GENDER DIFFERENCES IN LIPIDS AND LIPOPROTEIN (a) PROFILES IN HEALTHY INDIVIDUALS AND PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Background: This study aimed to assess gender differences in blood lipids and lipoprotein(a) levels in healthy individuals and patients with type 2 diabetes mellitus. **Methods:** This study was carried out at Department of Physiology, Army Medical College, Rawalpindi, Pakistan Sixty four patients suffering from type 2 DM and forty one healthy individuals were studied. The subjects were divided into healthy females, healthy males, type 2 DM females and type 2 DM males group. Fasting blood samples were analyzed for lipoprotein(a) [Lp(a)], total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), glucose and glycosylated hemoglobin (HbA1c). **Results:** When the lipid profile of healthy females was compared with the lipid profile of healthy males it was observed that HDL-C levels were significantly higher in healthy females as compared to healthy males (p < 0.05) while serum triglycerides were significantly raised in healthy males as compared to healthy females(p < 0.05). Diabetic females had significantly higher levels of LDL-C, HDL-C , TG and Lp(a) levels as compared to healthy females (p < 0.01, p < 0.05, p < 0.05 and p < 0.05). Diabetic males had significantly higher levels of TC, LDL-C, HDL-C and Lp(a) levels than healthy males (p < 0.01, p < 0.05, p < 0.01, and p < 0.05). The difference in lipid and Lp(a) profile was non significant between diabetic females and diabetic males. **Conclusions:** There are gender differences in lipid profile in patients with type 2 diabetes mellitus and well as healthy individuals. Diabetic individuals have raised levels of Lp(a) as compared to non diabetic subjects in case of both females and males. However in diabetic females and diabetic males there is no difference in lipid profile and Lp(a) concentrations. Further studies are needed to confirm these findings.
Key Words: Type 2 Diabetes Mellitus, Lipids, Lipoproteins, Lipoprotein (a) Glycosylated Hemoglobin.

INTRODUCTION

Heart disease is the number one cause of death in women, as it is in men; risk factors include high cholesterol, high triglycerides, low HDL-C, diabetes, hypertension, gender and cigarette smoking. The lipoprotein profile of a woman undergoes many changes during her lifetime because of the effects of endogenous hormones at pregnancy, the administration of oral contraceptives, and estrogen replacement at the menopause. Endogenous estrogen reduces the risk of heart disease in women as does unopposed estrogen replacement in the menopause. An increase in the incidence of coronary heart disease risk has commonly been reported in postmenopausal women.[1]
The incidence of coronary heart disease (CHD) is much lower in younger women than in age-matched men, and this has led to the popular misconception that cardiovascular disease is a disease of men, and is relatively rare in women. The incidence of CHD may be much lower in young women than in men of the same age, up to the age of 65. However after the age 65, the risk equalizes for both sexes.[2]
During the perimenopausal period there is a weight gain that does not seem to depend on the menopause

or HRT. Being overweight or obese during the menopausal transition is not necessarily associated with deterioration in coronary risk factors.[3]
Cardiovascular disease, may pose greater risk for women than for men. For example, the risk factors, testing modalities, presenting symptoms and the therapeutic choices made for women with coronary artery disease are significantly different from those for men. Low levels of high-density lipoprotein cholesterol (HDL-C), <35 mg/dL in men and <45 mg/dL in women, is associated with a greater risk of coronary artery disease and more progression of angiographically demonstrated disease in women, while increasing HDL-C has a more cardioprotective effect in the female than in the male population. The total cholesterol-to-HDL-C ratio is also more predictive of coronary artery disease in women than in men. Diabetes is particularly hazardous in women, and low HDL-C levels constitute a disproportionate risk for coronary artery disease in diabetic women compared with diabetic men.[4] Very high prevalence of dyslipidemia has been reported in healthy postmenopausal women.[5]
The misconception that women are at low risk of CHD has been influenced by many large studies of

cardiovascular disease in which women are under-represented, with the majority of trials being conducted in White, middle-aged men.[6-8] In addition to, or as a result of, the treatment bias, women with CHD also have a worse outcome than men. The precise reasons for the poorer outcomes are difficult to ascertain, but women tend to present at an older age and have more complicating factors such as diabetes, hypertension and heart failure than men.[9,10]

Most of the research on cholesterol and cardiovascular risk has been performed on men and the data extrapolated to women.[2] This approach has been questioned, since it is well-established that oestrogen affects lipid metabolism.[11,12] Lipoprotein levels strongly predict incident and recurrent CAD events in both sexes, and LDL particle size may be a better predictor of premature CAD in women than of CAD associated with advanced age. The effects of postmenopausal hormonal therapy on lipoprotein levels are complex, and the benefits of such therapy are not established. In contrast, lifestyle changes and pharmacological lipid-lowering therapy have been shown to favorably influence the natural course of atherosclerotic disease and reduce cardiovascular events in men and women.[13] Studies are needed to explore the differences in dyslipidemia of females and males. Moreover age related changes also need to be explored.

