EUROPEAN
ACADEMIC
RESEARCH

Effect of Hemodialysis on the Lipids Profile in Patients with Renal Failure - Khartoum State

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Abstract:

This study was conducted to measure serum levels of plasma lipids (TG, TC, HDL-c and LDL-c) in patients with chronic renal failure. Seventy fife random samples were collected from patients were already attended ALnaw Teaching Hospital during the period between January to march 2015 chosen randomly and seventy fife apparently healthy individuals were selected as controls.

Enzymatic method was used to estimate serum lipid profile levels manually using Biosystem kits and by using Mindary, and results were analyzed using (SPSS) computer program (T- test and A nova test).

The results showed that serum levels of TG, TC, and LDL were significantly increased (p-value = .000) (p-value = .001) (p-value = .000) respectively and the serum levels of HDL were significantly decreased (p-value = .000) in the Sudanese patients group.

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*TG serum level increased between (101.5±35.8) in control to
(132.2±56.3) in patients, TC also increased to (138.0±42.0) compared to
control (121.6±23.0). LDL showed the same pattern and significantly
increased to (133.8±43.1) in compare to control (92.8±37.8). HDL of
patients decreased to (31.8±4.0) while in control was (37.0±5.4).*

*According to causes of renal failure the results of this study
showed that; hypertension, diabetes and family history are the most
common causes in Sudan.*

*The results of this study showed that there is a weak
correlation between TG levels and duration of hemodialysis at (r =
.223, p-value=.054). No correlation between TC (r=.166, p-value =.154),
HDL-c (r=.166, p-value =.156) and LDL-c (r=.133, p-value =.254) levels
and duration of dialysis was observed.*

*In conclusion: the serum levels of TG,TC, and LDL
significantly increased in Sudanese patients with renal failure, but
serum levels of HDL significantly decreased in patients subjected them
to risk of many complications.*

Key words: Hemodialysis, Lipids Profile, Patients with Renal
Failure, Khartoum State

INTRODUCTION

Kidneys are paired, bean shaped organs located retro-
peritoneally on either side of spinal column.[1] Macroscopically
each kidney is enclosed by fibrous capsule of connective tissue,
when dissected longitudinally; two region can be clearly
discerned: the outer region called the cortex and the inner one
is named medulla. The pelvis of the kidney is a basin like cavity
at the upper end of the ureter into which newly formed urine
passes.[2]

Acute renal failure is a sudden sharp decline in renal
functions due to acute toxic or hypoxic insults to the kidneys.
Chronic renal failure is a clinical syndrome that occurs when
there is gradual decline in renal functions over time.[1] There

are different types of kidney dialysis, including: Hemodialysis is most commonly used to treat people with end-stage kidney disease, Blood is filtered using a dialyzer and dialysis machine.During a hemodialysis session, your blood flows a little bit at a time through a special filter inside the machine. The filter removes wastes and extra fluids from your blood, but retains the proper balance of minerals such as potassium and sodium. Once the blood is cleaned, it is returned to the body.[3] Peritoneal dialysis is filtering blood inside the body after the abdomen is filled with a special cleaning solution. This method allows your blood to be cleaned while you sleep, while you work, or while you perform your everyday activities.

The major forms of plasma lipids are: Fatty acids which are straight chain carbon compounds of variety lengths, they are be saturated, mono unsaturated or poly unsaturated.

Triglycerides are molecules consist of one molecule of glycerol with three fatty acid molecules.

Phospholipids are polar , ionic lipids composed of 1.2 diacylglycerol and phosphodiester bridge link the glycerol back bone to some base usually nitrogenous such as choline, serine or ethanol amine.**[4]** Cholesterol is saturated steroid of high molecular weight in it is esterifies form , it contain one fatty acid molecule. Lipoprotein are core of insoluble (non polar) cholesterol ester and triglycerides, surrounded by shell of protein, phospholipids and free cholesterol with their soluble (polar) group.[5]

Dyslipidemia is a very common complication of chronic renal failure (CRF) and are actively participate in the deterioration of renal function. In Sudan feeding products which constitutes high amounts of calories beside low physical exercise are main factors which subjects Sudanese individuals to diseases as CRF.[6]

MATERIAL AND METHODS

Study approach
A quantitative methods were used to measure plasma lipids
(TG, TC, HDL-c and LDL-c) in Sudanese patients with renal
failure in Khartoum state, during the period from January to
March 2015.

Study area:
This study was conducted in Alnaw hospital in Khartoum state.

Target population:
The study included patients with renal failure (males and
females) under hemodialysis.

Sample size:
A total of 75 patients with renal failure were enrolled in this
study, plus (75) non patients apparently healthy volunteers'
(age and sex matched with the test group) were included to
serve as control.

