

Comparison of Cholesterol and Vitamin D₃ Levels in Patients with End-Stage Renal Failure in Khartoum, Sudan

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Abstract

End-stage renal disease is associated with changes in, cholesterol and vitamin D₃ through therapeutic nutrition. The aim is to determine the level cholesterol and vit. D₃ in the blood serum in Sudanese patients with ESRD pre and post dialysis interims of nutrition and relation with renal failure. A cross-sectional included patients with ESRD of patients' renal failure on regular hemodialysis continually in three hospitals in the Khartoum area and Omdurman collected sampling the National Laboratory of Public Health (Stack) as well as the University Hospital of Rabat and medical arms in Omdurman by taking a sample of 31 patients pre and post hemodialysis and then worked laboratory analysis of these samples. Included search 31 patients (16 males, 15 females) with age ranged (mean was 47.05 years ± 32.05) and findings from this study indicate an increase in. Cholesterol means it showed a big change post dialysis (130.3 mg/dl) compared with that pre dialysis (124.13 mg/dl), as well as Vit. D₃ study mean which showed a significant increase markedly post dialysis (6.184 microgram/ml) compared with pre dialysis (4.977 microgram/ml). The study also showed that there is no obvious effect the age of the patients and the level of Cholesterol and vitamin D₃, but this study demonstrated that there is a clear impact of the gender on the level of patients variables.

Keywords: Cholesterol; Vitamin D₃; Patients; End-stage; Renal Failure; Khartoum; Sudan

Introduction

Kidney diseases are a serious and prevalent health problem and manifestation include changes in renal detoxification capacity, de-regulation of salt and water balance and altered endocrine functions. Overall, it affects significantly to the patient's short and long-term prognosis and his chances of survival. The path physiological spectrum of kidney diseases is broad. Chronic kidney disease characterized by a low glomerular filtration rate (GFR), is a steady loss of renal function over a period of time. The causes are manifold ranging from diabetes, hypertension and chronic glomerulonephritis to tubulointerstitial fibrosis. According to the Kidney Disease Outcomes Quality Initiative (K/DOQI), five stages of CKD exist. Stage-one is characterized by normal GFR values but with kidney damage, whereas stage-five at the other end of the spectrum with a GFR < 15 ml/min/1.37 m² finally leads to end-stage renal disease with the treatment options of dialysis or transplantation [1].

Healthy kidneys activate vitamin D. The activated form of vitamin D is called calcitriol which helps the body absorb calcium. Working together, calcitriol helps maintain normal parathyroid hormone (PTH) levels and they carefully balance calcium in the body system. When kidneys fail, they stop converting inactive vitamin D to calcitriol. The result is a body is unable to absorb calcium from food, so it "borrows"

the calcium it needs from the greatest calcium storage depot the bones [2]. vitamin D and parathyroid hormone and their interaction with the kidneys both of them a part in controlling the level of vitD₃ in the bloodstream (Jacobs., *et al.* 1974). The kidneys also turn vitamin D into an active hormone (calcitriol), which helps increase calcium absorption from the intestines into the blood.

The design of the health care system in Sudan is based on primary health care and is conceived as a decentralize health care system able to integrate, at district level, the existing vertical programmers, including fully developed but not yet universally applied. At village level, primary health care units represent the level of contact between the community and the health services. Secondary health care is available in small towns through rural hospitals and urban health centers. Tertiary care services provincial, regional, university and specialist hospitals (Yamamoto., *et al.* 1989). Later in the same year haemodialysis using a Keil machine was started is home dialysis for a non Sudanese patient. After his death the machine was donated to Khartoum Civil hospital to make the start of haemodialysis in Sudan. In 1975 the second renal unit was opened in Soba University Hospital with two peritoneal dialysis and two Ready recirculation haemodialysis machines [3]. The shortfall in the provision of renal services in Sudan although well recognized has not been highlighted in a well documented epidemiological studies and the incidence of end-stage renal failure is not known but is estimated to be very high (in excess of 300 per million population) due to the high prevalence of communicable disease and the unrecognized rise in non-communicable diseases (diabetes and hypertension etc). End-stage renal disease (ESRD) is associated with changes in bone minerals and parathyroid hormone (PTH).

Objectives of the Study

The main goal of this study is to assess the level of cholesterol and vitamin D₃ in Sudanese patients with ESRD and its relation to hemodialysis. In terms of nutrition.

General objectives

Effect renal failure on cholesterol and vitamin D₃ pre and post hemo dialysis.

Specific objectives

- Restrict the level of cholesterol and vitamin D₃.
- Determine the type of food required for the patient especially the cholesterol and modified diets for therapeutic care.
- Reduce the deterioration of the health condition of the patient.
- Assess food needs of the patient and the factors affecting them such as medicines and others.

Materials and Methods

Our study was conducted in Khartoum state, Omdurman province in Omdurman medical military hospital and Rabat Hospital (Haemodialysis Center) and other private centers.