Therefore we aimed to study gender based differences in lipid and Lp(a) profile in non diabetic healthy individuals and patients with type 2 DM.

PATIENTS AND METHODS

This study was carried out at Army Medical College and Armed Forces Institute of Pathology (AFIP), Rawalpindi. Sixty four patients suffering from type 2 DM and forty one healthy individuals were studied. The subjects were divided into four groups.

Group A consisted of healthy female subjects
Group B consisted of healthy male subjects
Group C consisted of type 2 DM female subjects
Group D consisted of type 2 DM male subjects

All patients were diagnosed cases of type 2 DM. Patients were selected on the basis that there were non significant differences in their clinical characteristics and glycaemic status. Thirty four patients were males and twenty six were females. Their height was measured in centimeters and weight in kilograms. Body mass index (BMI) was calculated by the following formula;

BMI = Body Weight in Kilograms / Height (square meters)

All the patients were in stable metabolic condition. History was taken regarding any disease that could affect the metabolic status of the body and the parameters studied like nephrotic syndrome, acute or chronic renal failure, thyroid disorders, acute infections, diabetic ketoacidosis and non ketotic hyperosmolar diabetes. The patients having any of the above mentioned disorders were excluded from the study. Those patients giving history of familial hypercholesterolemias, ischaemic heart disease or myocardial infarction were also excluded from the study. The history of medication was recorded and the patients taking lipid lowering agents, oral contraceptives, hormone replacement therapy and steroids were also excluded.

Blood pressure (SBP/DBP) was recorded in sitting position in the right arm in mmHg. The subjects included in the healthy group were age, sex and BMI matched healthy individuals. They were not suffering from any acute infection or any metabolic or psychological disorder. They had no family history of hypercholesterolemias or DM. Their lipid profile and fasting blood glucose were estimated. They had normal lipid profile and fasting blood glucose level less than 6.1 mmol/l (110 mg/dl).

Glucose was estimated by GOD–PAP (Glucose Oxidase Phenyl Ampyrone) method, an enzymatic colorimetric method with the kit supplied by Linear Chemicals (Cat No.GL-5083). Total Cholesterol was measured by CHOD-PAP (Cholesterol Oxidase Phenol Ampyrone), an enzymatic colorimetric method, using kits of Linear Chemicals, Spain (Cat No. CH 5054). GPO-PAP (Glycerol Phosphate Oxidase), an enzymatic colorimetric method was used for serum triglycerides estimation. The kit was supplied by Linear chemicals (Cat No TR 5046). The instrument used was Selectra 2 autoanalyzer. CHOD – PAP Method was used for HDL-C and LDL-C estimation with the kit was supplied by Merck Systems (Cat No; 28248). Ion exchange resin separation method was used for estimation of Glycosylated Haemoglobin. The kit was supplied by Stanbio Glycohemoglobin [Pre-Fil]. Serum Lp(a) levels were measured immunochemically with a Sandwich ELISA that uses a mouse monoclonal anti-apo(a) antibody as the solid phase antibody and a sheep antiapo B-100 polyclonal antibody (antibody against B-100) as the detection antibody. The antibodies used in this assay identify all known isoforms of apo(a). There was no cross-reactivity with plasminogen and LDL. The kits used were supplied by Innogenetics Biotechnology for Health Care, Gent, Belgium.

The data was analyzed by SPSS (version 10, Chicago). Data was expressed as mean and standard error of mean (SEM). The tests applied for statistical analysis were one way analysis of variance (ANOVA) and Bonferroni (Multiple comparisons) for comparison differences between studied groups. p value = 0.05 was taken as significant.

RESULTS

Both healthy subjects and DM patients were divided into male and female groups and their clinical characteristics were compared. There were non significant differences in the clinical characteristics among different groups. All subject were matched for age and BMI. Both SBP and DBP were significantly higher in diabetic females as compared to healthy females (p< 0.0001 and p < 0.05 respectively). Also in males both SBP and DBP were significantly higher in diabetics as compared to healthy subjects (p< 0.0001 and p < 0.001 respectively). There was no difference in SBP or DBP between males and females in case of both healthy individuals as well as diabetics.

