

Lipoprotein pattern in patients with chronic renal failure and those who had renal transplantation

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Abstract

Background: Vascular disease is common in patients with chronic renal failure as well as in patients with renal transplantation. It is a common cause of death in these patients. One possible factor for this increased incidence of vascular disease is hyperlipidaemia that frequently occurs in these patients.

Purpose: To determine the lipoprotein pattern in patients with chronic renal failure and patients who had renal transplantation in our community.

Methods: 78 patients, age ranging between 10-75 years (mean value of 42.7) were studied. They were 21 pre-dialysis patients, 42 dialyzed patients (20 peritoneal dialysis and 22 Haemodialysis), and 15 patients following renal transplantation. Fasting blood Cholesterol, triglyceride, high and low density lipoprotein (HDL and LDL) were determined.

Results: 2 patients of the pre-dialysis group had high cholesterol levels, while 6 patients had high triglyceride levels and 7 patients had high serum HDL levels. Only 9 patients had ischaemic changes on E.C.Gs. 3(7.3%) patients on dialysis group had high cholesterol levels, 11.9% had high triglyceride levels. 24 patients of the dialysis group had ischaemic changes in E.C.G. 7 transplant patients had high serum triglyceride levels and only two have elevated LDL.



Chronic renal failure is commonly accompanied by hypertriglyceridemia or combined hyperlipidemia with low levels of high density lipoproteins (HDL), but cholesterol levels in plasma are usually normal. Hepatic endothelial lipase activity is also low and there is commonly insulin resistance¹. After renal transplantation hypercholesterolemia appears probably due to the corticosteroid therapy². Atherosclerotic cardiovascular disease is the major cause of death among these patients. This may be related to their lipid abnormalities³. Patients undergoing Haemodialysis receive large amount of intravenous heparin. Heparin stimulates release of lipoprotein lipase into bloodstream. Repeated heparin infusions could deplete tissues of this enzyme².

Methods

The study was conducted in Ibn Sina Hospital and Khartoum Teaching Hospital, renal unit from Dec 1998 through March 1999. 78 patients were categorized as follows:

1. Pre-dialysis patients: 21 patients with ages ranged between 13-67 years. They were 76.2% males, and 23.8% females.

2. Patients on dialysis: 17 males and three females on peritoneal dialysis. Their age ranged between 21-60 years with 18 males and four females on haemodialysis. Their age ranged between 15-60 years.

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3. Transplant patients were 13 males and two females, their ages ranged between 15-60 years.

All patients had cholesterol, triglyceride, high-density lipoprotein (HDL) low density lipoprotein (LDL), very low-density lipoprotein (VLDL) and ECG done.

Five ml venous blood was collected after overnight fast. The blood was allowed to clot for one hour at room temperature, and then centrifuged at 5000 r.p.m for 10 minutes. Serum was withdrawn by pipette and kept into tightly covered containers at - 20°C till used for estimation of serum triglyceride, serum total cholesterol and serum lipoproteins.

Chemicals used were commercial kits obtained from Crescent Diagnostic (Saudi Arabia). Triglyceride kit Cat No. Cs 611. Cholesterol kit Cat No. CS 603. HDL precipitant Cat No. CS 606. MSE Micro centrifuge was used for the separation of HDL fraction in the serum. Corning colorimeter model 252 was used to estimate serum triglyceride, serum total cholesterol and serum HDL. Serum triglyceride concentration was measured using enzymatic methods⁴. The working reagent was prepared by dilution of one part of the enzyme concentrate with 100 parts of the buffer (1 ml/100 ml) mixed gently and allowed to equilibrate at room temperature. When combined as instructed, the working reagent contains the following: Buffer at PH 7.5 40 mmol /l, Lipase >150 JY.1, Glycerol kinas >0.4 KU/1, Glycerol-3-phosphate oxidase >1.5 KU/1, Peroxidase >0.5 KU/1, Magnesium 5 mmol/1, adenosine triphosphate (ATP) 1 mmol/1,

Chlorophenol 5 mmol/l, Aminoantipyrine 0.4 mmol/l, stabilizers and preservatives.

