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Lipid and lipoprotein status in hemodialysed patients

Profil lipidowy i lipoproteinowy u pacjentów hemodializowanych

INTRODUCTION

Dyslipidemia is a known cardiovascular risk factor in subjects without kidney disease. In patients with kidney disease, however, the relation of dyslipidemia to cardiovascular risk is confounded and underlying patomechanisms are undoubtedly complex [2, 17]. Recent studies have shown the spectrum of dyslipidemia in patients who have chronic renal disease (CKD) or end-stage renal disease (ESRD) to be different from that of the general population. There seems to be a shift to a uremic profile as the renal function deteriorates. Dyslipidemia is often observed in patients with chronic renal failure, resulting in abnormal concentrations and compositions of plasma lipoproteins. The prominent features of dyslipidemia in hemodialysed (HD) and peritoneal dialysis (PD) patients are an increase in plasma triglycerides in nearly all lipoproteins, and a reduction in high-density lipoprotein cholesterol [2, 11, 15]. Thus, those patients are at increased risk for atherosclerotic heart disease, which is due at least in part to atherogenic lipid and lipoprotein abnormalities [11, 18]. Atherosclerosis is recognized as the major cause of cardiovascular disease (CVD) in hemodialysis and it is responsible for 40% to 50% of deaths in this population. It has been suggested that dialysis itself could accelerate atherosclerosis and researchers agree that many patients enter dialysis with atherosclerosis, which can lead to a high risk of early mortality during the first years of dialysis [1, 3, 13]. Additionally, the change in high-density lipoprotein (HDL) concentration is favored by the common presence of microinflammation in uremic patients [3].

The magnitude of the abnormalities is not disclosed fully by routine laboratory chemistries that test only total cholesterol (TC), low-density cholesterol (LDL-C), high-density cholesterol (HDL-C), and triglyceride (TG) levels. Other modifications such as oxidation and glycation of lipoproteins may promote further atherosclerosis [9, 14].

The aim of this study was to measure lipid (TG, TC, LDL-C, HDL-C), lipoprotein (apoAI, apoB) profile parameters, OxyStat and count lipid and lipoprotein ratios for better understanding of the feature of dyslipidemia in hemodialysed patients.

MATERIAL AND METHODS

The studies were performed in 12 hemodialysed patients at the age between 21–60. Hemodialysed patients were without diabetes, liver disease, active inflammatory disease, malignancy, obesity, glucose intolerance. The healthy subjects (n=18) were chosen from among apparently normolipidemic individuals who were symptom free and had no evidence of previous cardiac, hypertensive or renal disease. They were without diabetes, liver disease, active inflammatory disease, malignancy, obesity or glucose intolerance. Venous blood was drawn after an 14-hour overnight fasting, and plasma was obtained by centrifugation at 3000 rpm at 4^{o} C immediately after blood collection. The samples were either used for measurements immediately or stored frozen at -80^{o}C. Routine laboratory parameters (the level of urea, creatinine, total protein, albumin) were determined using Au 400 analyzer (Olimpus), and hemoglobin using ADVIA analyser, Bayer. Lipids and lipoproteins were determined on Hitachi 902 analyser. The total cholesterol (TC) was estimated by the enzymatic-colorimetric method, triglycerides (TG) were determined using the standard enzymatic technique (BIOMAXIMA), HDL-C by the direct method with immunoinhibition (AB-WAKO - BIOMAXIMA). Total concentration of peroxides was measured as OxyStat by using ELISA method (Biomedica, Viena). ApoA-I and apoB were determined in turbidymetric method after the precipitation specific antiserum (Roche kits). Non-HDL cholesterol was obtained as TC minus HDL-C; LDL-C was calculated according to the Friedewald formula; TC/HDL-C, LDL-C/HDL-C, TG/HDL-C and apoA-I/apoB were also calculated.

Statistical analysis was performed using one-way analysis of the student t-test for comparison in RA patients and the reference group. The data are expressed as means ± SD. The statistical significance of all variables was established at the level $p<0.05$, and statistical analysis was performed using the STATISTICA program (Statsoft Polska, Krakow, Poland).