Inclusion criteria:
Sudanese patients with renal failure and apparently healthy
volunteers were included in this study.

Exclusion criteria:
Patients with renal failure and other disease that may affect
the parameters under study were excluded from this study.

Ethical consideration:
Consent was taken regarding acceptance to participate in the
study and reassurance of confidentiality. Before the specimen
was collected, the donor knew that this specimen was collected
for research purpose.

Data collection:

The Clinical data were obtained from history, clinical examination and hospital follow up records and were recorded on a questionnaire sheet.
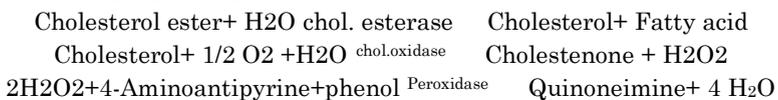
Sample collection and processing:

About 2.5 ml of venous blood were collected from each participant (both cases and control). The samples collected under aseptic conditions and placed in sterile heparin containers, and after mixing centrifuged for 5 minutes at 3000 RPM to obtain plasma, then The obtained plasma were kept at - 20°C till the time of analysis.

ESTIMATION OF TOTAL CHOLESTEROL

Principle of the method

Free and esterified cholesterol in the sample originates,by mean of the coupled reactions, acoloured complex that can be measured by spectrophotometry

Cholesterol ester+ H_2O chol. esterase   Cholesterol+ Fatty acid

Cholesterol+ 1/2 O_2 +H_2O chol.oxidase   Cholestenone + H_2O_2

2H_2O_2+4-Aminoantipyrine+phenol Peroxidase   Quinoneimine+ 4 H_2O

Assay procedure:

	Blank	Standard	Sample
Reagent (A)	1000 µL	1000 µL	1000 µL
Cholesterol standard(S)	─	10 µL	─
Sample	─	─	10 µL

The tubes were incubated for 10 minutes at room temperature, the absorbance of standard and sample were measured at 520 nm against the blank

Calculations: The cholesterol concentration in the sample is calculated using the following general formula:

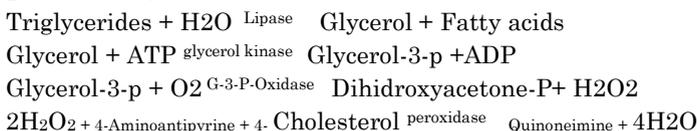$$\frac{\text{A Sample}}{\text{A Standard}} \times \text{C Standard} = \text{C Sample}$$

Reference value:

> Up to 200 mg/dL desirable
> 2oo- 239 mg/Dl borderline high
> >240 mg/dL high

ESTIMATION OF TRIGLYCERIDES

Principle of the method:

Triglycerides in the sample originates, by means of the coupled reactions, a colored complex that can be measured by spectrophotometry

> Triglycerides + H2O $^{\text{Lipase}}$ Glycerol + Fatty acids
> Glycerol + ATP $^{\text{glycerol kinase}}$ Glycerol-3-p +ADP
> Glycerol-3-p + O2 $^{\text{G-3-P-Oxidase}}$ Dihidroxyacetone-P+ H2O2
> 2H2O2 + 4-Aminoantipyrine + 4- Cholesterol $^{\text{peroxidase}}$ Quinoneimine + 4H2O

Assay procedure:

	Blank	Standard	Sample
Reagent (A)	1ooo uL	1000 µL	1000 µL
Triglycerides standard(S)	—	10 µL	—
Sample	—	—	10 µL

The tubes were incubated for 15 minutes at room temperature, the absorbance of standard and sample were measured at 520 nm against the blank

Calculations: The triglyceride concentration in the sample is calculated using the following general formula:

$$\frac{\text{A Sample}}{\text{A Standard}} \times \text{C Standard} = \text{C Sample}$$
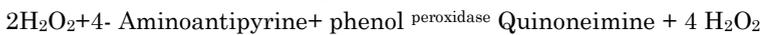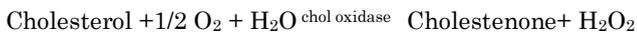
Reference value:

> Up to 150 mg/dl Normal

150- 199 mg/dl   Borderline – high
>500 mg/dl Very high

Estimation of HDL:

Principle of the method:
Very low density lipoproteins and low density lipoprotein in the
sample precipitate with phosphotungstate and magnesium ions,
the supernatant contains high density lipoproteins(HDL). The
HDL Cholesterol is then spectrophotometrically measured by
means of the coupled reactions described below

Cholesterol ester + H_2O chol esterase   Cholesterol + Fatty acid

Cholesterol +1/2 O_2 + H_2O chol oxidase Cholestenone+ H_2O_2

$2H_2O_{2+}$ 4-Aminoantipyrine+ phenol peroxidase Quinoneimine + H_2O_2

$2H_2O_2$+4- Aminoantipyrine+ phenol peroxidase Quinoneimine + 4 H_2O_2

Assay procedure

	Blank	Standard	Sample
Reagent (A)	1ooo uL	1000 µL	1000 µL
HDL cholesterol standard(S)	—	100 µL	—
Sample supernatant	—	—	100 µL

The tubes were mixed and incubated for 30 minutes at room
temperature. The absorbance (A) of the standard sample were
measured at 520 nm against the blank.

