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A Putative Nexus betweenDyslipidemiaand HbA1c and Significant Target Of Glycative Impact Towards Development Of Cardio-Metabolic Risk In Diabetic Patients.

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ABSTRACT

Diabetic patients with accompanied (but often unnoticed) dyslipidemia are vulnerable to cardiovascular complications. An early intervention to normalize circulating lipids has been shown to reduce such complications and associated mortality. Glycated hemoglobin (HbA1c) is a measure of the glycative stress and a routinely used marker for long-term glycemic control. The present study was undertaken to evaluate the predictive value of glycated hemoglobin (HbA1c) in diabetic dyslipidemia.Venous blood samples were collected from 100 diabetic patients and 70 healthy matched control subjects. The whole blood and sera were analyzed for HbA1c, fasting blood glucose (FBG) and lipid panel test. Dyslipidemia was defined as per the National Cholesterol Education Programme (NCEP) Adult Treatment Panel (ATP) III guidelines. Diabetes was defined as per American Diabetes Association criteria. One way analysis of variance (ANOVA) result showed mean differences in lipid panel markers to be significant among the study groups comprised of diabetic patients with poor glycemic control against euglycemic control individuals. Level of HbA1c showed direct and significant correlations with triglycerides as well as VLDL concentrations. The correlation of HbA1c with cholesterol and LDL was positive and with that of HDL was negative but these were statistically non-significant. These findings clearly suggest that besides its primary role in monitoring long-term glycemic control HbA1c can be valuable predictor of associated dyslipidemia. Further this study reinforces the view that triglyceride as a potentially significant target of glycative impact towards development of cardio-metabolic risk in diabetic patients.

Keywords: Diabetes mellitus, Dyslipidemia, Glycated hemoglobin, Triglyceride.

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INTRODUCTION

Diabetes mellitus is a disease with a typical "iceberg" effect of prevalenceand is as old as civilization. Although increase in both the prevalence and incidence of diabetes have occurred globally, these have been especially dramatic rise of non-insulin-dependent diabetes (NIDDM) in the societies with economic transition, in newly industrialized countries and in developing countries. The combined number of people with Type 1 and Type 2 diabetes was predicted to be 425 million in 2017[1]. Diabetes contributed 1.6 million fatalities in 2019, making it the tenth greatest cause of death worldwide, according to the World Health Organization (WHO), which estimates that noncommunicable diseases (NCDs) caused 74% of deaths globally in 2019 [2].

Due to an array of physiological changes induced by insulin resistance and/or hyperinsulinemia, normal glucose tolerance is maintained during these conditions. It's interesting to note that there is a direct link between insulin resistance and the likelihood of developing cardiovascular diseases (CVD). The link between insulin resistance and CVD is mediated by a number of biological pathways. The role of insulin resistance in the development of atherosclerosis, vascular function, hypertension, and macrophage accumulation is one of these processes [3-7] and is often result into lipoprotein disorders, rendering the affected persons soft targets of cardiovascular disorders (CVD). Most of these individuals have characterized by increased levels of plasma triglycerides and LDL cholesterol and decreased HDL cholesterol. Individuals with coexisting diabetes and metabolic syndrome (dyslipidemia + hyperglycemia + hypertension) have the highest prevalence of CVD [8]. Glycated hemoglobin (HbA1c) is a routinely used marker for long-term glycemic control. In accordance with its function as an indicator for the mean blood glucose level, HbA1c predicts the risk for the development of diabetic complications in diabetic patients [9]. The Diabetes Complications and Control Trial (DCCT) established glycosylated hemoglobin (HbA1c) as the gold standard of glycemic control, with a plasma level of6%deemed to be appropriate cut-off for the risk of vascular complications [10]. Elevated HbA1c has been regarded as an independent risk factor for coronary heart disease (CHD) [11] and stroke [12] in subjects with or without diabetes. Estimated risk of CVD has shown to be increased by 18% for each 1% increase in absolute HbA1c value in diabetic population [13].

With this perspective of intricate interplay of glycative stress and dyslipidemia in the diabetic patients the present study was undertaken with the objective of finding that whether the markers of lipid profile in diabetic population alters significantly with glycative stress in terms of increase in HbA1c and also to detect any particular lipid parameter that might be significantly associated with such change in the level of HbA1c.

MATERIALS AND METHODS

The present study was carried out in thedepartment of Biochemistry, Mahatma Gandhi Institute of Medical Science, Sevagram, during October 2021 to February 2022. The study was designed in case-control format.

Study population

Total 100 diabetes mellitus patients of non-insulin dependent type from both in-patients and out-patients under Medicine department were included in the study. The protocol of this study was approved by the Institutional Human Ethics Committee. The cut-off value for fasting blood sugar of the patient >126 mg/dl and postprandial blood sugar >200 mg/dl was taken as inclusion criteria following recommendation of WHO. Age and sex matched 70 normal healthy individuals, without any family history of diabetes mellitus and without evidence of any major illness and obesity on clinical examination, was included in control group. Diabetic patients were further classified into 2 groups either as moderate or severe group according to their glycemic index following standard guidelines; first group consists of patients with HbA1c value (>6 to<10%) and second group consists of patients with HbA1c value (10% onwards) respectively. Consequently 43 persons were there in moderate and remaining 57 persons were recruited in severe categories respectively.

