ORIGINAL ARTICLE

Pattern of dyslipidaemia and impact of increasing age and duration of type 2 diabetes mellitus on dyslipidaemia, insulin levels and insulin resistance

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Abstract

Objectives: To study the pattern of dyslipidaemia in Type 2 Diabetes Mellitus patients and to determine the correlation of increasing age and duration of the disease with dyslipidaemia, insulin level and insulin resistance in diabetic patients.

Methods: The cross-sectional case-control study was conducted at Combined Military Hospital, Rawalpindi, and Centre for Research in Experimental and Applied Medicine, Army Medical College, Rawalpindi, Pakistan from June 2011 to June 2012, and comprised patients of type 2 diabetes mellitus and healthy controls. Serum levels of total cholesterol, triglycerides, low density lipoprotein, high-density lipoprotein and insulin in both the cases and the controls. Insulin resistance was calculated by Homeostatic Model of Assessment of insulin resistance. Correlation between increasing age and duration of the disease was determined using biochemical parameters. SPSS 17 was used for statistical analysis.

Results: Of the 112 subjects in the study, 72(64%) were patients and 40(36%) were healthy controls. Among the cases, hypertriglyceridaemia was the commonest in 44(61%) followed by low-density-lipoprotein-hypercholesterolaemia 36(50%). Among the controls, 20(50%) subjects had low-density-lipoprotein-hypercholesterolaemia, followed by hypertriglyceridaemia in 17(42.5%). Duration of the disease was not found to be correlated with dyslipidaemia or insulin resistance ($p>0.05$). There was strong negative correlation of duration of the disease with serum insulin levels ($p=0.03$). Age showed no significant correlation with dyslipidaemia, serum insulin levels or insulin resistance on regression analysis ($p>0.05$ each).

Conclusion: In type diabetes mellitus, hypertriglyceridaemia was the commonest dyslipidaemia whereas hypercholesterolaemia was a risk factor in healthy individuals. Besides, the duration of disease was inversely correlated with serum insulin levels and positively correlated with dyslipidaemia.

Keywords: Diabetes mellitus, Dyslipidaemias, Insulin resistance. (JPMA 65: 928; 2015)

Introduction

The prevalence of diabetes mellitus (DM) worldwide was estimated to be 171 million people in the year 2000 and this is projected to increase to 366 million by 2030.[1] In Pakistan, the national diabetes prevalence is 6.76% and the comparative diabetes prevalence is 7.90%.[2] Obesity is strongly correlated to type 2 diabetes mellitus (T2DM). Dyslipidaemia, insulin levels and insulin resistance tend to worsen with progression of the disease and increasing age. Dyslipidaemia is found in metabolic syndrome and various patterns of dyslipidaemia in T2DM and obesity are reported in international[3] and national studies.[4-8] In Pakistani population, most of the studies found high levels of triglyceride (TG), total cholesterol (TC) and low-density lipoprotein (LDL)[7-13] whereas few studies reported only low high-density lipoprotein (HDL) levels in T2DM.[5,6,14,15] Few studies found normal HDL levels in DM.[16] Dyslipidaemia worsens with duration of the disease despite treatment and is the cause of diabetic complications. International studies report that increasing age and progression of the disease is a risk factor for dyslipidaemia, decreased insulin levels and enhanced insulin resistance in T2DM. A few studies so far reported the impact of increasing age on biochemical parameters in T2DM whereas no study in Pakistan highlighted the impact of duration of the disease on biochemical parameters in Pakistani subjects.

No study in Pakistan correlated the increasing age and duration of the disease with dyslipidaemia, insulin levels and insulin resistance though many studies reported the pattern of dyslipidaemia. The present study was conducted to evaluate not only the pattern of

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dyslipidaemia in T2DM patients and healthy subjects, but also the association of increasing age and duration of disease on dyslipidaemia, insulin levels and insulin resistance.

