Original Article

THE EFFECT OF HEMODIALYSIS SESSIONS ON VISFATIN, IL-6, TNF-ALPHA, VITAMIN-D AND SEVERAL BIOCHEMICAL PARAMETER IN PATIENTS WITH END STAG OF RENAL FAILURE IN RAMADI TEACHING HOSPITAL

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DOI: 10.47750/pnr.2023.14.S01.151

Abstract

Objectives: Adipokines, Cytokines and Biochemical parameters in patients' blood are very important indicator of the health status of patients with chronic kidney failure who undergoing dialysis.

Methods: In this study taken 600 blood samples from 100 patients, immediately in patients undergoing hemodialysis before and after three dialysis sessions, and then tested it for Visfatin, IL-6, TNF-alpha and Vitamin-D with 20 biochemical parameter.

Results: The results showed that the levels of serum interleukin-6, tumor necrosis factor-a, Visfatin, Cholesterol, TG, amylase and ALP, significantly increased in patients before and after three dialysis sessions compared to healthy group, correspondingly the level of direct Bilirubin recorded no significant difference before and after three dialysis sessions comparing with control group. On the other hand, the results indicated a significant decreased in serum Vitamin-D, albumin, HDL, Ca+2, Na+, AST, ALT and T.Bilirubin.Serum Urea, Creatinine, K+, Glucose and U.A, recorded a significant increase before dialysis while a significant decrease for S.TP compared with the control group. The results revealed the ability of dialysis membranes to change the levels of parameters after dialysis to 3 groups, first group showed a significant increase as S.LDH and second group showed a significanr decrease as S.UA, S.K+ and Cl-, finally the third group exhibited no significand difference comparing with control group as S.Urea, S.Creatinine and S.Glucose.The results demonstrate that there is a significant positive correlation between Interleukin-6, Tumor necrosis factor-a and Visfatin with each other, whereas a significant negative correlation between Vitamin-D with Interleukin-6, Tumor necrosis factor-a and Visfatin.

Conclusion: The study concluded that increased circulating serum Visfatin, Interleukin-6 and Tumor necrosis factor-a, dyslipidemia and electrolytes disturbances with Vitamin-D deficiency in CKD patients at 3-dialysis stage, probably promotes progression of renal disease and could contribute to accelerate the atherosclerosis and cardiovascular disease and may increase the morbidity and mortality in these groups of patients.

Keywords: CRF; CKD; Dialysis; ESRD; Hemodialysis; Cuperphan

Introduction

The urinary system composed of the renal, ureters, urinary bladder and urethra. The responsibility of kidneys are removing the waste products from blood and also maintaining pH balance and salt in the body. this vital work results forming the urine. Blood pressure regulation, red blood cell production, bone metabolism, and acid-base balance are further functions of the kidneys. All of these roles can be adversely affected by renal failure[1]. Chronic kidney disease (CKD) or also called chronic renal failure (CRF), is a progressive decrease of renal function that necessitates long-term treatment with renal replacement therapy. Hemodialysis is a type of kidney replacement therapy in which the body's waste products are eliminated, such as urea, creatinine and excess water[2].

In chronic renal disease and other chronic disorders, also the vitamin D deficiency (less than 30 ng /ml) is important and prevalent, associate, with adverse of outcomes, however, intervention research and randomized controlled trials, are still weak in this field. Because vitamin D levels vary seasonally due to sun exposure, serum levels of vitaminD should be evaluated on a regular basis. Nonetheless, nephrologists have long employed various forms of vitamin "D" in the treatment and prevention of hyperparathyroidism at renal failure. Selective active vitamin D metabolites, (paricalcitol and maxacalcitol), have been utilized to lower circulating hyperparathyroidism (PTH) with little changes in phosphate and calcium concentrations over the last two decades comparing with nonselective calcitriol[3]. Interleukin 6 (IL-6), is a pleiotropic cytokine that has a role in a variety of biological processes. The inflammatory response is orchestrated by a number of pro-inflammatory (TNF-alpha, interleukin-1, and interleukin-1) and anti-inflammatory (interleukin-10) cytokines, but the information at hand points to IL-6 and its soluble receptor sIL-6R, as the central regulators, of the inflammatory response. The IL-6 system stimulates inflammatory processes by stimulating lymphocyte activation and proliferation, B-cell differentiation, leukocyte recruitment, and liver synthesis of the acute phase protein response[4].

