Lipid profile and FFA in Iraqi patients with Chronic Renal Failure after Renal Dialysis

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الخلاصة

اجريت دراسة شملت ٣٠ مريضاً (١٧ ذكراً و ١٣ انثى)مصابين بمرض الفشل الكلوي المزمن وتم أخذ عينات من الدم قبل الديلزة الدموية وبعدها لقياس مستوى صورة الدهون والاحماض الدهنية الحرة . قورنت النتائج مع نماذج دم ٣٠ شخصاً (١٨ ذكراً و ١٢ انثى) اصحاء غير مصابين (مجموعة سيطرة) . اظهرت نتائج الدراسة وجود زيادة معنوية في مستويات TG وVLDL-C قبل وبعد الديلزة مقارنة بمجموعة السيطرة ، بينما كانت هناك تغيرات غير معنوية في مستوى LDL-C و TC لدى المرضى قبل الديلزة مقارنة مع مجموعة السيطرة ، بينما لوحظ هناك علاقة معنوية في مستوى TC بعد عملية الديلزة لاولئك المرضى مقارنة مع مجموعة السيطرة . اظهرت ايضاً هذه الدراسة بان هناك نقصان معنوي في مستوىHDL-C قبل عملية الديلزة مقارنة بمجموعة السيطرة وعدم تغير معنوي في مستوى LDL-C و HDL-C بعد عملية غسل الكلية مقارنة مع مجموعة السيطرة.اظهر مستوى FFA قبل وبعد عملية الديلزة زيادة معنوية مقارنة مع مجموعة السيطرة . أشارت النتائج الى ان هناك ارتباط ايجابي بين مستوى FFA ومستوى كل من TG وVLDL-C و LDL-Cوعلاقة موجبة غير معنوية مع TC وعلاقة سالبة معنوية مع مستوى HDL-C في مرضى الفشل الكلوي قبل عملية الديلزة .ولوحظ هناك علاقة موجبة معنوية بين مستوى FFA ومستوى كل من TC وLDL-C وعلاقة غيلر معنوية مع مستويات TG وVLDL-C وHDL-C في مرضى الفشل الكلوي بعد عملية الديلزة .

ABSTRACT

A study was carried out on 30 patients (17 male and 13 female) with chronic renal failure. Blood samples have been taken before and after hemodialysis to measure lipid profile and free fatty acids.

The results obtained have been compared with 30 (18male and 12 female) healthy subjects (control group). The results showed that there were significant increase in the level of TG, VLDL-C before and after hemodialysis , while there was non significant difference in the level of TC, LDL-C in pre-dialysis patients as compared to control group. The results also revealed a significant relation in the level of TC, in those patients after hemodialysis comparison with control group. This study also show a significant decrease in the level of HDL-C in pre dialysis patients as compared to control group and non significant changes in the levels of LDL-C and HDL-C in post-dialysis patients compared with control group. The level of FFA in patients before and after hemodialysis was significant increase as compared with control group. There is a significant positive correlation between serum FFA levels and TG, LDL-C, VLDL-C, whereas no significant positive correlation with TC and significant negative correlation with HDL-C levels in pre-dialysis patients. There is a significant positive correlation between serum FFA levels and TC, LDL-C, where as no significant correlation with HDL-C, TG, and VLDL-C in post- dialysis patients.

INTRODUCTION

Chronic renal failure (CRF) is defined as kidney damage for more than three months as evidenced by structural or functional abnormalities with or without decreased glomerular filtration rate (GFR) and manifested either as pathological abnormalities or kidney damage markers in blood or urine or in the imaging tests. Many people are unaware of the problem until more than 70% of kidney function has been lost (1, 2). Dialysis is a procedure that removes excess

fluids and toxic end products of metabolism such as urea from the plasma and corrects electrolytes balance by dialyzing the patients blood against fluid containing no urea but with appropriate concentrations of electrolytes ,free – ionized calcium and some other plasma constituents (3).

Hemodialysis relies on the principles of solute diffusion across a semi-permeable membrane. Movement of metabolic waste products takes place down a concentration gradient from the circulation into the dialysate, and in the reverse direction (4).