Calculations:
The HDL cholesterol concentration in the sample was
calculated using the following general formula:

   A sample/ A standard   × C standard × sample dilution factor = C sample

Reference values:
        Up to 35 mg/dl   High
        >60 mg/dl   LOW

Estimation of LDL

Principle: LDL precipitated by polyvinyl sulphate (buffered by poly-ethylene-glycol pH: 5.2) after centrifugation, chylomicrons, VLDL, HDL "other lipoproteins than LDL" are remain in supernatant and cholesterol of them estimated by cholesterol oxidase method, and after estimation of total cholesterol also, LDL_c calculated as follow:

LDL_c= Total cholesterol conc. – other lipoprotein cholesterol

PROCEDURE:

Step (1): precipitation of LDL:
In centrifuge tube add: 0.4mL from s/p +0.2 mL from polyvinyl sulphate of PEG

Mix, stand for 15' in R.T, then centrifuge for 10' at 4000 rpm, then obtain supernatant and continue as follow:

Step (2) estimation of other lipoproteins than LDL cholesterol:

	B	Std	T
working Reagent	1.0	1.0	1.0ml
std (200mg/dL)	-	0.02ml	-
Supernatant	-	-	0.02 mL

Mix, incubate for 10' in room tempreature then read at 520nm against reagent blank.

Calculation

Conc. of cholesterol of other lipoproteins than LDL = $\dfrac{A^0T}{A^0std} \times$ conc. of STD × D.F.

D.F = 1.5

R.V of LDL_c

Normal	Borderline	High risk
<130mg/dL	130 – 160mg/dL	>160mg/dL

RESULTS

Table (1): Ages, gender and family history of patients with renal failure disease:

Age of patients			Gender			Family history disease		
	No	**%**		**No**	**%**		**No**	**%**
25– 50	20	27	Male	45	60	Yes	28	37
51– 80	55	73	Female	30	40	No	47	62

Table (2): Body mass index (BMI) of patients with renal failure group and control group:

Variable	Patients group N=75	Control group N=75	p-value
BMI(Kg/m^2)	29±4.1 Kg/m^2 (19-30)	25±3 Kg/m^2 (19-30)	=0.02

Results given in mean ±SD, range between brackets.

p-value ≤o.o5 consider significant.

Table (3): The mean of plasma TG, TC, HDL-c and LDL-c levels in study groups.

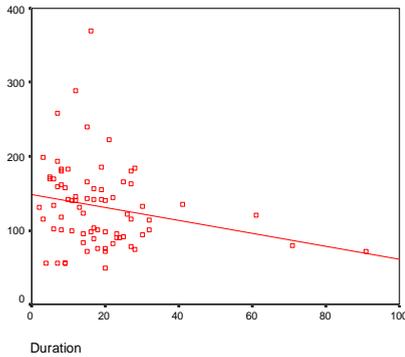| Variable	Case Mean ± SD	Control Mean ± SD	P-value
Triglyceride (mg/dl)	132.2±56.3	101.5±35.8	.000
Total cholesterol (mg/dl)	138.0±42.0	121.6±23.0	.001
HDL-cholesterol (mg/dl)	31.8±4.0	37.0±5.4	.000
LDL-cholesterol (mg/dl)	133.8±43.1	92.8±37.8	.000

Results given in mean ±SD.

Range between brackets.

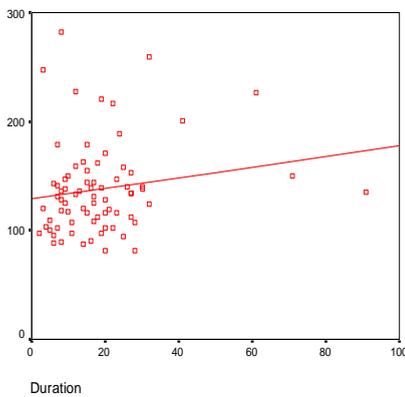**Figure (1):** Scatter plot of correlation between TG level and duration of dialysis (r=.223, p-value=.054).