Patients and Methods

The cross-sectional case-control study was conducted at Combined Military Hospital, Rawalpindi, and the Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College (AMC), National University of Science and Technology (NUST), Rawalpindi, Pakistan, from June 2011 to June 2012. Approval was obtained from the AMC ethical committee before undertaking the study which was conducted in accordance with the current Good Clinical Practices and the Declaration of Helsinki 1975; latest version 2008.

Using non-probability convenience sampling technique, T2DM patients were selected as per World Health Organization (WHO) criteria[1] with history of T2DM for at least one year with fasting blood glucose >7mmol/L and >11.1mmol/L 2 hours after breakfast. Patients with type 1 DM (T1DM), co-existent endocrine disease, chronic renal failure, already on insulin or lipid-lowering drugs, smokers and those suffering from acute or chronic inflammatory disease were excluded. Exclusion criteria was ensured on the basis of history, physical examination and laboratory investigations, including blood complete picture (CP), erythrocyte sedimentation rate (ESR) after one hour by Westergren's method,[16] and renal function tests. All subjects were advised not to do any exercise or change dietary habits at least for two weeks prior to the blood sampling. Written and informed consent was obtained from each participant.

Next, 10ml venous blood sample was drawn with a clean venipuncture from antecubital vein under aseptic conditions in a disposable syringe from each individual. Blood was allowed to clot for 30 minutes at room temperature. After retraction of the clot, the serum was separated by centrifugation at 3000 cycles per second for 15 minutes. Serum was then transferred to small sterile tubes and stored at -20°C prior to biochemical analysis. Blood CP was performed on sysmex automated analyser, ESR was determined by Westergren's method,[16] serum glucose by glucose-oxidase method, serum TC by immuno-inhibition method,[17] serum triglycerides (TGs) by calorimetric method,[18] Serum LDL by immune-inhibition method, serum HDL by Freidewald formula,[19] and serum insulin by Enzyme Linked Immunosorbent Assay (ELISA). Insulin resistance was calculated by Homeostatic Model of Assessment of insulin resistance (HOMA-IR).[17-21]

Statistical analysis was done using SPSS17.

Mean ± standard deviation (SD) of age of the patients, duration of the disease, serum TC, TG, HDL, LDL, insulin and insulin resistance by HOMA-IR was determined. Spearman's correlation analysis was done to assess the association of dyslipidaemia, insulin levels and insulin resistance with age and duration of the disease. $P<0.05$ was considered statistically significant.

Among the cases, hypertriglyceridemia was the commonest in 44(61%) followed by low-density-lipoprotein-hypercholesterolaemia in 36(50%). Among the controls, 20(50%) subjects had low-density-lipoprotein-hypercholesterolaemia, followed by hypertriglyceridaemia in 17(42.5%).

Results

Of the 112 subjects in the study, 72(64%) were patients and 40(36%) were healthy controls. Among the cases, 40(55.5%) were males and 32 (45.5%) were females. Among the controls, 27(67.5%) were males and 13(32.5%)

Table-1: Demographic and biochemical parameters in Type 2 diabetes mellitus patients and healthy controls.

S. No.	Parameter	Study group Mean ± SD (Range) n = 72	Control group Mean± SD (Range) n = 40	P value
1	Mean age (years)	42.92 ± 3.09	36.65 ± 4.54	0.6
	Range	(30-50)	(30-50)	
2	Male n (%)	40 (55.5%)	27 (67.5%)	-
	Female n (%)	32 (45.5%)	13 (32.5%)	
3	Mean duration (years)	4.3 ± 4.27	-	-
	Range	(1-18)		
4	BMI	27.29 ± 4.12	24.78 ± 5.16	0.006
	Kg/m^2	(19.23 – 38.4)	(15.69 – 35.6)	
5	Mean S.TC (mg/dl)	182.13 ± 37.12	167.82 ± 35.96	0.04
	Range	(113.68-261.79)	(85.84-242.45)	
6	Mean S.TGs (mg/dl)	200±102.65	148.67±90.27	0.008
	Range	(69.03-525.66)	(43.36-472.57)	
7	Mean S.LDL-C (mg/dl)	100.54±28.61	104.4±27.6	0.47
	Range	(23.2-178.65)	(42.54-163.95)	
8	Mean S.HDL-C (mg/dl)	42.53±9.66	39.44±8.12	0.04
	Range	(18.94-90.48)	(29-63.8)	
9	Mean S. insulin (mIU/ml)	13.2 ±6.93	13.47 ± 5.12	0.83
	Range	(4.9-43.1)	(7.1-26.98)	
10	HOMA-IR	4.78 ± 2.61	3.14 ± 1.2	0.001
		(1.45-18.8)	(1.58-6.28)	