RESULTS

Selected clinical and routine laboratory parameters are presented in Table 1. The lipid and lipoprotein profile, lipid and lipoprotein ratios and OxyStat concentration are presented in Table 2. Results showed that HD patients had normotriglicerydemia but HDL-C and apoAI concentration were significantly decreased when compared to the reference group, which corroborates in disturbed lipid and lipoprotein ratios. Significantly higher TC/HDL-C, LDL-C/HDL-C, and TG/HDL-C ratios and significantly lower HDL-C/apoAI, apoAI/apoB are noticed in the studied patients than reference group which suggests disturbed concentration and composition of high density lipoproteins particles and function as a consequence of rebuilding. An increased concentration of peroxides (increased OxyStat) reflects the presence of oxidative stress in HD patients.

Table 1. Clinical and routine laboratory parameters in HD patients and reference group

Parameter	HD patients n=12	Reference group n=18
Age (years)	55.8 ± 14.4	34.97 ± 14.4
BMI (kg/m^2)	22.06 ± 3.05	21.5 (18.5–25.3)
Hb (mg/dl)	11.3 ± 2.3	14.70 ± 1.60
Urea (mg/dl)	122.3 ± 36.2***	17.80 ± 1.3
Creatynine(mg/dl)	9.1 ± 1.8***	0.80 ± 0.2
TP (g/dl)	6.75 ± 0.75**	7.20 ± 0.30
Albumin (g/dl)	3.76 ± 0.43**	4.3 ± 0.40
Dialysis period	132.7 ± 93.7	-

***p<0.001 **p<0.01 *p<0.05 vs. reference group

Table 2. Lipids, lipoproteins concentration and lipids and lipoproteins ratios and OxyStat status in HD patients and reference group

Parameter	HD patients n=12	Reference group n=18
TG (mg/dl)	113.08 ± 33.84***	89.4 ± 14.4
TC (mg/dl)	171.42 ± 39.68	181 ± 25.13
LDL-C (mg/dl)	110.50 ± 35.44	99.54 ± 21.97
HDL-C (mg/dl)	35.0 ± 8.28***	57.42 ± 7.6
Non-HDL (mg/dl)	133.25 ± 38.72	116.0 ± 22.52
apoAI (mg/dl)	129.50 ± 23.53***	157.3 ± 10.8
apoB (mg/dl)	81.17 ± 15.02*	69.53 ± 11.09
TC/HDL-C	4.83 ± 1.19***	2.96 ± 0.60
LDL-C/HDL-C	3.23 ± 1.04***	1.72 ± 0.62
TG/HDL-C	3.28 ± 1.32***	1.48 ± 0.59
apoAI/apoB	1.69 ± 0.43**	2.19 ± 0.35
HDL-C/apoAI	0.27 ± 0.04***	0.37 ± 0.04
OxyStat (0,µmol/L)	286.44 ± 137.98 ***	126.17 ± 62

***p<0.001, **p<0.01, *p<0.05 vs. reference group

DISCUSSION

Compared with the general population, dialysis patients have a high incidence of cardiovascular morbidity and mortality [22]. Atherosclerotic vascular disease is the main cause of increased mortality in the end-stage renal disease and undergoing maintenance hemodialysis, and it appears to

be caused by a synergism traditional (body mass index (BMI), hypertension, hypercholesterolemia) and non-traditional (inflammation, malnutrition, oxidative stress, and abnormal mineral metabolism) risk factors [5, 20, 22]. Maintenance hemodialysis patients suffer from accelerated atherosclerosis, which is at least in part, resistant to conventional pharmacotherapy [20].