Adipose tissue is now recognized as an active endocrine organ that secretes adipokines, a type of inflammatory cytokine that interferes with insulin sensitivity, lipid and glucose metabolism, and also with inflammatory process. Adipokines play a role in renal and cardiovascular problems[5].

Visfatin, is a novel adipokine that is the recently identified as a ubiquitous adipokine first described by Fukuhara and colleagues in 2005[6]. The rise levels of Visfatin in patients with ESRD have been reported to significantly increase and be correlated with endothelial dysfunction. Thus, Visfatin may be associated with the progression of atherosclerosis and also found a close association between Visfatin and CVD[7].

End Stage Renal Disease (ESRD)

End-stage renal disease (ESRD), or what known as stage 5, is characterized by GFR values below 15 ml/min/1.73 m2, necessitating dialysis or kidney transplantation. Patients with ESRD are more likely to develop acute and chronic cardiovascular disease, other concomitant conditions, and pass away. In this stage, treatment modality is provided (hemodialysis or peritoneal dialysis) and patients are evaluated for transplantation[8].

Hemodialysis

When the kidneys are unable to eliminate waste and extra fluid from the blood, hemodialysis is used. Blood is extracted intravenously, after the invention of the Quinton–Scribner shunt (a tiny tube, usually made of synthetic material, used to connect an artery to a vein) permitted repeated access to the vascular, passed through a dialyzer, and then returned to the body via a blood artery. The blood is passed through a dialyzer membrane, which filters waste and fluid into a dialysate solution[9].

There are three types of hemodialysis methods:

a. Hemodialysis (HD) is the most popular clinical method for chronic or acute renal failure treatment. Diffusion

governs the removal of solutes and waste products, which in turn driven by the difference in concentrations of the solution between the blood and the side of the dialysate[10].

b. Hemofiltration (HF) is a method dependent on pure convective transport. Convection is dependent on solvent drag, where molecules are transferred through the membrane by bulk fluid flow regardless of size and molecular weight of the solutes compared to diffusive transfer. It will, therefore, lead to more effective removal of solutes/waste products of medium size[11].

c. Hemodiafiltration (HDF) is a method to overcome the disadvantages of HF and HD, which shows the limited capacity to remove small and big solvents, respectively. It is an extremely effective method based on concurrent diffusive and convective transport and needs to be inserted into the blood route, either before or after the dialyzer, as in HF (Lien, 2009). There are some types of dialysis machines and membrane, including[12]:

(Hemophane or polyamide dialyzers, Vitamin E-coated cuprophane (VE) dialyzer, Synthetic polyamide dialyzer, Regenerated cellulose membranes, and Biocompatible polymethylmethacrylate (PMMA) membrane)

In Iraq, hemodialysis represents the most popular method to treat end-stage renal disease (ESRD). Adequate and efficient hemodialysis (HD) increases the quality of life related to health and decreases morbidity and mortality of ESRD patients[13]. In January 2012, the total number of patients with ESRD in the regular hemodialysis in Iraq program was 2,445 patients across the country with a prevalence of 74 per million populations[14]. While By 2018, the total number of HD patients in Iraq was around 5,500 patients with a prevalence of about 193.8 patients per million[15].

Interleukin-6

Lymphocytes, epithelial cells, hepatocytes, and myeloid cells are just a handful of the target cells for IL-6, which was first discovered in 1986[16]. The IL-6 receptor (IL-6R) dimers and gp130, as well as a soluble form of the IL-6R, bind to IL-6. Classic signaling occurs when IL-6 binds to the IL-6R/gp130 dimer, resulting in an anti-inflammatory response. Whenever IL-6 interacts with the soluble IL-6R/gp130 dimer and triggers a pro-inflammatory response, this is known as trans-signalling[17]. Soluble IL-6R is produced via alternative splicing, proteolytic release of a membrane-bound IL-6R's ectodomain, or shedding, that can be induced by a variety of conditions such as cellular cholesterol depletion[16]. The members of the IL-6 cytokine family have a variety of helpful and pathogenic effects, which is why they are sometimes referred to as a "double-edged sword". Pathogenic consequences are frequently the result of signaling exceeding a key threshold. For example, when the degree of a renal damage grows, IL-6 expression rises, increasing the detrimental inflammatory response while also shielding the kidney against additional acute injury via its soluble receptor[18]. Therapies that target soluble IL-6R trans-signaling, such as the sgp130Fc protein, have been developed and may be useful in treating a variety of renal disorders,[19].