Serum triglycerides are hydrolyzed by the enzyme lipase to yield glycerol and free fatty acids. The released glycerol is assayed in reaction catalyzed by the enzymes glycerol kinase, glycerol-3-phosphate oxidase in a system that generates hydrogen peroxide. The hydrogen peroxide is monitored in the presence of peroxidase with 4- aminoantipyrine and chlorophenol as a chromogenic system. The end product is a quinone amine dye, the concentration of which at 540 nm is directly proportional to the concentration of triacylglyceride concentration in the sample.

One ml of the working reagent was pipetted into three test tubes labeled test, standard and blank. 0.1ml of the sample was pipetted into the test tube. 0.01ml was pipetted into the standard tube and 0.01 ml of distilled water was pipetted into the blank one using micro pipette. All the above tubes were incubated at 37°C for 5 minutes. Then the absorbency of the sample and that of the standard were measured against the reagent blank using the colorimeter at wavelength 540 nm within 60 minutes.

Cholesterol estimation: The enzyme reagent contains: Phosphate buffer (PH 6.5) 30 mmol/l, 4- aminoantipyrine 0.25 mmol/l, Phenol 5 mmol/l, Peroxidase > 10 KU/l, Cholesterol esterase >350 U/l and Cholesterol oxidase >140 U/l.

Cholesterol esterase catalyzes the hydrolysis of cholesterol esters to produce cholesterol and free fatty acids. The produced cholesterol is oxidized by the cholesterol oxidase to produce cholest 4-en one and hydrogen peroxide. Hydrogen peroxide couples with 4- aminoantipyrine and phenol in the presence of peroxidase to produce quinone amine dye which is a chromogen red in color. The absorbency of the dye at 540 nm is directly proportional to the concentration of cholesterol in the sample.

The absorbency of the sample and the absorbency of the standard were measured against the reagent blank using the colorimeter at wavelength 550 nm within 60 minutes.

Estimation of High Density Lipoprotein using phosphotungstic acid precipitation method. Reagent composition: Phosphotungstic acid 0.55 mmol /l. Magnesium ions 25.0 mmol/l.

Phosphotungstic acid and magnesium ions specifically precipitate LDL and VLDL. After centrifugation, the cholesterol contents of HDL

supernatant (HDL-Con) is determined using the cholesterol kit.

Reagent preparation: Four parts of the reagent were diluted with one part of distilled water (20 ml of distilled water were added to 80 ml of the reagent).

Precipitation into a micro centrifuge tube 0.5 ml of the reagent and 0.2 ml of the sample were pipetted, the tube was mixed, allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 r.p.m using micro centrifuge. Cholesterol content of the HDL supernatant was determined using Crescent Diagnostics kit Cat, No. Cs 603. Using micropipettes, 1 ml of the reagent and 0.1 ml of the sample were pipetted into a test tube labeled test, into another test tube labeled blank 1 ml of the reagent and 0.1 of distilled water were pipetted. Both the test tube and the blank were incubated at 30°C for 10 minutes. Then the absorbency of the sample was measured against the reagent blank using colorimeter at wavelength 540 nm within 30 minutes.

RESULTS

Pre-dialysis patients: 19 patients had value equal to or less than 200 mg/dl while 2 patients had high cholesterol level ranging between 250-300 mg/dl.

The normal value of serum HDL is more than 55 mg/dl in males, and more than 65 mg/dl in females. Serum HDL level in 10 patients ranged between 10-50 mg/dl. In four females it was 65 mg/dl and in six of both sexes it ranged between 80-100 mg/dl.

Out of the 21 patients there were 9 who had ischemic changes in E.C.G. Only 4 patients out of them had low serum HDL levels, 3 patients with borderline HDL levels and one had high serum HDL level. Most patients had normal serum LDL and only one patient exceeded the normal value (256.3 mg/dl).

The peritoneal dialysis patients:

11 females had high serum HDL levels between 60-65 mg/dl. Only 4 males had low serum HDL levels, and 4 females had borderline HDL. One female had low serum HDL level. Out of 21 patients 13 patients had ECG ischemic changes. Seven males had high serum HDL. Two women had serum HDL levels 68.5 mg/dl. All patients have normal value of LDL except one who exceeded the normal value. Most of the patients have normal very low density lipoprotein (VLDL) levels.