Uremic dyslipidemia may be present in at least 25% of hemodialysis patients [10, 22]. Multiple pathophysiological mechanisms that are responsible for the development of uremic dyslipidemia remain to be defined [21]. The mechanisms underlying the decreased catabolism of triglyceride-rich lipoproteins may involve both the down-regulation of the expression of key genes along with the direct inhibitory effects of uremic toxins or cytokines on the lipolytic enzymes. Regarding the reduction in HDL-levels, two main mechanisms have been proposed: a diminished activity of LCAT- the enzyme responsible for the estrification of free cholesterol in HDL-particles- as well as increased activity of CETP that facilitates the transfer of cholesterol from HDL to TG-rich lipoproteins thus reducing the serum cholesterol concentration of HDL [21, 22].

The effects of long term hemodialysis on lipolytic activities are not be clarified. There are many controversies about the effects of dialysis duration on plasma lipid metabolism. Some authors have found that lipid and lipoprotein compositions do not appear to be influenced by dialysis duration. Some authors have not established any relationships between plasma TG level and dialysis duration, whereas others have found correlation between hypertriglycerydemia, cholesterol, lecithin-cholesterol acyltransferase (LCAT) activity and hemodialysis duration [12, 13].

Studied hemodialysed normotriglyceridemic patients presented a more decreased HDL-C and apoAI concentration than reference group, which is proven by disturbed lipid (significantly higher values of TC/HDL-C, LDL-C/HDL-C, TG/HDL-C) and lipoprotein (significantly lower values of apoAI/apoB) ratios. Based on them we can suggest that concentration of cholesterol in HDL particle in decreased (low HDL-C/apoAI ratio) but TG content is raised (high TG/HDL-C). Moreover increased concentration of peroxides in HD patients reflects the presence of oxidative stress. Such a picture may suggest impaired HDL maturation and disturbance in reverse cholesterol transport (RCT) [4, 7, 8].

Similar findings were shown in our latest studies [4] made on healthy subjects and post renal transplant patients [7,8], where HDL-C and apoA-I concentration were decreased and increased TG content in lipoproteins was correlated with decreased HDL particle concentration – a risk factor for CVD.

In this study we showed decreased HDL-C and apoAI concentration. These parameters are in a group of agents with antioxidant properties [19]. It has been suggested that renal replacement therapy in uremic patients on hemodialysis or peritoneal dialysis may contribute to oxidative stress and reduce antioxidant levels in these patient [19].

Several authors reported an intensification of lipid peroxidation in patients with chronic renal failure treated with maintenance hemodialysis and suggested that hemodialiysis by itself can accelerate atherosclerosis by increasing lipid peroxidation. They suggest that oxidative modification of LDL might explain the accelerated atherosclerosis in HD patients in the absence of major increases in native LDL [19]. HDL isolated from dialysis patient is dysfunctional and less effective in protecting LDL from oxidation as compared with HDL obtained from normal individuals, while at the same time LDL from dialysis patients is more readily oxidized than LDL obtained from normal subjects

[6, 16]. Moreover, normally protective HDL are to some extent transformed by incorporation of serum amyloid A (SAA) into inflammatory acute phase HDL [17, 23]. Thus, hemodialysis by itself seems to improve the lipid profile in patients with previous prooxidative state like uremia but the administration of antioxidants substances is probably advisable [19].

CONCLUSIONS

Decreased HDL-C and apoAI concentrations suggest disturbed composition and function of HDL lipoprotein particle which together with an increased oxidative stress may accelerate premature atherosclerosis in hemodialysed patients.