Biochemical analysis

About 3 ml of blood was collected from antecubital vein from each individual after taking informed consent. The plasma was used to estimate Blood sugars, and the serum was used to estimate lipid profile. After separating the plasma, the buffy-coat was removed and the remaining erythrocytes were used to prepare the heamolysate, by washing it with normal saline for three times and then adding carbon tetrachloride. The hemolysate was used for estimation of glycosylated hemoglobin by spectrophotometric assay of Parker [14].

Fasting blood glucose (FBG),Postprandial blood glucose(PP), serum triacylglycerol (TG),total cholesterol (TC), high density lipoprotein(HDL) were determined using commercial assay kit (Erba kit; Transasia Biomedical Ltd., Mumbai using XL-360) following manufacturer's protocol. The LDL-cholesterol was calculated using the Friedwald formula.

Statistical Analysis

The data were analyzed by SPSS version 16.0. One-way analysis of variance (ANOVA) followed by post-hoc turkey's multiple comparison tests were used to examine the levels of significance for various biochemical parameters among described study groups, viz. severe and mild diabetics along with the control group. Level of alpha error is restricted to 5% for consideration of rejecting the null. Pearson correlation analysis was carried out to find any significant correlation between the lipid panel markers and also with the HbA1c level in the diabetic persons as well as in non-diabetic control population. Further a regression modeling was done to confirm the dependence of the most significant lipid parameter on the level of glycated hemoglobin.

RESULTS

The diabetic population, belonging both to the moderate and severe group (based on their HbA1c level) was found to have higher levels of all the lipid panel parameters except HDL cholesterol level which showed a lower level as opposed to non-diabetic control group (Table 1). One way analysis of variance (ANOVA) result showed all these differences in lipid panel markers to be significant among the study groups comprised of diabetic patients with poor and worse glycemic control along with euglycemic control individuals. Significant higher levels of cholesterol ($F = 34.201$, $P<0.001$), low density lipoprotein ($F = 12.445$, $P<0.001$), triglycerides ($F = 26.861$, $P<0.001$), VLDL ($F=24.505$, $P<0.001$)) and significantly lower levels of HDL ($F = 38.660$, $P<0.001$) was recorded. Tukey's Post Hoc test further confirmed such significant difference both in severe as well as moderate diabetics against control group ($P<0.05$). But there were no significant differences between severe and moderate diabetic groups.

Table 2a shows Pearson correlation analysis which demonstrated in diabetic population as a whole there was highly significant positive association of HbA1c with TG and also with its mathematical functional derivative VLDL(as VLDL is directly calculated from the former). The correlation of HbA1c with Cholesterol and LDL was positive and with that of HDL was negative but these were statistically non-significant. However **Table 2b** showing all the lipid panel markers failed to show any significant association with HbA1c in non-diabetic control population under study. Certain significant association among lipid panel parameters was found; total cholesterol showed significant association with all other markers in both cases as well as in control population except for HDL which did not show such significant association in diabetic cases. HDL cholesterol showed negative association with total and LDL cholesterol in cases and controls. Triglycerides also showed significant association with VLDL in both cases and control for obvious reason. Moreover, TG was found to bear a significant association with LDL cholesterol in control as well as in cases. Finally linear regression was carried out to determine the impact of glycation on the lipid panel parameters; we selected TG for the apparent significant correlation between this parameter and HbA1c in diabetic cases. HbA1c was found to be actually a significant predictor of high triglyceride ($R^2 =0.055$, adjusted $R^2 =0.046$, $\beta = 0.335$; $p = 0.019$).

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Table 1: Status of lipid profile average in moderate & severe diabetic and control subjects

GROUPS	T. Cho. (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control (n= 70)	152.61 ± 37.49	133.16 ± 64.37	55.16 ± 19.99	69.11 ± 3141	26.8 ± 12.76
Moderate diabetes (n= 43)	210.28 ± 60.18	218.58 ± 124.80	43.19 ± 21.38	105.30 ± 45.27	43.61 ± 24.69
Severe diabetes (n= 57)	224.88 ±59.97	273.37 ± 135.66	41.32 ± 22.50	100.79 ± 53.69	53.63 ± 27.62

Table 2a: Correlation among the studied parameters in the diabetic subjects

Diabetics	Cholesterol	TG	HDL	LDL	VLDL	HbA1c
Cholesterol	1	0.273**	-0.066	0.618**	0.235*	0.122
TG	0.273**	1	.051	0.223*	0.974**	0.235*
HDL	0.066	0.051	1	-0.046	0.070	0.001
VLDL	0.235*	0.974**	0.070	0.275**	1	0.227*
LDL	0.618**	0.223*	-0.046	1	0.275**	0.082
HbA1C	0.122	0.235*	0.001	-0.082	0.227*	1

