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Biochemistry Section

Fructosamine in Non-diabetic First Degree Relatives of Type 2 Diabetes Patients: Risk Assessor

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ABSTRACT

Introduction: A positive family history of diabetes increases the chances of developing the disease manifold. The earliest diagnostic marker for diabetes is elevated plasma glucose levels. Glycosylated haemoglobin (HbA1c) gives information on the long term control of diabetes, while the estimation of fructosamine (FA) depicts the short term glycaemic control. The specificity of the estimation of fructosamine and its comparison with the established markers in a group with high risk for the disease was the purport.

Methods: 23 non-diabetic first degree relatives of type 2 diabetics (Group 2) were compared with 20 healthy controls (Group 1) and 23 type 2 diabetic people (Group 3). Fasting plasma glucose (FPG), glycosylated haemoglobin (HbA1c), serum fructosamine and total proteins (TP) were estimated in fasting blood samples. The saliva was analyzed for fasting salivary glucose (SG), salivary fructosamine and total proteins. The body mass index (BMI), waist circumference (WC) and the blood pressure were recorded and compared.

Results: Group 3 had significantly higher mean FPG (150.26mg/dl), HbA1c (8.23 %) and salivary FA (202.05 mg/dl) values. Group 2 was associated with elevated serum FA levels (533.62 mg/dl), increased serum FA/ total protein (TP) ratio and a larger WC. On correlation analysis, FPG correlated significantly and positively with HbA1c in all three groups and with WC and BMI (0.613 and 0.400 respectively) in Group 2 only.

Conclusion: Serum FA foretells the development of diabetes in high risk populations, but it has less sensitivity in depicting chronic hyperglycaemia. Serum FA and WC could be useful predictors of the development of diabetes in 'high risk' individuals.

Key Words: BMI, Fructosamine, HbA1C, Saliva, type 2 diabetes, Waist circumference

INTRODUCTION

India currently harbours around 40 million people with diabetes as per 'The Diabetes Atlas 2006' which was published by the International Diabetes Federation. There is an imminent danger of a further rise in this number to 80 million by 2025, unless vital precautionary measures are taken. It has been established that subjects with a family history of diabetes or an increased body mass index [BMI], patients with hypertension, people who do stressful jobs and patients with dyslipidaemia [1] are the ones who are susceptible for type 2diabetes. Except the family history, all other factors which are mentioned above are co-morbidities which are associated with diabetes. Hence, the earliest target population to introduce preventive measures in or to study the risk predictors of would be the non-diabetic first degree relatives of the diabetes patients. The objective of this study was to compare the specificity of fructosamine [FA] estimation in the first degree relatives of diabetics with that in those without a family history and in known diabetics, so as to weigh the benefit of this glycaemic index in categorizing the high risk subjects.

METHODS

A cross-sectional, case control study was conducted at the Clinical Biochemistry laboratory of Kasturba Medical College Hospital [KMCH], Ambedkar circle, Mangalore, over a period of 6 months. Only 50 healthy people consented to participate in the current study as it required the sampling of blood and saliva, of which there were 7 dropouts. They were divided into two groups. Group 1 consisted of 23 healthy controls with no family history of diabetes and Group 2 consisted of 20 non-diabetic first degree relatives of type 2 diabetics. They were compared with 23 type 2 diabetes patients from the diabetes clinic, who were on treatment and who were marked as Group 3.

An informed consent was obtained from all the subjects. The protocol was approved by the institutional ethics committee.

People with FPG ≥ 110 mg/dL (For Group 1), FPG ≥ 126 mg/dL (For Group 2) and a history of infection in the past three months, chronic alcoholics and pregnant females were excluded from the study.

After an overnight fast of about 10 hours, blood was drawn from the ante-cubital vein and it was collected in vacutainers which contained fluoride and EDTA and also in plain bottles. The fluoride sample was used for evaluating the fasting plasma glucose, which was measured by the GOD POD method [2] and the EDTA sample was used for the estimation of HbA_{1C} by an automated Immuno Turbidimetric method [3], both of which were processed in a Hitachi 917 Autoanalyzer. Serum was obtained by centrifugating the samples for 15 minutes at 3000 rpm and it was used for the manual estimation of fructosamine and total proteins. Other details like age, sex, blood pressure, waist circumference and height and weight were documented. The BMI [body mass index] was calculated as follows. BMI = Wt. in Kg/Ht. in M^2.

Whole unstimulated saliva was collected from all the participants in sterile containers. Post collection, the saliva was centrifuged for a period of 5 minutes at 2500 rpm and the clear supernatant which was obtained was used for estimating salivary glucose, salivary total protein, and salivary fructosamine.