Tumor Necrosis Factor

TNF-a, a proinflammatory cytokine (17-k.D) originally linked with killing the tumor cells, also plays a key function in the regulation of both pro- and anti-inflammatory mediators. TNF- has been dubbed the "master regulator" of the cytokine cascade, since it provides a quick form of host defense against infection but may be lethal in high doses[20]. In uremia, deterioration of renal function may be one of the most important factors associated with a significant increase in TNF-a activity[21]. Indeed, correlations between renal function and TNF-a and its soluble receptors have been demonstrated in patients with varying degrees of renal failure[22]. Further, reduced renal function has been shown to affect TNF-a clearance in rats[23]. The importance of the renal in TNF-a handling is further emphasized by findings from previous studies, which showed that the Tamm-Horsfall glycoprotein may regulate TNF-a activity. However, because TNF-a has a short half-life and local tissue degradation may possibly contribute to cytokine inactivation, additional study is needed to clarify the kidney's proportional involvement in TNF clearance[24,25].

Visfatin

Visfatin is a recently identified adipocytokines and was revealed to be predominantly secreted by the visceral adipose tissue and to exert insulin-mimetic effects. As, its discovery in 2005, Visfatin has been a focus of extensive research to characterize its pathophysiological relevance to visceral obesity, insulin resistance, type 2 diabetes and cardiovascular disease[26]. Visfatin mimics the action of insulin, but the insulin-mimetic role of Visfatin was recently asked.Some studies on human subjects have led to incompatible results regarding the insulin mimetism of Visfatin.Circulating Visfatin is associated with HDL-cholesterol, and it seems that Visfatin could influence lipid metabolism through its role in the biosynthesis of NAD because the NAD precursor nicotinic acid is able to increase HDL-cholesterol significantly[26,27]. Recent studies, showed that prevalent levels of Visfatin at patients with CKD have been reported to significantly increase and be correlated with endothelial dysfunction. Thus, Visfatin may be associated with the progression of CKD[28]. These recent studies, also showed that plasma Visfatin levels were associated with infarct-related artery occlusion and the progression of atherosclerosis. Thus, there is association between Visfatin and CVD[29].

Cholecalciferol (Vitamin D3)

Vitamin D3 is the natural, which is created by the body from cholesterol (cholecalciferol). A small range of solar ultraviolet (UV) light (290–315 nm) converts 7-dehydrocholesterol to pre-vitamin D3, which is then isomerized to vitamin D3 via the body's temperature[30]. Renal dysfunction, which is frequently seen in patients with renal disorders, contributes to the vitamin D insufficiency that results in hypocalcemia and secondary hyperparathyroidism, both of which are known to put people at risk for secondary osteoporosis. It has been noted that renal illnesses with severe disease stages have higher levels of vitamin D insufficiency. Guidelines for the diagnosis and treatment of CKD recommend that the level of VitaminD be kept at (30 ng/ml) or above since it has a reverse association with the development of CKD[31].

Materials and Methods

Research design

This study used a cohort study descriptive correlational design. The data were collected from February 2021 to September 2021.

Sampling and setting

This study was carried out in School of Distance Education, Universiti Sains Malaysia, and was conducted at Ramadi Teaching Hospital, in Ramadi city, Iraq.

Measurements instruments

In this study, as in table 1. several biochemical tests, Visfatin, Vitamin D, IL-6 and TNF-α study were performed for patients for three dialysis seasons, and the control group were conducted. For comparing and standardization, Controls were 100 healthy persons. By using enzyme-linked immunosorbent immunoassay (ELISA) and with spectrophotometry for measurements all the parameters used in this study. The biochemical tests are Urea, Creatinine , Aspartate aminotransferase, Alanine aminotransferase, glucose, albumin, Lactate Dehydrogenase, Amylase, Cholesterol, High-density lipoprotein, Triglycerides, Uric acid, Direct-Bilirubin, Total-Bilirubin, Alkaline phosphatase, Total-protein, Calcium, Sodium, Potassium and Chloride in patients undergoing hemodialysis before and after three dialysis sessions.