Materials

Cholesterol oxidize' enzyme, Cholesterol esterase enzyme and Hydrogen oxidase was used. Acetonitrile (PH 5.19), methanol (PH 4.7; 95.5%) and methanol with 0.1% formic acid (PH 3.0)/water with 0.1% formic acid (PH 2.83; 95:5%) 2-propanl ratio 80:20 (v/v) 1 ml hexane, are also used.

Devices

Atomic absorptions, full automatic analyzer selecta (E) and HPLC was used.

Sample collection (Methods)

Blood sample (5 ml) were obtained pre and post hemodialysis from a patients with chronic renal failure the serum were kept frozen at 20 until analyses Serum was analyzed for cholesterol and vitamin D₃.

Patients

Thirty one patients with chronic renal failure (CRF) who commend regular hemodialysis treatment participated in this study and randomly selected and based on questionnaire and examination of many biochemical changes was done. The males 16 and females' 15 patients varied in age from 16 to > 75 years. The levels of each class of studied character (cholesterol and vitamin D₃ were measured for each class of age (15 - 25, 26 - 35, 36 - 45, 46 - 55, 56 - 65 and > 75 years) so as to determine the effect of age on these characters and completely randomized design according to previous studies. All patients underwent dialysis three time weekly four hours per session the study included also comparison with pre and post in all test which is reported of previous for patients with end stage renal failure.

Principle

Cholesterol esters are hydrolyzed by cholesterol esterase enzyme to free cholesterol and free fatty acids. free cholesterol is oxidized by cholesterol oxidase enzyme to form cholesta - 4-on 3 one and hydrogen peroxide the hydrogen peroxide is reduced by hydrogen oxidase in enzyme to water and oxygen that is received by oxygen acceptor (4-aminophenzone) (4 AA) and in the presence of phenol - as indicator - quinone monoimimino red is formed and it is measured at 250 nm green filter [4].

Cholesterol esterase

Cholesterol ester + H₂O → free Cholesterol + FFA.

Cholesterol oxidase

Cholesterol + O₂ → Cholesa -4-en-3-one + H₂O₂

Hydrogen peroxides

H₂O → H₂O + O₂

Phenol

O₂ + 4AA → H₂O + Quinone imine red (That absorbs at 250 nm).

Samples: Serum or plasma collected by standard procedures.

Hazardous materials: This procedure uses phenol, which is caustic. Do not swallow and avoid contact with skin and mucous membranes.

Limitations: Haemolysis and lipaemia cause elevated Cholesterol Levels. Do not report results from specimens with suspected interference. Inform the requesting physician problem.

Determination of vitamin D₃ by HPLC (Ultimate 3000, Thermo fischer scientific, USA 2010).

Standard preparation: A stock solution mixture of Vit.D₃ standard was prepared (1 mg mL⁻¹) in methanol and stored at -20°C 3 working solution mixture was prepared by diluting the stock solution in acetonitrile [5].

Vitamin D extraction: Vitamin D extraction was done by slight modification in the method of to 0.5 ml of sample/matrix, we added 350 ul of methanol and 2-propanl in the ratio of 80:20 (v/v) the contents were mixer for 30s Vitamin D was extracted by mixing two times (60s each time) with 1 ml of hexane, the phases were separated by centrifugation and the upper organic phase was transferred to a conical tube and dried under nitrogen, the residue was dissolved in appropriate volume of mobile phase.

Concentration sample = (Peak height sample X concentration of the calibrator)/(peak height calibrator)

Statistical methods

Descriptive baseline characteristics were summarized as frequencies mean values standard deviation (as mean ± SD) we compared (the cholesterol and VitD₃) between patients with end stage renal disease (ESRD) under hemodialysis pre and post interims of nutrition for the analysis of the parametric variable we used the student T- test for comparison between the different parameters. Statistical analyses were performed by using SPSS and Result was considered statistically significantly for (p-value < 0.05).

Results and Discussion

Distributions of patients

The age of patient under study group ranges from 16 - 95 years and divided to different age groups (16 - 25, 26 - 35, 36 - 45, 46 - 55, 56 - 65 and > 75) years. Table 1 showed the distribution of age groups in studied patients. The age group 36 - 45 was the most prevalent while the age group greater than 75 was the least prevalent. The present study was done to assess cholesterol and vit. D₃ in Sudanese patients with end-stage renal disease pre and post hemodialysis interims of nutrition and relationship with renal failure.

Variable	Age group	N	Mean	Std. Dev	T- test	p- value
Cholesterol (post)	16 - 25	4	136.75	40.442	.449	0.81**
	26 - 35	6	129.00	8.462		
	36 - 45	11	135.27	38.764		
	46 - 55	2	103.00	4.243		
	56 - 65	6	128.17	16.774		
	66 - 75	2	129.00	14.142		
	Total	31	130.39	28.217		

Table 1: Distribution of male and female patients according to age.

The age group 16 - 25 and 66 - 75 was no a significant difference even though there is difference at pre and post dialysis higher means of cholesterol (132.72 and 130.00 mg/dl) following by age group 36 - 45 and 26 - 35 (124.64 and 124.00 mg/dl) respectively and 56 - 65 (123.83 mg/dl) table 3 and eventually 46 - 55 which recorded (99.50 mg/dl). Table 2 the age group 16 - 25 recorded higher mean at post (136.75 mg/dl), 36 - 45, (135.27 mg/dl), 46 - 55, recorded lower mean (103 mg/dl).