SD: Standard deviation
BMI: Body Mass Index
S: Serum'RC: Total cholesterol
RG: Triglyceride
LDL: Low-density lipoprotein
HDL: High-denity lipoprotein
HOMA-IR: Homeostatic model of assessment of insulin resistance.

Table-2: Comparison of dyslipidaemia, insulin levels and insulin resistance between study and control group.

Parameters	T2DM	Healthy
Obesity n (%)	46 (63.8%)	20 (50%)
Hypercholesterolaemia-total n (%)	22 (30.5%)	7 (17.5%)
Hypertriglyceridaemia n (%)	44 (61%)	17 (42.5%)
Hypercholesterolaemia-LDL n (%)	36 (50%)	24 (60%)
Low HDL n (%)	10 (13.8%)	12 (30%)
Hyperinsulinism n (%)	50 (70%)	08 (20%)
Insulin resistance n (%)	74 (88.8%)	*23 (59%)

*In obese persons only
T2DM: Type 2 diabetes mellitus
HDL: Hig-density lipoprotein.

Table-3: Correlation between serum insulin with duration of disease and insulin resistance.

Spearman's Correlation	p value	R coefficient	R^2coefficient
Duration vs. serum insulin	0.03	-0.247	0.057
Serum insulin vs. HOMA-IR	0.0001	0.864	0.775

HOMA-IR: Homeostatic model of assessment of insulin resistance.

were females. Mean values of age, duration of the disease, BMI, serum TC, TGs, LDL, HDL, insulin and insulin resistance of both study and control group were noted (Table-1). Mean duration of disease was 4.3±4.27 years. The BMI of cases was 27.29±4.12Kg/m^2) and that of the controls was 24.78±5.16Kg/m^2 (p=0.006). Serum TC, TG, insulin resistance (IR) was significantly higher and HDL was significantly lower in T2DM (p<0,05 each) compared to healthy controls whereas serum LDL and insulin levels were comparable in both groups (p>0.05).

In both groups, the subjects were divided into obese and non-obese, having hypercholesterolaemia, hypertriglyceridaemia, LDL-hypercholesterolaemia, and low HDL. The cut-off value for BMI was >25 kg/m^2 for obesity, >200 mg/dl for TC, >150 mg/dl for TG, >100 mg/dl for LDL and <40 mg/dl for HDL. The cut-off value for normal insulin was 10 μIU/L and for insulin resistance by HOMA-IR was 2.5.

Among the case, 46(63.8%) were obese and 26 (36.2%) were non-obese. Among the controls, 20(50%) were obese and 20(50%) were non-obese. Hypercholesterolaemia was found in 22(30.5%)cases versus.7(17.5%)controls, hypertriglyceridaemia in 44(61%) cases versus 17(42.5%) controls, LDL-hypercholesterolaemia in 36(50%)cases versus 24(60%) controls, and low HDL in 10(13.8%) cases versus 12(30%) controls (Table-2).