Table 1 The chemicals were used and their companies supplied

N	Chemicals	Supplier
1	(Visfatin) hormone ELISA kit	Cusabio, China
2	(Vitamin D3) hormone ELISA kit	Cusabio, China
3	(TNF-α) hormone ELISA kit	Cusabio, China
4	(Interleukin 6) hormone ELISA kit	Cusabio, China
5	(Glucose, Urea, Calcium, AST, ALT, TP) kit	BioMerieux (France)
6	(Creatinine, albumin, LDH, Amylase, Cholesterol, HDL, TG, UA, direct Bilirubin. Total Bilirubin) kits	Biolabo , France
7	(Sodium, Potassium, Chloride) kits	Spinreact, Spain

Data collection

This segment included selection 100 patients with CRF from the dialysis unit at Ramadi Teaching Hospital as a case study between (34 - 68) years old with an average age of (48.86 ± 10.21) who were clinically diagnosing by a nephrologist as chronic kidney failure in the fifth stage (end stage renal disease), depending on patient history, a clinical diagnosis, kidney function testing, and other laboratory testing. The standard four-hour hemodialysis sessions were performed three times a week with Cuprophan membranes (CU; mean membrane surface, 1.3 m2, thickness, 8 μ; sterilization, ethylene oxide; filters manufactured by Bellco, Mirandola, Italy).

Statistical analysis

Data were coded and analyzed using SPSS version 22.0 for win- dows (SPSS Inc., Chicago, IL, USA). Descriptive statistics methods was used to determine the mean and standard deviation for numeric variables (variables measured in the study and the ages of the patients and the control group). On-way ANOVA table was using to compare the results of various all parameters of patients with the control. Where the study groups were divided into six sections, this division was done for each test. Correlation coefficient (r) was calculated to identify the relationships between different parameters, using Pearson's correlation model. Sample Size Calculator.

Results

The data concerning with Chronic Renal Failure in patients on hemodialysis and control group shown in table 2. at order a clarify the role of hemodialysis, where the results of this study showed a significant increase in the levels of Visfatin, interleukin-6 and tumor necrosis factor-alpha, with a significant decrease in vitamin D levels for three dialysis sessions. This study didn't record the effect of Cuperphan membranes used in hemodialysis filtration on interleukin-6, Visfatin, tumor necrosis factor-alpha and vitamin D as shown in Fig 1. This research recorded a significant decrease for (S.TP) and a significant increase for (Urea, Creatinine, K+, Glucose and U.A) before the three dialysis sessions. This study showed the effect of Cuperphan membranes in reducing (S.Urea, S.Creatinine, S.Glucose, S.UA, S.K+ and Cl-) levels after hemodialysis for three sessions, while the results showed a significant increase in (S.LDH) levels after hemodialysis for three hemodialysis sessions. There was no significant difference in (S.Bilirubin direct) levels before and after three dialysis sessions

compared with the control group.