Age group	Male	Female	Total
16 - 25	2	2	4
26 - 35	3	3	6
36 - 45	4	7	11
46 - 55	1	1	2
56 - 65	5	1	6
> 75	1	1	2
Total	16	15	31

Table 2: Cholesterol Levels at pre dialysis according to patient's age.

*Significant different at the 0.05 level.

** Not Significant different at the 0.05 level.

Variable	Age group	N	Mean	Std. Dev	T- test	p- value
Cholesterol (pre)	16 - 25	4	132.75	41.404	1.331	0.28**
	26 - 35	6	124.00	12.570		
	36 - 45	11	124.64	31.219		
	46 - 55	2	99.50	6.364		
	56 - 65	6	123.83	18.005		
	66 - 75	2	130.00	14.142		
	Total	31	124.13	25.248		

Table 3: Cholesterol Levels at post dialysis according to patient's age.

*Significant different at the 0.05 level.

** Not Significant different at the 0.05 level.

Normal range cholesterol

Total cholesterol: Less than 200 mg/dl

Border line: 200 - 240 mg/dl

High risk: More than 240 mg/dl.

Cholesterol has important role like vit.D₃ manufacturing, bile acid, Steroid hormones manufacturing such as estrogens androgen and progesterone. Cholesterol is very necessary for body, even though, its non-essential nutrition because the body able to manufacturing Cholesterol at pre and post dialysis less than that. (200 mg/dl) due to all patients has them problems in livers and also has them deficiency vit.D₃ because vit.D₃ from fat soluble vitamin [6].

Table 5 showed that VitD₃ at pre was no significant difference higher at age group 66 - 75 (6.433 microgram/ml) and respectively. The age group 56 - 65 (6.433 microgram/ml) while the age group 26 - 35 recorded (5.183 microgram/ml) and the age group 16 - 25 and 36 - 45 (4.475 microgram/ml and 4.745 microgram/ml) but the age group 46 - 55 less than all age group. Table 5 showed that vit.D₃ at post dialysis higher mean in age 56 - 65 (7.083 microgram/ml), 16 - 25, (6.800 microgram/ml) and the age group 46 - 55 recorded decrease (1.700 microgram/ml).

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Variable	Age group	N	Mean	Std. Dev	T- test	p- value
Vit D ₃ (Pre)	16 - 25	4	4.475	3.6564	1.331	0.28**
	26 - 35	6	5.183	3.5835		
	36 - 45	11	4.745	2.7700		
	46 - 55	2	0.750	.3536		
	56 - 65	6	6.433	2.2277		
	66 - 75	2	6.500	.7071		
	Total	31	4.977	2.9520		

Table 4: Vit.D3 Levels at pre dialysis according to patient's age.

*Significant different at the 0.05 level.

** Not Significant different at the 0.05 level.

Variable	Age group	N	Mean	Std. Dev	T- test	p- value
Vit D ₃ (Post)	16 - 25	4	6.800	2.9710	.989	0.44**
	26 - 35	6	6.717	3.3648		
	36 - 45	11	6.255	3.5909		
	46 - 55	2	1.700	.8485		
	56 - 65	6	7.083	2.5015		
	66 - 75	2	4.750	4.5962		
	Total	31	6.184	3.2538		

Table 5: Vit.D3 Levels at post dialysis according to patient's age.

*Significant different at the 0.05 level.

** Not Significant different at the 0.05 level.

Normal range vit.D₃, Level: Total (3 - 10 microgram/ml).

vit.D₃ at pre and post dialysis in age group 46-55 showed a lower than normal range at pre (0.750 microgram/ml) as compared to post (1.700 microgram/ml), this age has them problem in liver like hepatitis B and do not gives them nutrition as supplement [7].

Effect of area in studied patients

Table 6 showed that there is strong relationship with area and disease based on statistical analysis. The majority come from south 52% followed by that from the west 29% then from east 13% the minority come from north 7% [8-11].

Area	Number of Patients/Gender		Total
	Male	Female	
North Khartoum	1	1	2
South Khartoum	8	8	16
West Khartoum	5	4	9
East Khartoum	2	2	4
Total	16	15	31

Table 6: Area or residence of patients.

Conclusion

Our study achieved many hypotheses reduce the number of time dialysis in some patients when controlling food and cholesterol our study also achieved adjust the level of cholesterol in the blood. This study showed that relationship of medical nutrition therapy to renal failure and confirms the relationship between vit. D₃ and cholesterol, with ESRD. Cholesterol and vit. D₃ increased at post dialysis when compared with that at pre dialysis. The study noticed unify diets for all patients with end stage renal disease this from the bag problem and major mistakes. This study confirms that dialysis of cost higher than cost kidney transplantation, and also showed that nutrition needs its different based on type of dialysis and CKD stages.

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