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ASSOCIATION OF DYSLIPIDEMIA AND INFLAMMATORY MARKERS IN TYPE 2 DIABETES MELLITUS

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ABSTRACT: Background: The interplay between inflammation and lipids has recently been the focus of research aimed at understanding the development of Type 2 diabetes mellitus and the process of atherogenesis. Inflammatory markers particularly Tumor necrosis factor-α and high sensitive C-reactive protein interfere with lipid homeostasis and activates proinflammatory mechanisms in type-2 Diabetes mellitus. Our aim of the study was to analyze and correlate the inflammatory markers with lipids in Type2 Diabetes mellitus. 50 diabetic subjects and 50 normal healthy controls were recruited from the out-patient department of Narayana Medical college and hospital, Nellore, A.P). Serum TNF-alpha concentration was measured using Sandwich ELISA kit method. commercial enzymatic methods were used in the determination of serum total.cholesterol, HDL cholesterol and Triacylglycerides. LDL cholesterol was estimated using friedewald equation. Turbidimetric immunoassay (QUANTIA CRP- US) is used for ultrasensitive determination of high sensitive C-reactive protein in serum. Results: serum levels of TNF-α and hs-CRP were significantly elevated in type 2 diabetes mellitus and correlated positively with triacylglycerides, total cholesterol and LDL cholesterol but negatively with HDL cholesterol. We conclude from our study that there is a strong association between proathergenic lipid profile and inflammatory markers in Type2Diabetes mellitus and suggests a significant role of the inflammatory markers in the pathogenesis of dyslipidemia in Type2diabetes mellitus.

Key words : C-reactive protein , Tumor necrosis factor-α, lipids, Type 2 Diabetes mellitus.

INTRODUCTION

Metabolically triggered inflammation has been proposed as a key step in the pathogenesis of Type2Diabetes mellitus (T2DM) and accelerate atherosclerosis and premature death in subjects with T2DM [Pradhan et al 2007] . Atherosclerosis in T2DM can be attributed to an increased frequency of traditional risk factors such as obesity, dyslipidemia hypertension etc.[Doria et al 2005] . Apart from these traditional risk factors recent research has shown some novel disease related risk factors that may account for increased frequency of atherosclerosis and T2DM which includes proinflammatory cytokines and high sensitive C-reactive protein (hs CRP), an acute phase reactant (Bays et al 2008). The interaction between the inflammatory response and disrupted lipid homeostasis is implicated in several diseases and has recently been the focus of research aimed at understanding the development of T2DM and atherosclerosis (Klementia et al 2007).

TNF alpha is a multifunctional proinflammatory cytokine which exerts a myriad of biological actions in different tissues[William P.Cawthorn et al 2008]. hs CRP is a well known marker of inflammation (Noriyuki et al 2003). TNF-α and the acute phase protein hs-CRP interferes with lipid homeostasis and activates harmful proatherogenic and related pathological processes (Harold Bays et al, 2004). The most prominent alteration represent decreased HDL, increased LDL, and raised TAG [Klementia et al 2007].

TNF-α can directly alter lipid metabolism through inhibition of FFA uptake and lipogenesis and stimulate free fatty release via lipolysis and therefore contributes to the development of dyslipidemia and resultant metabolic complications (Jaswinder K.sethi 1999). Dysregulation of TNF-alpha production could be one facet in the development of disorders associated with glucose and lipid metabolism{warne}. TNF alpha and hs-CRP are key components in a number of metabolic diseases like obesity, type 2 diabetes, dyslipidemia , atherosclerosis etc(M. Cesari et al 2003). The novel insights into the interactions between the inflammatory markers and lipids might serve as a therapeutic targets to treat disorders associated with chronic inflammation, dyslipidemia and Type2 Diabetes mellitus (Calin Popa et al , 2007). Ultrasensitive measurements of CRP and TNF-α are extremely valuable markes for underlying systemic inflammation(Ridker et al ,1997). The Purpose of the present study was therefore to analyze and correlate the levels of TNF-α and hs-CRP with other covariates like blood glucose and lipid profile in T2DM and to study the impact of inflammatory markers on lipid metabolism in T2DM.