Table 2 The Results of this research

Parameters	Control group	Dialysis 1		Dialysis 2		Dialysis 3	
		Pre	Post	Pre	Post	Pre	Post
Visfatin	3.95±1	12±2.2	11.9±2.3	11.9±2.3	11.3±2	11.6±2.6	10.9± 1.7
IL-6	38.8±3	65±10	63.6±10.8	63.3±11	62.3±10.8	65.4±15.4	62.8± 13.1
TNF-α	43.8±6.3	160.7±14.8	159.8.±30	167±24	161.7±24	161±36	160±20
Vit. D3	41±14	29±11	28±11	27 ± 10	27 ± 9.9	26 ± 9	25 ± 8
Urea	34 ± 6	116 ± 29	21 ± 7	118± 27	18.8±5.2	116 ±26.6	18.6 ± 4.6
Creatinine	0.8 ± 0.12	7.1 ± 2.5	1.1 ± 0.19	7 ± 1.2	1.1 ± 0.2	7.2 ± 1.6	1 ± 0.2
Cholesterol	129 ± 44	174 ± 36	179± 21	176 ± 9.5	175 ± 27	175 ± 16	171 ± 24
Tg	100 ± 26	183 ± 20	188 ± 19	188 ± 14	180 ± 26	178 ±25	177 ± 29
HDL	46 ± 4.1	38.7 ± 8.3	38 ± 5.2	37 ± 8	36 ± 6.7	38.9 ± 9.9	36 ± 8
Ca+2	8.93±0.3	8.19±0.46	8.24±0.44	8.1±0.5	8.19±0.42	8.1±0.5	8.2±0.48
Na-	140±2.8	132±2	132±2.5	134±2.6	134±2.5	134±1.88	134±1.81
K+	4.1±0.3	5.4±0.3	2.3±0.4	5.3±0.17	2.1 ± 0.3	5.4±0.24	2.0±0.08
Cl-	101±2.9	101±3.3	96±3.3	101± 3.0	98±4	101±3.7	98±5
Glucose	91±11	107±13	91±8	106±19	92±11	106±12	91±6
Bili- direct	0.2±0.08	0.21±0.07	0.21±0.09	0.2±0.08	0.2±0.08	0.21±0.08	0.2±0.08
Bili-Total	0.7 ± 0.2	0.45±0.12	0.47±0.11	0.46±0.11	0.45±0.12	0.46±0.12	0.45±0.12
AlkP	114±38	176±33	184±48	187±47	189±51	183±38	189±36
ALT	29.7± 6.6	20.34± 3	20.9± 5.7	21.18± 5.5	21.3 ± 5.4	20.8± 5.6	21.5± 5.4
AST	25.7±5.9	22±3.7	22.5±3.4	21.8±4.1	22.32±5	23±3.4	22.12±3.9
Albumin	4.3± 0.48	3.5 ± 0.25	3.5 ± 0.14	3.6 ± 0.33	3.6 ± 0.22	3.5 ± 0.24	3.6 ± 0.19
UA	4.4 ± 0.8	6.7±1.1	1.6±0.7	6.5± 1	1.4±0.4	6.5±0.94	1.4±0.4
LDH	185±35	190±28	212±29	187±29	203±34	186±40	208±43
TP	7.32±0.5	6.88±0.63	7.29±0.8	6.93±0.63	7.31±0.70	6.9±0.45	7.3±0.65
Amy	81.8±22	109±33	115±38	109±32	112±35	109±21	114±32

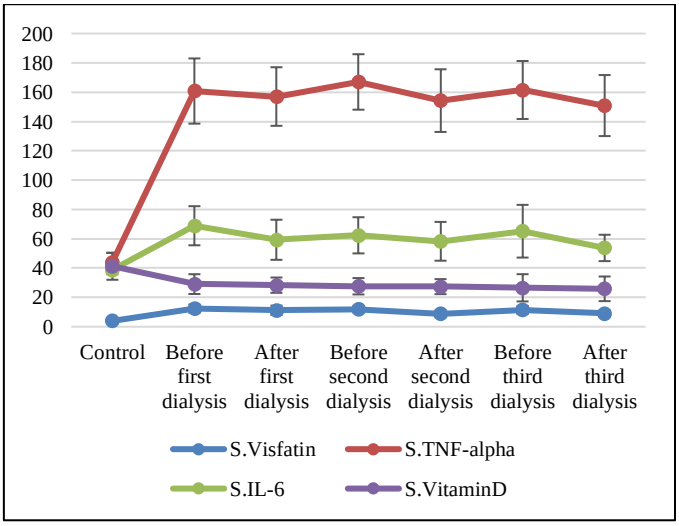
* Significant $p \leq 0.05$, $p > 0.05$ non-significant

In this correlation study as in table 3. revealed a significant positive correlation of Visfatin exhibited a significant positive correlation of S.Visfatin with IL-6, TNF-alpha, S.Glucose, B.Urea, S.Creatinine, Cholesterol

and Amylase, while it showed a significant negative correlation of S.Visfatin with VitaminD, HDL, Calcium, Sodium, Total-Bilirubin, Direct-Bilirubin, Aspartate aminotransferase, AlbG, Uric acid, Lactate Dehydrogenase and total-protein. This study also revealed, a non-significant correlation of S.Visfatin with T.G, K+, Cl-, Alkaline phosphatase and Alanine aminotransferase.

