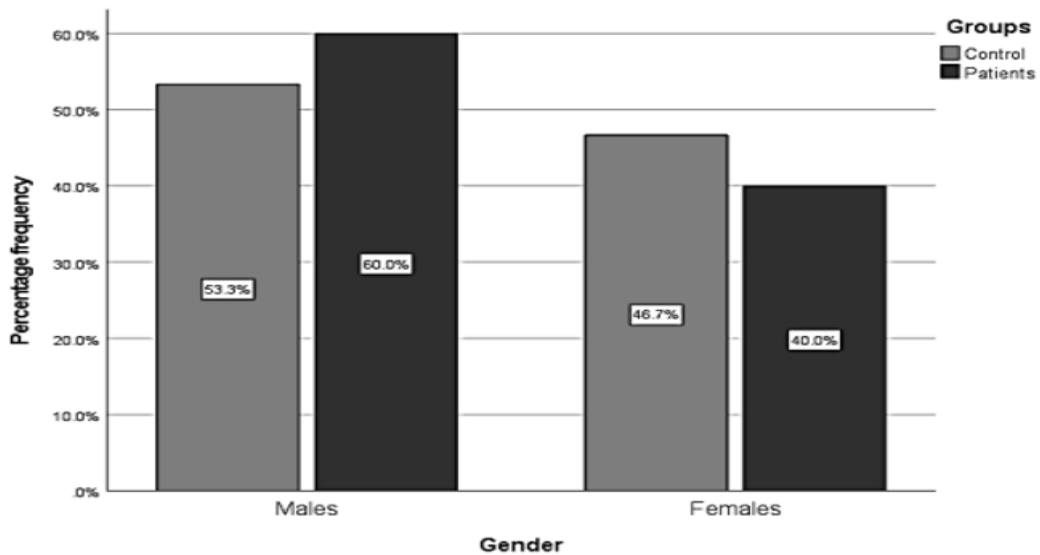**Figure 1: The distribution of study population according to the age**

Figure 2: The distribution of study population according to the gender

Assessment of impact B lymphocytes (CD19+) levels in HD patients

In Table 1 and figure 3, the results of the patients group, where the mean CD19 marker was (1.72 ± 0.10) pg/ml while in healthy individuals, it was (4.62 ± 0.19) pg/ml. The results showed significantly decreased in mean serum CD19 levels (P < 0.001) between patients and healthy volunteers.
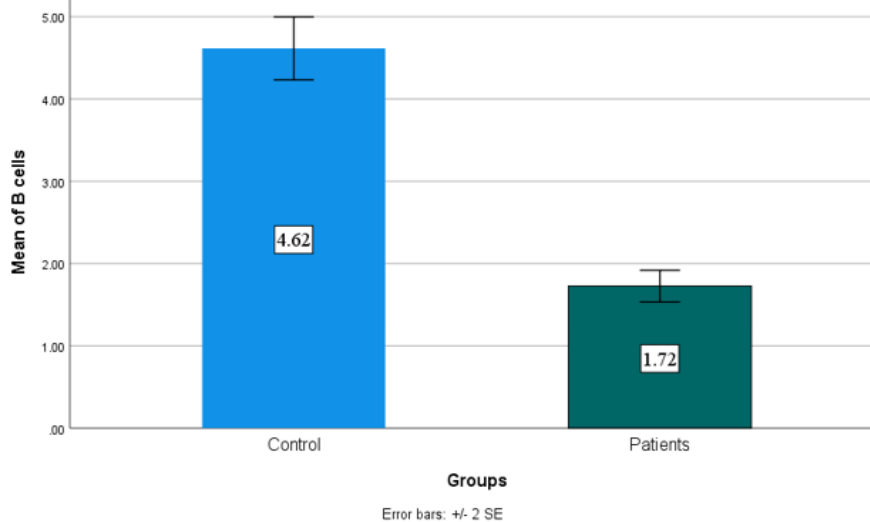
Error bars: +/- 2 SE

Figure 3: Compare between the studied groups according to CD19 B- cells

Table 1: Distribution of CD19+ B-cells levels between the studied groups

Groups	CD19+ mean ± SE (pg/ml)		Probability
	Patients	Control	
Total	1.72 ± 0.10	4.62 ± 0.19	P < 0.001