MATERIALS AND METHODS

The study was carried out on 50 newly diagnosed T2DM and 50 age and sex matched healthy controls recruited from the outpatient department of General Medicine, NMCH, Nellore, A.P. All subjects underwent a complete medical evaluation by a physician. Informed consent was obtained from all the subjects.

Both male and females between 20-65 years of age were included in the study. Individuals with severe inflammatory diseases, infections, cancers, hepatic or renal diseases and persons on antihyperlipidemic drugs, or insulin or other drugs that would affect blood glucose or lipid levels were excluded from the study. Pregnant and lactating women were also excluded from the study.

Blood samples were obtained in the morning by a venepuncture after overnight fasting. Serum was separated and stored at -20^{0}C. All samples were processed and examined according to principles of good laboratory practice at central laboratory,NMCH, Nellore, A.P. Glucose was measured by glucose oxidase method using automated chemistry analyzer(HUMASTER-300, GMBH, Germany) using Human kits(GmbH). Serum TNF-alpha concentration was measured using Sandwich ELISA kit method (e- Bioscience, Bender med systems) which has an interassay coefficient of variations of 7.5-10.5% and a lower limit of detection of 0.5pg/ml. commercial enzymatic methods were used in the determination of serum total. cholesterol, HDL cholesterol and Triacylglycerides. LDL cholesterol was estimated using Friedewald equation (Friedewald et al). Turbidimetric immunoassay (QUANTIA CRP-US) is used for ultrasensitive determination of hs CRP in serum with a lower detection limit of 0.015mg/dl.

Statistical analysis : Statistical analysis was done using SPSS-13 software version. Data was presented as mean ±S.D. Statistical comparison of cases and controls is performed by students t test. Spearmen's correlation analysis were used to analyze the correlation among the variables. $p< 0.05$ was considered to be statistically significant.

RESULTS

Table -1 demonstrates the mean ± SD values of the main characteristics of the cases and controls. T2DM subjects had significantly elevated levels of TAG , Total Cholesterol, and LDL lipoprotein ($p<0.005$) while HDL lipoprotein levels were significantly decreased ($p<0.005$) compared to healthy controls. TNF-α and hs CRP levels were also significantly elevated in T2DM compared to controls.Table -2 shows Spearman's correlations between TNF-alpha and lipid profile.

Strong positive correlation was seen with TNF-α and Triacylglycerides (R=0.34 P<0.05). A negative correlation between TNF-α and HDL cholesterol (r=-0.09 p<0.005) and between hs CRP and HDL cholesterol (r=-0.02 p<0.05) was observed.Fig-1 shows the negative correlation between TNF-α and HDL cholesterol.

Table I: Main Characteristics of the cases and controls

Variable (n)	Cases (50)	Controls (50)	P value
Fasting Blood Sugar mmol/lit	8.54 ± 3.6	4.587 ± 0.946	0.0001
Total cholesterol (mg/dl)	202.5 ± 42.6	172.8 ± 44.2	0.0009
LDL cholesterol (mg/dl)	119.16 ± 31.6	104.04 ± 21.8	0.0138
HDL cholesterol (mg/dl)	45.34 ± 11.7	50.26 ± 12.4	0.0444
Triacyglycerides (mg/dl)	154 ± 47.2	122.6 ± 61.6	0.0052
hs -CRP (mg/dl)	1.68±0.852	0. 14±0.068	0.0001
TNF α (pg/l)	215.180 ± 119.5	22.940 ± 25.2	0.0001

Data are expressed as mean ± S.D P<0.05 were significant

Table 2: Correlations among TNF-α, hs CRP and lipid profile in T2DM subjects

Compounds	TNF-α	hs CRP
	r	r
Triacylglycerides (mg/dl)	0.34**	0.50**
Total cholesterol (mg/dl)	0.48**	0.65**
HDL cholesterol (mg/dl)	-0.009***	-0.02**
LDL cholesterol (mg/dl)	0.045**	0.42*

*p<0.05, **p<0.005, ***p<0.0001 by sperman's correlation coeffe cient.