Furthermore the correlation study for IL-6 exhibited a significant positive correlation of IL-6 with S.Visfatin, TNF-alpha, S.Glucose, B.Urea, S.Creatinine and Amylase, while it showed a significant negative correlation of IL-6 with VitaminD, HDL, Calcium, Sodium, Chloride, T.Bilil, D.Bili, AlkP, ALT, AST, AlbG, UA, LDH and TP.

Figure 1. Interleukin-6, Visfatin, tumor necrosis factor-alpha and vitamin D for pre and post 3 dialysis sessions.



This study also revealed, a non-significant correlation of IL-6 with Cholesterol, T.G and K+.

In the same fashion for over three hemodialysis stages has been study of the relationships between concentration of serum TNF-α and Vitamin D with other clinical variables and biochemical parameters in patients with ESRD.

Where the correlation study for TNF-α revealed a significant positive correlation of TNF-α with S.Visfatin, IL-6, S.Glucose, B.Urea, S.Creatinine, Cholesterol and Amylase, while it showed a significant negative correlation of TNF-α with VitaminD, T.G, Ca+2, Na+, T.Bilil, D.Bili, AlbG and TP. This study alsos howed a non-significant correlation of TNF-α with HDL, K-, Cl-, AlkP, ALT, AST, UA and LDH.

Table 3 Correlation of serum TNF-alpha Visfatin, IL-6 and Vitamin D levels with each other and biochemical parameters in patients under hemodialysis.

Correlations								
Parameters	**TNF**		**Visfatin**		**Interleukin 6**		**Vitamin D**	
	r	***P value***	***r***	***P value***	***r***	***Pvalue***	***r***	***P value***
S.Visfatin	0.58**	0.001			0.47**	0.001	-0.24**	0.001
IL-6	0.56**	0.001	0.42**	0.001			-0.18**	0.01
TNF-alpha			0.58**	0.001	0.56**	0.001	-0.28**	0.001
Vitamin D	-0.28**	0.001	-0.24**	0.001	-0.18**	0.01		
Glucose	0.27**	0.001	0.24**	0.001	0.24**	0.001	-0.08	0.14

Urea	0.37**	0.001	0.43**	0.001	0.34**	0.001	-0.06	0.23
Creatinine	0.45**	0.001	0.46**	0.001	0.42**	0.001	-0.05	0.25
Chole.	0.14*	0.03	0.13*	0.04	0.07	0.17	0.15*	0.02
Trigl.	-0.13*	0.03	0.00	0.48	0.08	0.15	0.00	0.49
HDL	0.04	0.31	-0.14*	0.03	0.19**	0.001	0.07	0.2
Ca+2	-0.34**	0.001	-0.29**	0.001	-0.13*	0.04	0.61**	0.001
Na+	-0.46**	0.001	-0.37**	0.001	-0.33**	0.001	0.03	0.35
K+	-0.01	0.43	0.09	0.12	0.12	0.06	0.25**	0.001
Cl-	0.01	0.44	0.04	0.32	-0.08	0.14	-0.22**	0.001
BilD	-0.17*	0.01	-0.16*	0.02	-0.19**	0.01	-0.2**	0.001
BiliT	-0.27**	0.001	-0.22**	0.001	-0.25**	0.001	-0.07	0.17
AlkP	0.09	0.11	0.10	0.1	-0.12	0.06	-0.25**	0.001
ALT	0.09	0.12	0.04	0.31	-0.16*	0.02	-0.09	0.11
AST	-0.06	0.23	-0.01	0.43	-0.18**	0.01	-0.07	0.17
AlbG	-0.32**	0.001	-0.28**	0.001	-0.29**	0.001	0.491**	0.001
Uric acid	-0.12	0.06	-0.03	0.34	-0.03	0.36	0.18**	0.01
LDH	-0.02	0.41	-0.10	0.1	-0.03	0.33	0.12	0.06
TP	-0.15*	0.02	-0.21**	0.001	-0.2**	0.001	0.3**	0.001
Amy	0.26**	0.001	0.038	0.11	0.2**	0.001	0.13	0.12