Haemodialysis patients:-

Six out of 22 patients had history of hypertension. 19 out of 22 patients had serum cholesterol levels within normal value i.e. equal or less than 200 mg/dl. Two had values ranging between 200-240 mg/dl. Only one patient had high cholesterol value (300 mg/dl). All this group of patients had serum triglyceride levels within the normal value, which ranges between 30-165 mg/dl. The majority of patients (18) had low serum HDL levels. 4 patients (all of them females) had high serum HDL levels. Out of 22 patients there were 11 patients with ischemic changes in the E.C.G, 8 of them had low serum HDL values. Three patients had ischemic changes in the E.C.G with normal or high serum HDL value. Out of those 11 patients there were 4 with minor ischemic changes. 7 Patients had major ischemic changes. Most of the patients had normal serum LDL levels; only one exceeded the normal value. All patients had normal VLDL levels.

Transplant Patients:

The majority of patients (60%) had their transplant for more than 3 years. 3 patients had it for 7-12 months. 2 patients had it for 25-30 months. Only one patient had it for less than 6 months. 53.3% of the patients were not diabetic two patients developed diabetes mellitus after the transplantation. Five patients had history of DM before transplantation. 33.3% are hypertensive.

11 patients had normal value of blood glucose levels. Three patients who were diabetic had blood glucose levels 260-325 mg/dl. 5 male patients had major ECG schematic changes, but, their serum HDL levels were normal.

Most patients had serum cholesterol level equal to or less than 200 mg/dl. Only one patient had serum cholesterol level 233.3 mg/dl.

Serum triglyceride in eight patients was within normal. Seven patients had hypertriglyceridaemia ranging between 180-330 mg/dl.

Discussion:

Vascular disease is common in patients with chronic renal failure as well as in patients with renal transplantation. It is a common cause of death in these patients. Disturbance of lipid profile is a well known risk factor for these vascular diseases. A lot of work was done to determine lipid disorder in different populations of renal disease. Seventeen out of nineteen patients in the predialysis group in this study had high cholesterol level. This finding is similar to

literature and earlier studies⁵. Unlike previous reports most patients in this group had normal value of HDL and only 5 patients exceeded the normal value of triglyceride⁶. Most of our patients have normal VLDL levels but four exceeded the normal value. This is not consistent with the others findings. In the haemodialysis group only one patient had high cholesterol value. All this group of patients had serum triglyceride levels within the normal value. This observation is rather different from other researches. In agreement with earlier studies, 18 patients in this study had low serum HDL levels but 4 (all of them females) had high serum HDL levels⁷. Out of 22 patients there were 11 patients with ischemic changes in the E.C.G, 8 of them had low serum HDL values. However all patients had normal VLDL levels; which is different from a similar work done elsewhere^{2,8}. Consistent with other studies, most patients in the transplant group had serum cholesterol within normal limits apart from one patient. Never the less, different results were found by other workers^{2,5}.

Serum triglyceride in eight patients was within normal but seven patients had hypertriglyceridaemia ranging between 180-330 mg/dl. This finding is in agreement with literature^{2,4}.

Conclusion

We conclude that most patients with chronic renal failure who are on conservative management have normal values of lipoproteins.

The majority of patients on peritoneal dialysis had normal values of lipoproteins. Seven of them had ECG ischemic changes while two had ischemic changes with borderline levels.

With the exception of one, patients on haemodialysis had normal value of lipoproteins, (this one patient had high cholesterol level). All patients had normal triglyceride and serum VLDL levels. Most patients had normal serum LDL levels, only one exceeded the normal value.

Although corticosteroids and cyclosporine are the major causes of hyperlipidaemia in transplant patients, in this study all patients had normal levels of cholesterol, LDL, triglyceride and VLDL except 4 patients who exceeded the normal VLDL value. Seven patients had high HDL levels three of them had ECG ischemic changes.

Although high HDL levels protect against the risks of coronary heart disease we found that 17 patients out of 78 had ECG ischemic changes with high HDL value.

Our hypotheses is that the major causes for these results of lipoprotein pattern are related to the Sudanese diet which tends to be low in fat as well as an unidentified role for genetic factors e.g. more potent lipase enzyme, or may be due to some abnormalities in lipoprotein's carriers.

Recommendations

Lipoproteins investigation should be done for all patients who suffer from chronic renal failure as well as for patients who had renal transplant as a routine test. E.C.G has to be done for those patients from time to time for early diagnosis of ischemic heart disease. The patients must be referred to the dietician for dietary management and follow up.

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