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Table 2b: Correlation among the studied parameters in the control subjects

Control	Cholesterol	TG	HDL	LDL	VLDL	HbA1c
Cholesterol	1	0.538**	0.366**	0.752**	0.542**	0.040
TG	0.538**	1	-0.034	0.244*	0.992**	0.064
HDL	0.366**	-0.034	1	0.132	-0.009	0.016
VLDL	0.542**	0.992**	-0.009	0.232	1	0.086
LDL	0.752**	0.244*	0.132	1	0.232	0.012
HbA1C	0.040	0.064	0.016	0.012	0.086	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

The present study showed significant alteration in lipid profile markers in diabetic populace as compared to the healthy control individuals which clearly reflected the derangement in lipoprotein metabolism due to diabetic pathology. However significant associations were found among lipid panel markers both in cases as well as in controls, which might possibly be attributed to the expected complex relationships among lipoprotein metabolites. Both of the subgroups comprising of patients with moderate and severe diabetics (classified based on glycemic index) mirrored the impact of formidable glycative stress. Among the study groups, diabetic populace with higher glycated hemoglobin level showed highest significance among all the groups as per post hoc statistical test. This indicates the underlying significance of glycative burden as a possible determinant of this metabolic pathology.

Correlation analysis displayed a significant association between TG and glycated hemoglobin in cases but not in control subjects, which further suggests possible impact of glycative stress responsible for alteration in lipoprotein metabolism. Hemoglobin glycation might be considered as a representative of the overall glycative force in operation in diabetic pathology. The non-enzymatic glycation of proteins and lipoproteins is linked to hyperglycemia and the vascular complications of diabetes. Earlier workers observed a direct correlation between HbA1c and the severity of coronary artery disease (CAD) in diabetic patients [15]. Conversely, improving the glycemic control can substantially reduce the risk of cardiovascular events in diabetics [16, 17]. Previous study reportedly observed significant elevation of soluble form of receptor for advanced glycation end products (sRAGE) in type 2 diabetic patients with

CAD and also demonstrated significant association between sRAGE and HbA1c as well as with serum lipid levels [18].

In the face of high glycation potential, atherogenicity of lipoproteins also increases. Particularly glycated LDL, which evades detection by classic LDL receptors, enhances accumulation of cholesteryl ester and results conversion of macrophages into foam cells along with proliferation of smooth muscle cells following binding of glycated LDL to macrophage receptors [19]. As an ensuing vicious cycle, impairment of degradation of LDL, sequestration of LDL in the arterial intima is prolonged culminating into additional glycation and oxidation [20]. In the present study we observed significant direct association of TG with the level of glycated hemoglobin. However, in this context it is worth mentioning the intricate inter-relationship among the lipoproteins involving TG and cholesteryl ester exchange between the forward and reverse transporter of cholesterol (LDL & HDL respectively) to accomplish the cholesterol disposal to liver (Harper)[21]. Hence, in this light, the observed association particularly of TG with the glycative force might have underlying pathological consequence. Moreover, glycation of HDL impairs its binding to HDL receptors, delaying HDL clearance and decreasing receptor mediated cholesterol efflux [8,22]. Glycation of VLDL as well as of apo-lipoproteins E and C can prolong the persistence of VLDL or apo-lipoprotein remnants in the circulation [23, 24]. Thus development of fierce interplay of glycation and altered lipoprotein metabolisms in overall diabetic pathology may be envisaged.

Triglyceride emerged as the most significant lipid panel marker in diabetic populace due to its significant link with the pathogenic glycation. Regression analysis also confirmed that higher triglyceride level is dependent on glycated hemoglobin, underscoring the significance of glycative stress in diabetic pathogenesis. However more importantly it highlights the putative pivotal role of triglyceride in the diabetic dyslipidemia. Interestingly, this parameter is also recorded to be significantly associated with the LDL level, another direct contender in atherogenic cascade. This reverberate the view of the patho-physiologic nexus between LDL and HDL through triglyceride factor. Our previous study with cardio-metabolic stress also highlighted the crucial role of triglyceride (communicated elsewhere). This view is also supported by the reported study in Japanese population that found insulin resistance, the major component of metabolic syndrome, to be significantly associated to both visceral fat as well as serum triglyceride level [25]. Moreover, such visceral fat was actually shown to be reflected by triglyceride and consequently VLDL level [26]. Therefore, evidence suggests more deep-seated involvement of triglyceride in the entire pathogenesis of cardio-metabolic pathology. Quite predictably, this parameter figures in both National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) as well as in WHO criteria of metabolic syndrome [27].

CONCLUSION

Hence, we conclude that the present study underscores the significance of triglyceride as the key parameter of the diabetic dyslipidemia which bears a direct causal relation reflecting the level of glycative stress as its major determinant. We advocate the measurement of triglyceride alone might be considered as a valid prognostic marker for assessing the cardio-metabolic risk profile in diabetic patients.

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