Parathyroid hormone and vitamin D3 in CKD patients undergoing HD

The current study included in Table 2 a comparison of parathyroid hormone levels with healthy individuals and patients undergoing HD, where the mean was (39.90 ± 2.65 g/dl ,426.71 ± 47.77 g/dl) respectively, the level was significantly increased (P < 0.001) in HD patients than in the healthy control, but the serum levels of vitamin D3 in patients were (14.95 ± 1.41) mg/dl, and in healthy control were (38.13 ± 3.25) mg/dl, the results showed significantly decreased in levels of D3 in the serum (P < 0.001) between patients and healthy group.

Table 2: Clinical parameters data for PTH and VitD3 between patients and control groups

Groups	PTH level mean ± SE (g/dl)		Probability
	Patients	Control	
Total	426.71 ± 47.77	39.90 ± 2.65	P < 0.001
Groups	Vitamin D3 level mean ± SE (mg/dl)		Probability
	Patients	Control	
Total	14.95 ± 1.41	38.13 ± 3.25	P < 0.001

The correlation between studied parameters in patients

There was a highly significant positive correlation between B-cells levels in serum and vitamin D3 level at the (0.01 level) as well as showed this study a highly significant negative correlation between B-cells and Para Thyroid hormone. Moreover these findings showed a significant negative correlation at the (0.05 level) between Vitamin D3 and PTH, as shown in Table 3.

Table 3: The correlations among studied parameters in HD groups

		Vitamin D3 level (mg/dl)	Para Thyroid hormone level (g/dl)	B cells
Vitamin D3 level (mg/dl)	Pearson Correlation	1	-0.214[*]	0.569[**]
	Sig. (2-tailed)		0.043	0.000
Para Thyroid hormone level (g/dl)	Pearson Correlation		1	-0.399[**]
	Sig. (2-tailed)			0.000
B cells	Pearson Correlation			1
	Sig. (2-tailed)			
*. Correlation is significant at the 0.05 level (2-tailed).				
**. Correlation is significant at the 0.01 level (2-tailed).				

Discussion

Chronic kidney disease (CKD) is a significant and growing health-care challenge(17) .In CKD patients, lymphocyte-mediated immune responses are known to be impaired(18). Several studies conducted over the last few decades have discovered abnormalities in various immune system components and function in HD patients (19).

The present results showed the incidence of HD patients was higher in males compared to the females and this results was in agreement with the study of Abou-Bakr et al who stated that males percentage were (62.7%) and females were (37.3%) (20). Sex has been linked to the evolution of kidney disease. In patients with CKD, regardless of blood pressure or plasma lipid levels, men developed renal insufficiency faster than women (21)Furthermore, sex hormones have significant effects on cellular apoptosis, which may influence renal disease progression(22).

There are many studies that indicated immunological parameters, including B lymphocytes, but the focus was on CKD patients at specific stages or at all stages not only HD patients while in our current study those undergoing HD and factors were aggravated the disease. The result revealed that the concentration of CD19 was significantly decreased in HD patients compare with the healthy control; this was in agreement with a

previous study that recorded that low CD19 B-cell counts can lead to higher all-cause and cardiovascular (CV) mortality in a group of HD patients(23). In another study, Lin et al. found that B cell counts were decreased in all stages of CKD, and reduced B lymphocyte numbers and antibody-producing capacity have been linked to CKD-related immune deficiency (24) .Immune deficiency reduces the effectiveness of vaccinations and raises the likelihood, severity, and unfavorable outcome of microbial infections. In patients with advanced CKD, these abnormalities account for a sizable portion of morbidity and mortality (25). Higher levels of B cells may be protective in CKD, according to a study(26). B-cell lymphopenia (Bcl-2) is prevalent in HD patients and is caused by increased apoptosis caused by decreased Bcl-2 expression and resistance to interleukin 7 (IL-7) and B-cell-activating factor of the Tumor Necrosis Factor (TNF) TNF family, all of which are required for B-cell differentiation and survival(11). The dialyzer membrane's biocompatibility causes a variety of negative changes in the peripheral blood during dialysis (18).