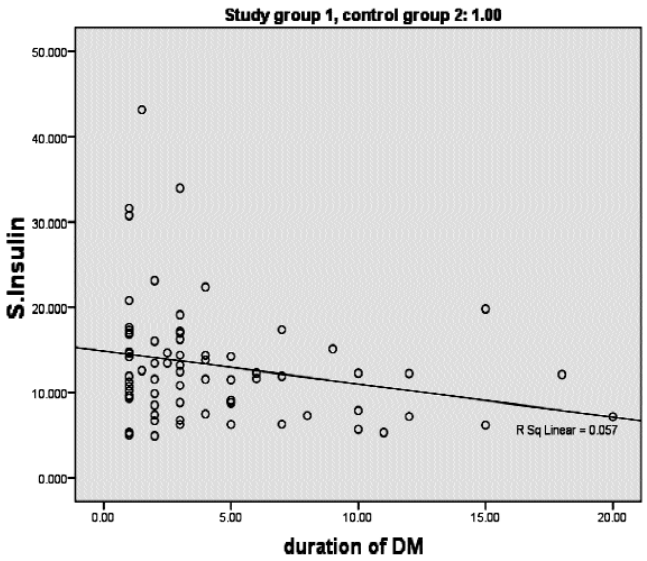

Figure-1: Correlation of serum insulin levels with duration of the disease in type 2 diabetes mellitus.

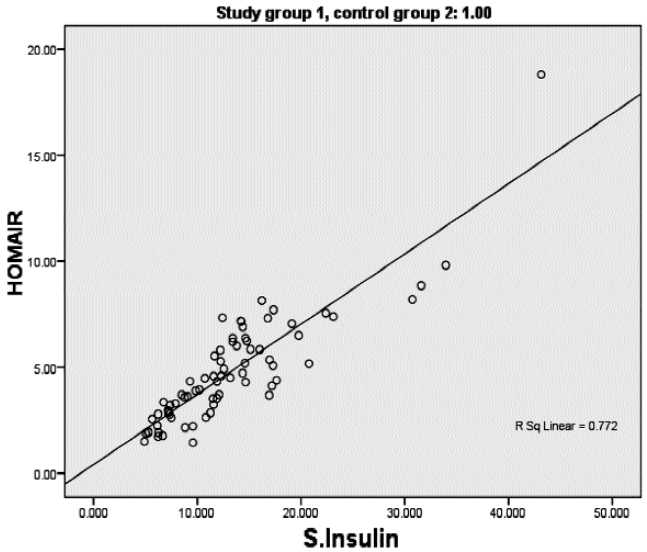

Figure-2: Correlation of serum insulin levels with insulin resistance in type 2 diabetes mellitus.

There was a definite increase in the percentage of patients having more than 150mg/dl of serum TG from 23.6% at two years of the disease to 37.5% after 5 years of the disease. Similarly, patients having LDL-hypercholesterolaemia increased from 18% after 2 years to 32% after 5 years of the disease. There was no definite increase in serum TC and HDL with duration of DM. On Spearman's correlation analysis, duration of the disease was not found to be correlated with dyslipidaemia or insulin resistance, but there was weak negative correlation of duration of the disease with serum insulin

levels (p<0.03; R coefficient=-0.247) (Table-3; Figure-1). Age showed no significant correlation with dyslipidaemia, serum insulin levels or insulin resistance on statistical analysis.

Hyperinsulinaemia was found in 50(70%) and insulin resistance in 64(88.8%) cases. In the study group, 42(58%) had severe and 30(42%) had mild insulin resistance. In obese but otherwise healthy controls, 11(59%) had mild insulin resistance by HOMA-IR index, indicating risk of T2DM in obese individuals. On Spearman's correlation analysis, in T2DM patients, serum insulin showed strong positive correlation with insulin resistance (p<0.0001; R coefficient=0.864) (Figure-2).

Discussion

The current study is the first one reporting association of increasing age and duration of disease on dyslipidaemia, insulin levels and insulin resistance in Pakistani T2DM patients. Types of dyslipidaemia and their frequencies have been reported in literature in T2DM as well as in healthy obese and non-obese persons in a number of international and national studies. In Pakistani patients, no study has reported correlation of duration of the disease with dyslipidaemia, insulin levels and insulin resistance.