**. Correlation is significant at the 0.01 level (1-tailed).*. Correlation is significant at the 0.05 level (1-tailed).

As well as the correlation results for Vitamin D where revealed a significant positive correlation of Vitamin D with Cholesterol, Calcium, K-, AlbG, UA, TP and Amylase, while it showed a significant negative correlation of Vitamin D with S.Visfatin, IL-6, TNF-alpha, Cl-, D.Bili and AlkP. This study also showed a non-significant correlation of Vitamin D with S.Glucose, B.Urea, S.Creatinine, T.G, HDL, Na+, T.Bilil, ALT, AST and LDH.

Discussion

The results describe here, in this observational study of patients with end session of renal failure undergoing hemodialysis, the prevalence of abnormalities of vitamin D and Ca.

On the other hand, inadequate dietary intake, vitamin loss into dialysate, and altered metabolism in uremia may lead to vitamin deficiencies in dialysis patients. Serum levels of certain vitamins have been reported to be low in dialysis patients[32,33]. The results of this study disagreement with mentioned above that vitamins loss during hemodialysis, as the outcomes of this study inducted that no significant change in vitamin D levels between pre and post dialysis for three sessions of hemodialysis that mean no effect to dialysis membranes for decrease of vitamin D levels in patients with ESRD. Data presented here also indicate that ESRD patients retain capacity for 1α-hydroxylase activity, where the results of this study showed the potential for synthesis of vitamin D in ESRD patients in response to cholecalciferol supplementation but not within the normal reference range (41.28±9.24).The results of this study showed that high levels of cytokines (IL-6 and TNF-a) and Visfatin not significantly affect the liver to produce vitamin D in patients with ESRD. Therefore, according to the results of this study and another studies which showed a significant correlation between decrease the level of vitamin D and increase the levels of Visfatin, IL-6 and TNF-a[34,35], it is suggested to give oral cholecalciferol to patients for reduce the level of of Visfatin, IL-6 and TNF-a and to treat vitamin D deficiency.

In this study markedly elevated circulating IL-6 levels are found in ESRD patients, this significant increase of IL-6 concentrations was elevate generally in hemodialysed patients with CRF whether before or after dialysis compared with normal healthy individuals (control group). In this study the increased in IL-6 in CKD patients may be due to impaired removal of cytokines, and increased synthesis due to various infectious processes , co-morbid conditions such as coronary heart disease[36], chronic heart failure, increased body fat mass, as well as other as yet unknown factors[37]. Probably intermittent stimulation by endotoxins originating from the dialysis

water supply and artificial vein grafts or bioincompatibility may play a role. The latter might activate an inflammatory process in chronic hemodialysis patients, but the available data are inconsistent. Although some studies found an inflammatory response during hemodialysis with bioincompatible membranes[38], others did not detect any differences in acute phase reactants during treatment with biocompatible or bioincompatible membranes[12]. In this study, the patients were treated with regenerated cellulose (cuprophan) natural membranes which is considered less biocompatible than modern synthetic membranes.

As shown above, this study recorded high levels of TNF-α concentration in the patients undergoing hemodialysis. the results showed consecutive dialysis of patients in the end session of renal failure had no significant effect on the levels of TNF-α. Renal function decline may be one of the most prominent variables contributing to a marked rise in TNF-a activity in CKD[39]. In fact, in patients with chronic renal failure, connections between renal function and TNF-a and its soluble receptors have been shown [40].

Dialysate contaminants of microbial origin, such as endotoxin (the main component of the outer membrane of the cell wall of Gram-negative bacteria), can unequivocally induce IL-6 and TNF synthesis and secretion. Endotoxin monomers, low molecular weight substances derived from dialysate-borne bacteria, can permeate across dialysis membranes and stimulate cytokine production by monocytes[41].

A previous study revealed that the Tamm-Horsfall glycoprotein may control TNF-a activity, further emphasizes the significance of the kidney in TNF-a management. TNF-a levels in dialysis patients have also been linked to surrogate markers of malnutrition and anorexia[42].