The current study demonstrated a significant increase in PTH levels among patients and controls. Moreover, there was a significant difference in serum vitamin D3 levels between patients and the control group. Also, these results revealed a significant negative correlation between Vitamin D3 and PTH, in a study that reported a higher significance in serum PTH levels in ESRD patients compared to controls, this result was consistent with the current research, and also revealed a decrease and a negative correlation, but it was not significant in the concentration of vitamin D3 and also its correlation with PTH and this does not agree with our results (27,28). Hyperparathyroidism is the overproduction of PTH, which results in excess calcium in the blood. Hyperparathyroidism can be primarily due to gland cell hyperplasia for unknown reasons or secondary to a vitamin D deficiency caused by other diseases (29).In ESRD patients, an increase in parathormone is induced by acidosis, calcitriol resistance, an increase in blood phosphorous, and a decrease in blood calcium due to a decrease in

1,25(OH)2D3 production (the active form of vitamin D)(30).Vitamin D has both direct and indirect effects on the parathyroid gland. For example, in chronic renal failure, the regulatory capacity of the vitamin D3 receptors in the parathyroid gland is reduced due to low levels of vitamin D or high levels of phosphate, resulting in vitamin resistance and loss of the reactive feeding mechanism for the reaction of the vitamin on the gland (31), by this direct mechanism the gene expression of PTH is stimulated, and then the secretion of PTH is increased(32). Since the kidneys are the main site of production of 1,25(OH)2D3, the production of vitamin D3 decreases in patients on hemodialysis (31).

The most probable mechanism for the effect of vitamin D receptor activators (VDRAs) is decreased PTH levels, but growing evidence suggests that vitamin D may have health benefits other than suppressing PTH secretion (33). Supplement usage is widespread among dialysis patients (34) because metabolic abnormalities in vitamin D status and PTH in hemodialysis patients are linked to higher mortality and a decline in quality of life (35). PTH has a more direct impact because its receptors are identified on B cells; this has been demonstrated in multiple clinical studies where PTH was discovered to impact various B-cell function aspects. The current study is the first to record the relationship between CD markers for B-cells and PTH, finding a highly significant negative correlation between the two. Researchers discovered that HD patients' B cells had decreased proliferative potential, but stimulation with S. aureus revealed both the intact molecule of PTH and its amino-terminal fragment (1-34). PTH caused dose-dependent inhibition of B-lymphocyte proliferation in normal subjects ,and this was a specific dose of PTH(36). We analyzed important factors to investigate the persistent decrease in CD19 and mortality in HD patients. The existing study has some limitations, including a small sample size and difficulty following the immune development of B-cells in HD patients, particularly the percentage of patients who die during a period and the influence of these cells. There is a need for studies on these results to know more about the mechanisms and methods of B cells

that stimulate disease progression.

Conclusion

The existing results reveal the levels of CD19 were a significantly decreased in HD patients compare with the healthy volunteers. And there is a significant increase in PTH levels among patients and controls. Also, there was a significant difference in serum vitamin D3 levels between patients and the healthy volunteers; moreover, there was a highly significant correlation between serum B-cells levels and D3, PTH levels We can conclude that CKD-associated immunodeficiency has been associated with a decrease in B cells and maybe a significant proportion of mortality with advanced conditions; therefore, we can support the immune system of the HD patients before deterioration. In future studies, monthly examination of CD19 immune biomarker correlations with laboratory parameters may improve outcomes and disease prediction, it is also necessary to verify the primary immune source that affects the disturbance of the natural system of the kidney, more studies are needed to shed light on antibody therapies.

Disclaimer: None

Conflict of interest: There is no conflict of interest to declare in this article.

Abbreviations

CRF Chronic renal failure
CKD chronic kidney disease
ESRD end-stage renal disease
HD hemodialysis
PD peritoneal dialysis
B-cells B lymphocytes
PTH parathyroid hormone
VitD3 vitamin D3
CD Cluster of Differentiation
SE standard error
CV cardiovascular
VDRAs vitamin D receptor activators
Bcl-2 B-cell Lymphoma 2

IL interleukin
TNF Tumor Necrosis Factor

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