In our study, hypertriglyceridaemia (61%) was the commonest followed by LDL-hypercholesterolaemia (50%), hypercholesterolaemia (30.5%) and low HDL (13.8%) in diabetic patients. This finding is in accordance with many other national studies in which Pakistani patients with T2DM reveal hypertriglyceridaemia the most common dyslipidaemia.[8-14] One study reported hypertriglyceridaemia followed by LDL-hypercholesterolaemia in males, while hypercholesterolaemia followed by LDL-hypercholesterolaemia in female Pakistani T2DM patients the most common dyslipidaemia.[11] In another study, S.TG was highest in DM patients followed by LDL as in our case although in the other study percentage of patients having dyslipidaemia was higher.[8] In contrast with our results, some studies[4,6,14,15] have reported low HDL as the commonest dyslipidaemia in DM patients, while a few reported hypercholesterolaemia as the commonest finding in Pakistani T2DM patients.[10,12] In one study,[22] none of DM patients had low HDL, unlike our findings. A study in Karachi reported combination of high LDL and low HDL the commonest finding.[23] In another study,[24] raised LDL was found to be more common than high TC in female Pakistani T2DM patients. Various pattern of dyslipidaemia in T2DM Pakistani patients could be due to varying sample size, variable duration of the disease and BMI of the study samples. Large mass-scale cohort should be investigated after catering to confounding factors to reach a definitive conclusion regarding the most common dyslipidaemia pattern in Pakistani population.

International studies also show varying results regarding pattern of dyslipidaemia in T2DM. In Chinese T2DM patients, hypertriglyceridaemia was found the commonest, followed by raised TC and then LDL, as was the case in our study.[3] Dyslipidaemia was found more pronounced in females.[3,5]

In healthy obese and non-obese controls in our study, commonest dyslipidaemia was LDL-hypercholesterolaemia (60%) followed by hypertriglyceridaemia (42.5%), low HDL (30%) and total hypercholesterolaemia (17.5%). In few studies,[6,25] high serum TG and low serum HDL were the commonest dyslipidaemia in healthy controls.

In our study, there was a definite worsening of dyslipidaemia with progression of the disease, but correlation was not found significant on statistical analysis. We found a weak negative correlation between duration of the disease and serum insulin levels (p<0.003; R coefficient: -0.247) (Figure-1). Other studies reported positive correlation of dyslipidaemia with duration of the disease.[5,10,26] A study reported that serum TC, HDL and LDL were not, but serum TG and very low-density lipoprotein (VLDL)were positively correlated to duration of DM.[5] In the same study,[5] serum TC, TG, HDL and VLDL were correlated to increasing age, while in our study that was not the case. In another study, dyslipidaemia was found to be worsened by increasing age in Iranian population.[27] Duration of the disease was not correlated to insulin resistance in our study. International data report that insulin resistance increases while insulin level decreases with increasing age and longer duration of the T2DM.[3]

Hyperinsulinism and insulin resistance found in majority of T2DM patients in our study is in accordance with other studies.[2-4] There was a strong positive correlation between insulin resistance and serum insulin levels in T2DM in our study (p<0.0001; R coefficient: 0.864) (Figure-2) as found in other studies.[6,11,24] Among healthy controls, insulin resistance was found in obese, but not in lean persons.

Future studies with large sample size can highlight the prevalence of dyslipidaemia and insulin resistance in healthy Pakistani population. Our study indicated that serum insulin levels decline and dyslipidaemia worsens with increased duration of the disease in T2DM. Dyslipidaemia along with hyperglycaemia rather than hyperglycaemia alone is the cause of diabetic

complications. Dyslipidaemia tends to worsen and should be treated along with control of blood sugar levels in patients of T2DM.

Limitation of our study was its small sample size. Moreover, subgroups based on BMI were also not studied. The external validity can be checked by replication of the study in a larger sample-sized study. Generalisability of targeted population with studied population cannot be reached due to the small sample size and can be achieved by replication of the study with larger sample size.

Conclusion

In T2DM, hypertriglyceridaemia was the commonest dyslipidaemia whereas hypercholesterolaemia was a risk factor in healthy individuals. Duration of the disease is inversely correlated with serum insulin levels and positively correlated with dyslipidaemia in T2DM.

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