Patients with end-stage renal disease, whether or not they are undergoing dialysis therapy, frequently present evidence of dyslipoproteinemia (5,6) and have an increased risk of developing cardiovascular disease and of suffering from its complications. The frequent findings are increased concentrations of triacylglycerols and very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) (7), and decreased concentrations of high density lipoproteins (HDL) (5). By contrast, total cholesterol and low density lipoproteins (LDL) appear to be little affected by renal disease, except in nephritic subjects (8) .Total serum free fatty acid (FFA) levels provide an important measure of the physiologic state (9). Low level of FFA occurs in all tissues but substantial amount can sometimes be found in the plasma, particularly during fasting or starvation. Plasma FFA (transport by serum albumin) is "en route" from their point of origin (triacylglycerol of adipose tissue or circulating lipoproteins) to their site of consumption (most tissues) (10).

Plasma concentrations of FFA are very variable, being influenced by hormonal, metabolic and nutritional status. Abnormally high plasma concentrations of FFA are implicated in increased risk ventricular fibrillation (10) and sudden cardiac death (11) and more controversially of coronary heart disease (12, 13). The important of FFA in renal disease seems to have been neglected in the last years; nevertheless, many studies have in the past, established that FFA concentrations in end stage renal failure patients are increased following treatment by hemodialysis (14, 9).

MATERIAL AND METHODS

Serum total cholesterol, triglyceride, HDL, were measured by colorimetric assay using kits supplied by Spinreact in spain. Free fatty acid was estimated by colorimetric assay using soap formation (15).

Calculations:

a- LDL-C was calculated according to Bairaktary *et al* equation (16):

$$\text{LDL-C} = 0.94 \times \text{Total cholesterol} - 0.94 \times \text{HDL-C} - 0.19 \times \text{TG}$$

This equation is more accurate than Friedewald equation because Friedewald's equation considerably inaccurate even at TG concentrations of 200-400 mg/dL (17) .

b- $\text{VLDL-C} = \text{TG}/5$

Sample Collection

Thirty patients (17 male and 13 female) were involved in this study. The patients were referred to Baghdad Teaching Hospital, Al-Kadhimiya Teaching Hospital, and Al -Hakeem Hospital, Baghdad, Iraq. All patients with CRF were diagnosed by clinical examination (urea & creatinine) in those medical centers. The mean age of the patients was 45±10 years. All those patients were treated with hemodialysis (twice in a week). Control group consisted of 30 healthy subjects (18 male and 12 female) with mean age (40±10).

Preparation of Blood Samples

Ten milliliters samples of venous blood were taken from all fasting patients before and after hemodialysis. Blood samples were left for 20 minutes at room temperature. After blood coagulation, the sera were separated by centrifugation at 3000 xg for 15 minutes and then sera stored at -20 ºC. Hemolyzed samples were discarded.

Statistical Analysis

The data was analyzed on the computer statistical programme SPSS version10. The mean ±SD was also computed for the comparison of results. The comparison of mean between two groups was tested by Student's't' test. Results were considered statistically significant if P value is less than 0.05.

RESULTS AND DISCUSSION

The results in table 1 referred to non significant increased in the serum level of TC in pre-dialysis patients and significant increased in the serum level of TC in post-dialysis patients with CRF may be attributed to either a highly significant increase in FFA. The increase in the latter component leads to increased formation of acetyl-CoA with a subsequent increase of cholesterol synthesis . The increase in the level of cholesterol in post- dialysis patients with CRF as compared with pre- dialysis patients with CRF due to haemoconcentration and selective retention of high molecular weight compounds after hemodialysis (18).

Table - 1: Lipid profile and FFA levels in pre-and post dialysis patients and control group.

Parameters	Control	Pre-dialysis	Post-dialysis
TC (mmol/L)	4.327±0.235	4.575±1.216	4.772±1.013*
TG (mmol/L)	1.41 ± 0.127	2.45 ± 1.006***	2.267 ± 0.623***
HDL-C (mmol/L)	1.24 ± 0.168	1.08 ± 0.174***	1.27 ± 0.309
VLDL-C (mmol/L)	0.28 ± 0.025	0.49 ± 0.205***	0.453 ± 0.125***
LDL-C (mmol/L)	2.634 ± 0.124	2.822 ± 1.145	2.86 ± 1.027
FFA (mmol/L)	0.353 ± 0.08	1.016± 0.717***	1.514± 0.626***