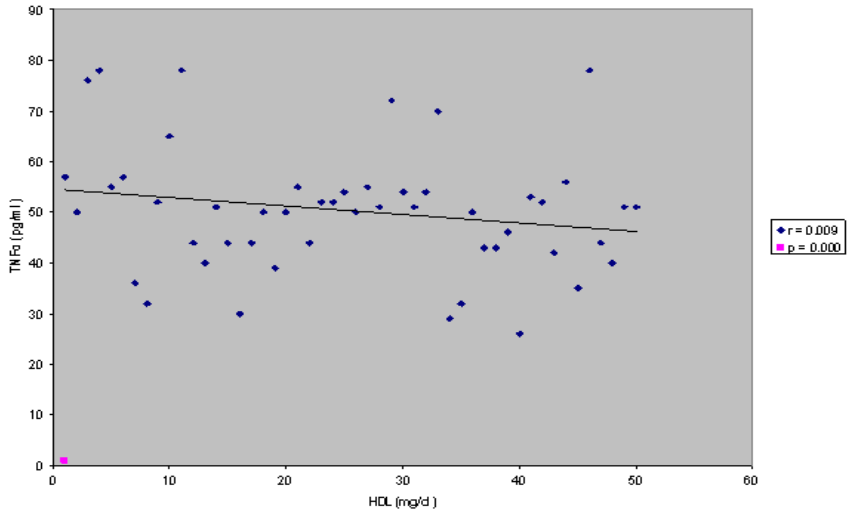

Fig-1: Correlation between TNF-α and HDL cholesterol

DISCUSSION

Previous studies in humans have shown that T2DM is associated with low grade inflammation, characterized by increased plasma levels of inflammatory markers like hs CRP and TNF-α [Ridker et al 1997]. Both TNF-α and hs CRP have been shown to cause disturbances in circulating and hepatic lipid metabolism (Klementia 2007). In our study we evaluated the relationship among lipids , TNF-α and hs CRP in T2DM. Results from our study confirmed previously reported associations between lipid profile and inflammatory markers (Pradhan et al 2001, Klementia et al 2007). TNF-alpha and hs CRP correlated positively with Triacyglycerides, total.cholesterol and LDL cholesterol but negatively with HDL cholesterol. This is in accordance with the observations of Nilsson et al 1998 which showed increased TNF-α levels in elderly diabetic subjects significantly correlated with fasting blood sugar, Triacylglycerides , and negatively with HDL cholesterol. Demimbras et al also observed that in subjects with myocardial infarction TNF-α correlated positively with Triacylglycerides and inversely with HDL. Consistent with these findings ,we observed significant association among lipids and inflammatory markers in T2DM. it has been previously shown that lack of TNF-α function results in significant reduction in obesity induced hyperlipidemia which would contribute to changes in insulin sensitivity (Ayo P. Doumatey et al 2010). The association between inflammatory markers and lipids has been shown to be a risk factor not only for the development of atherosclerosis but also for Cardiovascular disease in T2DM.[Noriyuki et al, 2003]. Our observations may help explain the coexistence of metabolic syndrome and cardiovascular disease in T2DM. Our results indicates that acute inflammatory response activates harmful metabolic pathways that are involved in chronic proatherogenic and related pathological changes and therefore suggests that inflammation contributes to dyslipidemia in T2DM .

Conclusion

We conclude from our study that there is a strong association between proatherogenic lipid profile and inflammatory markers in T2DM . our observations add further support to the hypothesis that the inflammatory markers, TNF-alpha and hs CRP are the key components in developing dyslipidemia in T2DM, and may serve as markers of the disease and might offer novel potential strategies and drug targets for dyslipidemia therapy in T2DM.

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