Figure (2): Scatter plot of correlation between TC level and duration of dialysis (r=.166, p-value=.154).

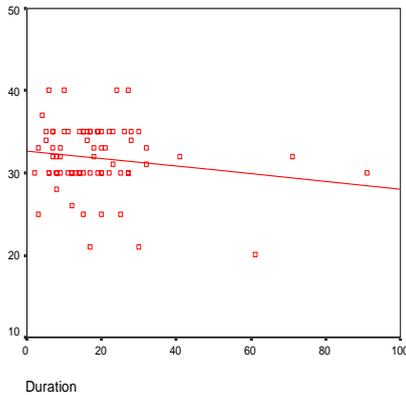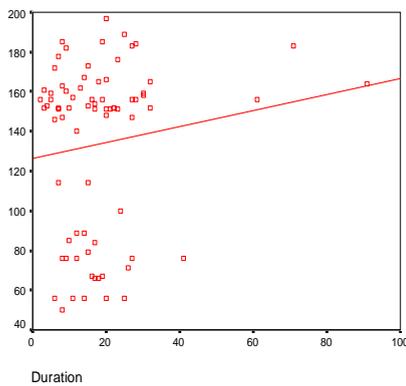**Figure (3):** Scatter plot of correlation between HDL level and duration of dialysis. (r=.166, p-value=.156).

Figure (4): Scatter plot of correlation between LDL level and duration of dialysis (r=.133, p-value=.254).

DISCUSSION

From the finding of this study it appears that that the majority of patients under dialysis participated in this study were in the average ages of about 55 years. This agreed with previous published results of many authors.[7], whose finding confirmed that, after the age of 30 years, glomerular filtration rate (GFR) progressively declines at an average rate of 8 mL/min/173 m²

per decade , and the risk of renal failure increased with age. This result also was agreed with result carried by; (Christian)[8] showed that the average age of a British person with the renal failure is 77 years. Sex distribution in patients under hemodialysis of this study revealed that 60% were males. This agree with the previous study which documented in the field of nephrology, showed that women seem to be somewhat protected from developing end stage renal failure; the cumulative incidence of the disease remains low during the reproductive ages and begins to rise 10 years later.[9] .

Social clinical history index of patients under the study indicated that appositive family history of renal failure of first degree relatives found to be in 37% of cases. These findings may indicate that hereditary play a role in the pathogenesis of renal failure patients. This result agreed with previous study showed that, There is a high prevalence of family history –end stage renal disease among US population, about 23%. [10] The findings of this study showed that , there was a significant differance in the body mass index (BMI; determined by dividing the weight in kilograms by the height in meter square.) between patients and control, the patients with renal failure susceptible to be more obese than control group. This made the BMI is independent factor of renal failure. This agreed with previous study which found positive correlation between increased (BMI) and risk of renal failure disease.[11] . Another study examined the relationship between increased weight (BMI) and renal function evaluated by the estimated glomerular filtration rate, Increased BMI was consistently associated with reduced glomerular filtration rate.[12]

In this study serum levels of TG,TC, and LDL were significantly increased and the serum levels of HDL were significantly decreased in patients under hemodialysis group in compare to control group. TG (132.2±56.3 versus 101.5±35.8, p-

value = 0.00).TC (138.0±42.0 versus 121.6±23.0, P-value=0.001).
LDL (133.8±43.1 versus 92.8±37.8, p- value =0.00)

HDL (31.8±4.0 versus 37.0±5.4,p-value=0.00) .This results agreed with a study carried by (Sathyian *et al*)[13] , which showed that ; Plasma TG, TC and LDL concentration frequently elevated in patients with CRF under hemodialysis because heavy proteinuria alone or in combination with chronic renal insufficiency results in acquired LDL receptor deficiency, which play a central role in the genesis of the associated hypercholesterolemia (Sathyian *et al*).[13]

Also the results was in agreement with another studies carried by many authors (Dipika *et al;*)[6], (Weam)[14], (Nzere et *al*)[15], which finding confirmed that Chronic renal failure patients with hemodialysis are at greater risk of development of dyslipidemia characterized by hypertriglyceridemia, elevated TC and LDL-c levels and decreased HDL-c levels generated during the course of CRD which place them at risk of developing cardiovascular diseases.

According to figure (1) showed that, there was a weak correlation between TG levels and the duration of hemodialysis. this result disagreed with another result, which showed that, there was a strong correlation between TG levels and the duration of dialysis.[14]

Also the findings of this study showed that there were no correlation between duration of dialysis and concentration of TC, HDL-c and LDL-c as appeared in figure (2) (3) (4). This results agreed with previous results which revealed that no significant correlation between serum lipid profile levels and the duration of hemodialysis.[14]

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