Values significantly different from the controls *p<0.05, **p<0.01, ***p<0.001

The significant increased in the serum level of TG and VLDL-C in patients with CRF as compared to control group can be explained in reference to Klin M. etal (19), observations .Those investigators found that the downregulation of hepatic triglyceride lipase, and the VLDL receptor can, in part ,account for the associated hypertriglyceridemia, elevation of plasma VLDL-C, and impaired clearance of triglyceride-rich lipoproteins in CRF. Our results were in a good agreement with Ikewaki *et al* (20), Miida *et al* (21). Tzanatos *etal* (18), found non significant decrease in the serum levels of TG and VLDL-C in post- dialysis patients with CRF as compared with pre-dialysis patients with CRF . This decrease can be attributed to heparin given during dialysis activate endothelial lipoprotein lipase , leading to enhancement of hydrolysis of circulating TG (22).

The present study showed decreased in concentration of HDL-C in pre-dialysis patients with CRF as compared to control group. Fuh *et al* (23), found the decrease in HDL-C concentration in CRF patients is associated with decreases in both the fractional catabolic rate (FCR) and total synthetic rate of apoAI/HDL. Furthermore, it appears that the worse the renal function , as estimated by either blood urea nitrogen or creatinine , the slower the FCR and the lower the total synthetic rate of apo AI /HDL. Castelli *et al*, found that low levels of HDL-C are strongly associated with high risk of atherosclerotic heart disease (24) .Thus, cholesterol esterification by lecithin-cholesterol acyltransferase may play a key role in the prevention of cardiovascular disease, improving cholesterol exportation in biliary acids (25). AL- Rashidi *et al* (22) and Gillett *et al* (9), attributed the increase in the level of HDL after hemodialysis to haemoconcentration and changes in some chemical components of dialysate which lead to increased synthesis APoE ,APo cII, APoA.

The results in table 1 showed there was a non significant increase in the serum levels of LDL-C in pre- and post-dialysis patients with CRF as compared to control group. The alternative equation for LDL-C yield slightly better results than the Friedewald equation especialy in hypertriglyceridemia . The results of this equation is more accurate and nearest to the results ultercentrifugation procedure (16). In this study there was non significant increase in the level of LDL-C in post-dialysis patients in comparison with that of pre-dialysis .The changes in lipoproteins and their carrier protein (apoliproteins) could not have been due to solely to haemoconcentration caused by fluid withdrowal during maintaining hemodialysis , it would appeare that selective retention of high molecular weight apoliproteins by dialysis membranes caused the increase in apolipoprotein levels , which inturn , resulted in retention of lipoproteins (22). There was a highly significant increase in the serum levels of FFA in pre- and post-dialysis patients with CRF as compared to control group. The results also revealed highly significant increase in the levels of FFA in post - dialysis patients with CRF compared with pre- dialysis patients with CRF ($p< 0.001$).

The increase of FFA in blood may affect metabolic pathway and endocrine disturbance, it may lead to metabolic syndrome and insulin resistance

.It may also affect the levels of total different type of lipoproteins, is associated with increased risk of vascular disease (11). Gillett *et al*, explained this result by heparinization of patients during hemodialysis and the consequent release into the circulation of lipoproteins and hepatic lipases has been thought to cause raised level of FFA concentrations. Other factors such as carnitine deficiency and the presence of acetate in the dialysis buffer solutions may lead to those subsequent changes in FFA (9).

Table (2) shows a significant positive correlation between FFA and each of TG , VLDL-C and LDL-C in pre-dialysis patients and the same trend of significant were found in TC ,LDL-C in post-dialysis patients while there was a significant negative correlation between FFA and HDL-C in pre-dialysis patients.

Table – 2 : Correlation coefficients and the significance levels of different serum chemical components in patients with CRF.

Component *VS* FFA	Pre-Dialysis		Post-Dialysis	
	r	P	r	P
TC	0.317	0.088	0.397*	0. 03
TG	0.517**	0.003	-0.141	0.458
HDL-C	-0.487**	0. 006	0.061	0.750
VLDL-C	0.517**	0.003	-0.141	0.458
LDL-C	0. 366*	0.046	0.372*	0.043

* Correlation is significant at the level 0.05
**Correlation is significant at the level 0.01

FFA is one of the best indicators of metabolic changes (especially with lipid profile). FFA significantly increase in post-dialysis patients due to heparin infusion. This increase is an independent risk factor for CRF patients. Thus, regular determination of FFA in CRF patients is important for monitoring the treatment.

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