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Research Article

MEAL INDUCED OXIDATIVE STRESS LEVELS IN CONTROLLED AND POORLY CONTROLLED DIABETES

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ABSTRACT

Objective: The postprandial (PP) metabolic derangements are accentuated in Type 2 diabetes and are important risk factors for cardiovascular disease since they induce oxidative stress and endothelial dysfunction. The aim of the study was to analyze meal induced oxidative stress levels in controlled and poorly controlled diabetes.

Methods: Total 60 Type 2 diabetic patients on oral hypoglycemics of duration 5-15 years were divided into two groups based on hemoglobin A1c values (≤7.5% for controlled/Group 1 and ≥7.6% for poorly controlled/Group 2). They were assayed for serum/plasma glucose, thiobarbituric acid reacting substances (TBARS) and oxidized low-density lipoprotein (ox-LDL) parameters both in fasting and 2 hrs post meal in both groups. Statistical analysis was performed using independent t-test between the groups and paired t-test within each group.

Results: The PP TBARS were found significantly higher in both groups, whereas no significant difference between the two groups. The ox-LDL levels were found similar at two points of time in both groups.

Conclusion: An exaggerated PP oxidative stress levels are associated with diabetes and its complications including endothelial dysfunction.

Keywords: Thiobarbituric acid reacting substances, Oxidized low-density lipoprotein, Hemoglobin A1c, Diabetes.

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INTRODUCTION

Diabetes mellitus is a group of common metabolic disorders that share the phenotype of hyperglycemia as a consequence of defect in insulin secretion and or insulin action [1,2]. The prevalence of diabetes is increasing the worldwide which need more measures for prevention and control [3]. Oxidative stress in diabetes, as in other ailments, is a result of the hyper-production of reactive oxygen forms on one hand and hypoactivity of antioxidative system on the other [4]. Numerous studies indicate that postprandial (PP) metabolic derangements, most notably hyperglycemia and hypertriglyceridemia, are exaggerated and prolonged in Type 2 diabetes and are important cardiovascular disease (CVD) risk factors since they induce oxidative stress and endothelial dysfunction [5].

Studies are notably conducted using a 75 g glucose load. It is also well-documented that physiological variations in response to oral glucose load and a mixed meal exist. Hence, this study was undertaken to observe the effect of regular diet on oxidative stress levels in Type 2 diabetic patients.

METHODS

The study was conducted at Kasturba Medical College and Hospital, Mangalore. Protocol was approved by Institutional Ethics Committee. Informed consent was obtained from all the patients. Patients enrolled into the study were Type 2 diabetic patients of duration 5-15 years on oral hypoglycemics. Patients on insulin were excluded from the study. Patients were divided into two groups of 30 individuals each based on hemoglobin A1c (HbA1c) values (Group 1 with HbA1c≤7.5% and the Group 2 HbA1c ≥7.6%). A brief history of diabetes was taken and 5 ml plain blood and 2 ml fluoride blood was collected in sterile vacutainer from each subject in fasting as well as 2 hrs after mixed meal. Patients were provided a meal (breakfast) amounting approximately 300-500 kcal. They were allowed to choose the food of their liking but restricted to the calories. Approximate calorie content of common breakfast items available was considered. For example dosa (plain) - 120 kcal, idli (3 big) - 130 kcal, uttapam (2 big) - 330 Kcal, sambar (1/2 cup) - 105 kcal, bread (2 slices) - 60 kcal, and egg boiled (1) - 55 kcal. The samples were assayed for plasma glucose (PG), thiobarbituric acid reacting substances (TBARS), and oxidized low-density lipoprotein (ox-LDL).

Malondialdehyde estimation was done manually by TBARS method [6-8]. 0.5 ml serum was precipitated with 2.5 ml of 10% phosphotungstic acid. After standing for 10 minutes was centrifuged at 3000 g for 10 minutes. The sediment was suspended in 4 ml distilled water. 4 ml distilled water treated similarly was used as the blank. This was followed by addition of 0.5 ml glacial acetic acid and 0.5 ml 0.33% thiobarbituric acid. It was kept in a water bath at 97°C for 45 minutes. The tubes were cooled and 1 ml 5 M HCl was added to the tubes to lower pH of the solution to <2 (1.6-1.7). The pink color was extracted with 5 ml butanol. The butanol layer was transferred to a spectrophotometer cuvette. Absorbance was read at 535 nm. TBARS (μmol/L) = $A \times V_s \times 1000/\varepsilon \times V_t$, where, ε (molar absorbance of TBARS)=1.56×10^5, A=Absorbance, V_s=Volume of solution in cuvette, V_t=Volume of serum. TBARS values were expressed as μmol/L. The ox-LDL was done with simple precipitation method [9,10].

Comparison between both groups was done using independent t-test and comparison within each group at two points of time was done using paired t-test.

RESULTS

Patients in Group 1 and Group 2 were comparable with respect to age and duration of disease. The mean calorie intake was also similar between the two groups (Table 1). Significant difference in mean fasting PG and mean PP-PG values were found. An increase in PP-PG by 60 mg/dl in Group 1 and nearly 78 mg/dl in Group 2 from the baseline values was noted. Poorly controlled Group 2 displayed significantly higher levels of

all the mean glycemic indices measured (Table 2). Markers of oxidative stress TBARS and ox-LDL were measured in fasting and PP state and compared. Fasting values of TBARS were compared with PP within each group which showed a statistically significant increase in both groups. Such variation was not observed in the case of ox-LDL (Table 3 and Fig. 1).