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SUMMARY

The main cause of increased morbidity and mortality among patients on maintenance hemodialysis is a cardiovascular complications due to accelerated atherosclerosis. The aim of this study was to evaluate parameters of lipid and lipoprotein profile, lipid and lipoprotein ratios and oxidative stress parameter for better understanding the feature of dyslipidemia in hemodialysed patients. The studies were performed in 12 hemodialysed patients at the age between 21–60 and a reference group (n=18). The venous blood after 14-hour fasting was taken to commercial tubes and centrifuged at 3000 rpm at 4^{o}C immediately after blood collection. Routine laboratory parameters (the level of urea, creatinine, total protein, albumin) were measured using Au 400 analyzer (Olimpus), and hemoglobin using ADVIA analyser, Bayer with using standard techniques. Lipids and lipoproteins were determined on Hitachi 902 analyser with standard techniques. The total concentration of peroxides was measured as Oxy-Stat by using ELISA method (Biomedica, Viena). The total cholesterol (TC) was estimated using the enzymatic-colorimetric method, triglycerides (TG) were determined using the standard enzymatic technique (BIOMAXIMA), HDL-C with the direct method with immunoinhibition (AB-WAKO - BIOMAXIMA). Non-HDL-C and a TC minus HDL-C, LDL-C with using Friedewald formula, lipids (TC/HDL-C, TG/HDL-C, LDL-C/HDL-C) and lipoprotein ratios (apoAI/apoB, HDL-C/apoAI) were calculated. Studied HD patients presented normotriglicerydemia but HDL-C

and apoAI concentrations were significantly decreased when compared to the reference group, which corroborates in disturbed lipid and lipoprotein ratios. Significantly higher TC/HDL-C, LDL-C/HDL-C, TG/HDL-C ratios and significantly lower HDL-C/apoAI and apoAI/apoB and are noticed in the studied patients than for the reference group. An increased concentration of oxidative stress parameter-Oxy Stat was also presented. Decreased HDL-C and apoAI concentrations suggest disturbed composition and function of HDL lipoprotein particle which together with increase oxidative may accelerate premature atherosclerosis in hemodialised patients.

Key words: lipids, lipoproteins, hemodialysis, cardiovascular disease

STRESZCZENIE

Choroby sercowo-naczyniowe, u których podłoża leżą zmiany miażdżycowe, stanowią obecnie główną przyczynę zgonów pośród pacjentów hemodializowanych. Celem pracy była ocena parametrów profilu lipidowego (TC, TG, LDL-C, HDL-C, nieHDL-C) i lipoproteinowego (apoA-I, apoB), wskaźników lipidowych i lipoproteinowych oraz markera stresu oksydacyjnego, jakim jest całkowite stężenie nadtlenków u pacjentów hemodializowanych. Badaniami objęto grupę 12 chorych hemodializowanych w wieku 21–60 lat oraz odpowiednio dobraną wiekowo 18-osobową grupę osób zdrowych. Materiałem do badań była surowica krwi otrzymana po odwirowaniu krwi pełnej pobranej na czczo (14 godzin od ostatniego posiłku). Rutynowe parametry laboratoryjne (stężenie białka całkowitego, albuminy, mocznika i kreatyniny) wykonano na analizatorze Au 400 (Olimpus), a stężenie hemoglobiny oznaczono na analizatorze ADVIA (Bayer) z wykorzystaniem metod referencyjnych. Całkowite stężenie nadtlenków oznaczono przy wykorzystaniu testu OxyStat techniką ELISA (Biomedica, Viena). Oznaczenia lipidowe i lipoproteinowe wykonano przy użyciu analizatora Hitachi 902 z wykorzystaniem metod referencyjnych. Na podstawie uzyskanych wyników obliczono stężenia nie-HDL-C, LDL-C oraz wartości wskaźników lipidowych i lipoproteinowych. U badanych pacjentów stwierdzono istotnie niższe stężenia HDL-C i apoA-I oraz normotriglicerydemię w stosunku do grupy kontrolnej. Potwierdzeniem zaburzeń lipidowych i lipoproteinowych są podwyższone wartości wskaźników TC/HDL-C, TG/HDL-C i LDL-C/HDL-C oraz obniżenie się wskaźnika apoA-I/HDL-C i apoA-I/apoB. Zaobserwowano również wzrost statusu oksydacyjnego na podstawie wzrostu OxyStat. Obniżenie stężenia HDL-C i apoA-I u pacjentów hemodializowanych może wskazywać na zaburzoną budowę i funkcję lipoproteiny HDL, co łącznie z obecnością stresu oksydacyjnego może przyczyniać się do przedwczesnego rozwoju miażdżycy i jej następstw.

Słowa klucze: hemodializa, lipidy, lipoproteiny, choroby sercowo-naczyniowe