The present study, based on a large series of 100 patients, provides strong evidence that significant TNF-a plasma activity is detectable in long-term HD patients before and after hemodialysis.

Since circulating IL-6 and/or TNF-a are present in nondialyzed and long-term HD patients regardless of the type of the membrane equipping the dialyzer, and given the above-mentioned in vitro studies, and according to the results of this study, it was conclude that the type of membrane does not exert a pivotal role on the presence of these circulating cytokines, because no significant differences in TNF-a and IL-6 levels before and after dialysis were found. Also, TNF-a and IL-6 levels remained high even after three days after dialysis.

In this present study, it was found a prevalence of high levels of serum Visfatin in CKD patient with end session renal disease on HD. The concentrations of Visfatin were significantly elevated generally in hemodialysed patients with CRF whether before or after dialysis compared with normal healthy individuals (control).These results These results were in agreement with the results of other previous and recent studies done in other countries[43,44]. However, these results are in disagreement with the results of other study [45] which found decreased serum Visfatin among ESRD patients treated by HD. The explanation that elevated Visfatin in CKD patients may be due to renal failure and/or inflammation. Chronic renal failure has been associated with impaired immunity and subclinical inflammation involving Visfatin and other inflammatory adipocytokines derived from adipose tissue. Deteriorating renal function may increase overall inflammatory responses because of the decreased renal clearance of factors that are directly or indirectly involved in inflammation[46]. Another study suggested that Visfatin is localized to foam cell macrophages within unstable atherosclerotic lesions, which might play a role in plaque destabilization[47]. Since Visfatin displays potential pro-inflammatory action and has been found to be expressed in human atherosclerotic plaques, this adipocytokine …may directly participate in the development of atherosclerosis in CKD patients. Therefore, this study demonstrated a positive correlation between IL-6, TNF-a and Visfatin levels, and the previous and recent studies revealed a positive correlation between coronary lesion severity score and plasma Visfatin level[48].

Conclusions

From this present study, we can conclude that:

Both serum Adipokine (Visfatin) Cytokines (IL-6 and TNF-alpha) are significantly elevated in chronic kidney diseases patients and they are independent risk factors for the development of atherosclerosis and hence for

cardiovascular disease.

Significant decrease in vitamin D levels in end-stage renal failure patients undergoing dialysis and this decrease significantly affected the gastrointestinal absorption of calcium in patients.

The dialysis membranes of the type (Cuprophan) had no significant effect on the levels of Visfatin, IL-6, TNF-alpha, and vitamin D in dialysis patients for three sessions. Measurements of s.Visfatin, IL-6, TNF-alpha, and vitamin D levels have much greater values in the diagnosis, prognosis of nephropathy and cardiovascular diseases, and they may have therapeutic value of providing new targets for the treatment of such diseases. Serum IL-6, TNF-alpha and Visfatin are significantly correlated with each other's, in addition to their association with dyslipidemia, electrolyte imbalance, malnutrition or hypoproteins, direct and total bilirubin problems, high amylase level, hyperglycemia, and others. The use of IL-6, TNF-alpha and Visfatin may help in the identification of higher risk individuals for diabetes and cardiovascular disease with a better comprehension about the complex intercorrelation between, glucose and lipid metabolism and vascular disease. CKD, even if treated with hemodialysis, is an independent risk factor for the development of atherosclerosis and hence for cardiovascular diseases.

Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

The authors have no conflicts of interest to declare.

Ethical approval

This study approved by the ethical committee of University of Anbar in Ramadi city on 8th July 2021, (Approval no.104).

Author contribution

The authors confirm that all persons designated as au- thors qualify for authorship and have verified the article for plagiarism. If plagiarism is detected, all authors will be held equally responsible and will bear the resulting sanctions imposed by the journal thereafter. MRA, MTCO, and ZZ, participated in the study's design. MRA collected the samples from patients and then performed all the immunological and biochemical parameters and analysis interpretations were done. MTCO conducted the manuscript preparation and participated in the data editing. ZZ performed the statistical analysis and substantively revised the manuscript. The authors read and approved the final manuscript.

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