When the lipid profile of healthy females was compared with the lipid profile of healthy males it was observed that there was a significant difference in serum HDL-C and serum triglyceride levels while there was no difference in TC, LDL-C and Lp(a) levels. HDL-C levels were significantly lower in healthy females as compared to heathy males (p < 0.05) while serum triglycerides were significantly raised in healthy males as compared to healthy females(p < 0.05).

When heaithy females were compared with diabetic females we observed significantly higher levels of LDL-C, HDL-C , TG and Lp(a) levels in diabetic females (p < 0.01, p < 0.05, p < 0.05 and p < 0.05 respectively).

When heaithy males were compared with diabetic males we observed significantly higher levels of TC, LDL-C, HDL-C and Lp(a) levels in diabetic males (p < 0.01, p < 0.05, p < 0.01, and p < 0.05 respectively). In case of diabetic females and diabetic males the difference in lipid and Lp(a) profile was non significant.

DISCUSSION

Women have significantly different lipid, apoprotein, and lipoprotein profiles than men.regardless of menopausal status. In a population sample of women there were lower values of BMI, TG, TC/HDL ratio and higher levels of HDL-C than men.[14] Gender affects lipid parameters and this effect is independent of age and menopausal status.[15] Presumably these differences are due to the different levels of circulating sex hormones, specifically estrogens and androgens in women versus men. It has been reported that women have higher production rates of apoA-I, the major HDL apoprotein, than do men, and that levels of apoA-I and production rates of apoA-I and Lp A-I can be increased with estrogen administration.[16]

For screening purposes, one measurement of serum total cholesterol in a woman gives a good estimate of the long-term level. The current data indicate that repeated measurements of serum total cholesterol do not improve the predictability of future cholesterol levels. The data also suggest that, at least in postmenopausal women with an elevated level of serum total cholesterol, one should proceed immediately to lipoprotein analysis for further risk assessment.[17]

Table-1: Clinical characteristics, Glycaemic status and insulin levels in healthy and diabetic subjects (Data is expressed as Mean ± SEM).

	Healthy Females N=25	Healthy Males N=16	Diabetic Females N=29	Diabetic Males N=35
Age (years)	47.36 ± 1.14	46.62 ± 1.78	47.17 ± 1.72	48.24 ± 2.30
BMI (kg/ m2)	25.81 ± 0.65	24.96 ± 0.44	26.36 ± 0.99	25.32 ±.70
SBP (mmHg)	129.60 ± 2.56	125.31 ± 2.06	144.13 ± 2.45 ###	143.62 ± 2.96 ¶¶
DBP (mmHg)	79.60 ± 1.62	78.75 ± 1.67	85.34 ± 1.70 #	88.10 ± 1.72 ¶
FG (mmol/l)	5.02 ± 0.14	5.08 0 ± 0.12	10.75 ± 0.45	9.08 ± 0.65
HbA1c %	4.74 ± 0.08	4.82 ± 0.10	7.35 ± 0.24	6.95 ± 0.27

\# p< 0.05 compared to healthy females   # # # p< 0.0001 compared to healthy females
¶ p< 0.05 compared to healthy males   ¶¶ ¶p< 0.0001 compared to healthy male

Table-2: Fasting lipid and Lp(a) profile in healthy and diabetic subjects (Data is expressed as Mean ± SEM)

	Healthy Females N=25	Healthy Males N=16	Diabetic Females N=29	Diabetic Males N=35
T Chol (mmol/l)	4.28 ± 0.13	4.32 ± 0.18	4.65 ± 0.18	4.97 ± 0.19 ¶¶
LDL-C (mmol/l)	2.61 ± 0.12	2.59 ± 0.17	3.03 ± 0.13 ##	3.10 ± 0.19 ¶
HDL-C (mmol/l)	**1.20 ± 0.02**	1.08 ± 0.04*	0.95 ± 0.04 #	1.02 ± 0.04 ¶¶
TG (mmol/l)	1.12 ± 0.10	**1.46 ± 0.009***	2.05 ± 0.30 #	1.69 ± 0.12
Lp(a) [mg/dl]	20.82 ± 5.3	18.34 ± 4.3	47.58 ± 7.60 #	54.67 ±10.19 ¶

* p< 0.05 compared to healthy females   # p< 0.05 compared to healthy females,
\# # p< 0.01 compared to healthy females   ¶ p< 0.05 compared to healthy males
¶¶ p< 0.01 compared to healthy males