Table 1: Age, duration of diabetes and calorie intake in both groups

Parameters	Mean±SD		p value
	Group-1	Group-2	
Age (years)	55.3±12.3	54.6±11.4	0.207^{ns}
Duration of diabetes (years)	6.4±1.4	6.9±2.7	0.817^{ns}
Calorie intake (kcal)	398.8±104.1	440.2±120	0.160^{ns}

Ns: Not significant, SD: Standard deviation

Table 2: Comparison of glycemic indices between the groups

Parameters	Mean±SD		p value
	Group-1	Group-2	
HbA1c (%)	6.68±0.47	8.86±1.17	<0.001***
FPG (mg/dl)	120.9±18.07	161.7±47.48	<0.001***
PP-PG (mg/dl)	180.7±55.66	240.5±82.23	0.002**

**Highly significant, ***Very highly significant. FPG: Fasting plasma glucose, PP-PG: Postprandial plasma glucose, SD: Standard deviation, HbA1c: Hemoglobin A1c

Table 3: Comparison of oxidative stress markers in fasting and postprandial states

Parameters	Group-1		Group-2	
	Mean difference (F vs. PP)	p value	Mean difference (F vs. PP)	p value
TBARS (µmol/L)	2.729	<0.001***	2.046	<0.001***
ox-LDL (µmol/L)	0.026	0.821^{ns}	0.004	0.948^{ns}

***Very highly significant. ns: Not significant, F: Fasting, PP: Postprandial, TBARS: Thiobarbituric acid reacting substances, ox-LDL: Oxidized low-density lipoprotein

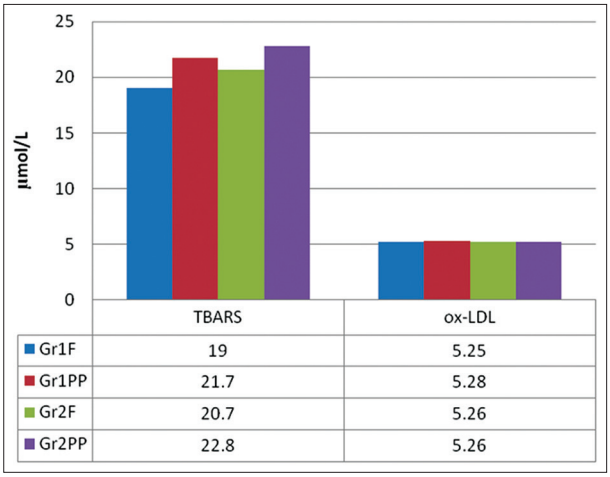

Fig. 1: Comparison of mean thiobarbituric acid reacting substances (TBARS) and oxidized low-density lipoprotein (ox-LDL) levels. Gr1F=Group 1 fasting state, Gr1PP=Group 1 postprandial state, Gr2F=Group 2 fasting state, Gr2PP=Group1 postprandial state

DISCUSSION

Correlation of HbA1c with both fasting and PP glucose is well proven [11-14]. In this study, both groups had PG levels comparable to their HbA1c values.

Mean PG showed a rise by 78 mg/dl in Group 2 from fasting to PP state. The rise was 18 mg/dl higher than that seen in Group 1 (60 mg/dl). This could be attributed to the result of poor glycemic control (mean HbA1c 8.8%).

Studies by Ceriello *et al.* [15] in diabetics reported PP increase in PG, triglyceride TG, and TBARS after a standard meal. This study with regular diet also showed a significant increase in PP glucose and TBARS in both groups.

Studies have demonstrated a positive correlation between PP TBARS and TG [16-18] and that with PG [19]. This marker of oxidative stress has also been found to have a positive correlation with endothelial dysfunction [18].

High serum TG has been found to augment the oxidation of low-density lipoproteinLDL in fed state. Exaggerated LDL oxidation seen in PP state in diabetes may be a decisive contributor to CVD risk. Sensitivity of LDL to oxidation has been well studied *in vitro* and the maximal oxidation occurs at 120 minutes following the peak oxidative stimulus [20]. However, the time frame to measure peak ox-LDL *in vivo* has not been defined [21]. In this study, ox-LDL values were measured in fasting and 2 hrs post meal and were found comparable. In animal studies, it was observed that after injecting highly oxidized and acetylated LDL intravenously, it disappeared from plasma within a matter of minutes indicating very short half-life. This reflects extremely rapid uptake of modified LDL by hepatic Kupffer cells and sinusoidal endothelial cells [22].

In this study, PP TBARS were high but contrary to this ox-LDL levels remained unaltered from their fasting values in both groups. Low PP ox-LDL levels despite a significant increase in mean PP TBARS levels could be attributed to short half-life of ox-LDL due to rapid uptake or longer time required for oxidation which needs further evaluation.

CONCLUSION

Regular diet with identical calorie intake has caused higher post prandial glycemia in poorly controlled Type 2 diabetes. Post meal oxidative stress as measured by TBARS was found to be present and greatly increased in Type 2 diabetes irrespective of glycemic control.

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