Increased rates of coronary heart disease (CHD) occur with advancing age in both sexes, although CHD rates in women lag behind those of men by about 10 years. There is a sharp increase in CHD rate among women after approximately 50 years of age. The reasons for this are not completely understood and are undoubtedly multifactorial. Cross-sectional data from large-scale population studies suggest that around the time of the menopause, low-density lipoprotein (LDL)-cholesterol levels increase by approximately 15 to 25%. Because this increase is larger than that observed in men over the same age span and closely approximates that observed in women after oophorectomy, it is likely that reduced circulating estrogen levels associated with menopause play a role in the adverse changes in both blood lipid levels and CHD incidence. There is clear evidence that treating hypercholesterolemia reduces cardiovascular risk in women, as well as in men. The favourable effects of statins on high-density lipoprotein (HDL)-cholesterol and triglyceride levels are more modest, and statins are not known to decrease lipoprotein (a) [Lp(a)] levels. Estrogen or hormone replacement therapy (ERT/HRT) and nicotinic acid improve LDL- and HDL-cholesterol levels and also decrease Lp(a) levels. Treatment should be individualised for each patient. It is important to evaluate the primary form of dyslipidaemia, other CHD risk factors, comorbidities, and the extent of lipid improvement needed in order to reach treatment goals.[18]

Hypertension is one of the major risk factors for the development of CHD. The incidence of hypertension increases with age and is higher in men than in women up to the age of about 50. Beyond middle age, however, blood pressure in women exceeds that in men. It has been suggested that menopause may potentiate the age-related increase in systolic pressure, perhaps as a result of reduced arterial compliance. Staessen et al[19] measured the blood pressures of 315 women and followed them up for a median of 5.2 years. Those women who were post-menopausal had a 4–5 mmHg higher systolic blood pressure than their pre- and perimenopausal counterparts, and also, while there was no change in systolic blood pressure in premenopausal women during follow-up, the systolic blood pressure increased by 4 mmHg in 5 years in the postmenopausal and perimenopausal women.

Large epidemiological studies have found a sex difference in lipoprotein distribution. These differences may in part explain the difference in the incidence of CHD between the sexes. Younger women, however, have lower LDL- cholesterol and higher HDL-cholesterol compared to men.[20] In contrast, elderly women have higher total cholesterol and younger men have lower HDL-cholesterol.[21] In a cross-sectional study, Weiss et al[22] found that postmenopausal women had significantly higher serum cholesterol compared to pre-menopausal women. In the Framingham Study[23] women between the ages of 29 and 62 years who were followed-up in a longitudinal study for 18 years demonstrated a significant rise in serum cholesterol levels between premenopausal and postmenopausal examinations, with the rise taking place within a short time of the onset of the menopause, thus suggesting a causal effect.

Nevertheless, not all studies have confirmed this relationship. For example, in a study of Pima Indian women, no association was found between the menopause and serum cholesterol levels.[24] It has been suggested that this is due to the diet of Pima Indians, which is generally lower in cholesterol than that of Whites and thus, environmental factors may play an additional and important role in the increase in cholesterol seen in older White women.

Lipoprotein (a) is a lipoprotein which has been related to thrombogenesis and atherogenesis. Clinical data, mainly from cross-sectional studies, have shown that this molecule can be positively associated with an increased risk of CHD and stroke.[25] In a study by Lip et al[26] plasma levels of lipoprotein (a) were significantly increased compared to base-line, following hysterectomy and bilateral oophorectomy. In the Framingham Offspring study,[27] however, no significant association was found between the menopause and lipoprotein(a) levels. Lipoprotein(a) is an independent risk factor for CHD and there is some evidence that lipoprotein (a) increases with age in women.[28] Another study has shown that Lp(a) was a significant risk factor for CAD in both pre- and postmenopausal women.[29] Lp(a) measurement is of value in investigation of patients at risk for CAD and that it is a particularly useful predictor of risk in women.[30]

Diabetes increases the risk of CHD threefold in women, and puts them at the same risk of CHD as men of the same age. Much of this excess risk is due to the excess in other coronary risk factors which occur in diabetics.[31]

In the Nurses' Health Study[32] body mass index was strongly associated with death due to CHD, with the risk of CHD over three times higher among women with a body mass index of 29 or higher. Much of this increased risk can be attributed to influences on blood pressure, glucose tolerance and lipid levels. However, the presence of diabetes seems to negate any cardioprotection that a woman may have. The findings of the present study are in line with these findings.

Conclusions

There are gender differences in lipid profile in patients with type 2 diabetes mellitus and well as healthy individuals. Diabetic individuals have raised levels of Lp(a) as compared to non diabetic subjects in case of both females and males. However in diabetic females and diabetic males there is no difference in lipid profile and Lp(a) concentrations. Further studies are needed to confirm these findings.

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