Salivary glucose was assayed spectrophotometerically by the GOD POD method by using the Aggape Diagnostic kit. The salivary total protein was estimated by Lowry's method [4] and fructosamine was estimated by the NBT method [5].

STATISTICS

Statistical analyses were performed by using the SPSS software (version 16.0; SPSS Inc, Chicago). The data was presented as mean ± standard deviation and it was analyzed by ANOVA. Pearson's correlation coefficient ('r' value) was used to compare the data. All the *p*-values were based on 2-sided tests, and the cut off for the statistical significance was 0.05.

RESULTS

[Table/Fig-1] illustrates the anthropometric measures of the study groups. The 3 groups showed no significant difference in the BMI, whereas the waist circumferences of Group 2 and Group 3 were significantly higher than that of Group 1. Group 3 also showed significantly higher systolic blood pressure [SBP] and diastolic blood pressure [DBP] levels in comparison to those of other groups.

Of the glycaemic indices which were measured, the FPG and the HbA_{1C} values were significantly higher in Group 3, and the serum FA values were higher in Group 2 [Table/Fig-2]. The values of serum TP did not differ between the groups. On adjusting the serum FA to the serum TP levels by taking a ratio, the significance still persisted in Group 2 in comparison to the diabetic group and the controls.

The salivary parameters viz., salivary glucose, and TP showed comparable results in all the three groups and significantly high salivary FA values in Group 3.

On comparison of FPG with other measured indices of glycaemia [Table/Fig-3]; it was found that FPG correlated with HbA_{1c} in all the three groups and with serum FA in the diabetic group only.

A significant correlation of FPG with both BMI and WC was observed only in Group 2 [Table/Fig-4].

DISCUSSION

The first degree relatives were comparable in age with the control group and they were normotensive, but a significantly higher WC was observed [Table/Fig-1]. The type 2 diabetic group had a significantly higher WC and higher systolic and diastolic blood pressure levels. Earlier studies [6] which were conducted in India stated that though the BMI in the Indian population was low or near normal, the waist circumference – the measure of central adiposity, was higher. This has been explained by the fact that Asian Indians have more total abdominal and visceral fat for any given BMI and that for any given body fat, they have increased insulin resistance [7-10].This excess abdominal fat is in turn a high risk factor for diabetes due to the insulin resistance which arises from the release of free fatty acids [11].

There exists a difference of opinion regarding the usage of HbA_{1c} for the screening or the diagnosis of diabetes [12]. The data from previous studies have suggested that the combined use of FPG and HbA_{1c} helps in detecting undiagnosed diabetes, especially in high risk individuals [11, 13]. Some studies [14,15] have shown that HbA_{1c} is not an optimal screening test for diabetes in individuals with impaired glucose tolerance (IGT). The current study included high risk individuals [first degree relatives] with FPG levels which ranged between 85–111mg/dL [Table/Fig-1]. This group had a mean HbA_{1c} of 6.03% as compared to 5.8% in the controls, which

Parameters	Group 1 Controls n = 23	Group 2 First degree relatives n =20	Group 3 Diabetics n =23
Age (yrs)	39.1 ± 1	38.4 ± 7.4	60.2 ± 10.3
BMI	22.3 ± 4	24.9 ± 5.3	25 ± 3.2
WC (cm)	79.9 ± 9.6	87.5 ± 9.0*	90.5 ± 11.1*
SBP (mm of Hg)	118.2 ±12.9	118.1 ± 11.3	135.4 ± 19.7*†
DBP (mm of Hg)	75.9 ± 9.1	77.4 ± 9.2	85.5 ± 8.1*†

[Table/Fig-1]: Anthropometric measures of the study groups (Values are Mean ± SD)

BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; WC: Waist circumference; *p ≤0.05 (Group 1 V/s Group 2 & 3), † p ≤ 0.05 (Group 2 V/s Group 3)

	Group 1 Controls n = 23	Group 2 First degree relatives n =20	Group 3 Diabetics n =23
FPG (Range mg/dL)	94 ± 5.4 (85-106)	98.5 ± 6.7 (85-111)	150.3 ± 61.3*† (95-302)
HbA_{1C} (Range)	5.8 ± 0.4 (5-7 %)	6.0 ± 0.4 (5-7 %)	8.2 ± 1.7*† (6-13 %)
Serum FA (Range nmol/L)	406.7 ± 160.1 (211-708)	533.6 ± 98*† (413- 708)	462.6 ± 155.3 (217- 708)
Serum TP	9.6 ± 2.3	8.6 ± 2.3	9.7 ± 1.7
Serum FA/TP	45.8 ± 24	68.6 ± 29.6*†	45.6 ± 18.8
Salivary glucose (Range mg/dL)	9.4 ± 6.8 (3-22)	9.2 ± 5.9 (3-24)	8.8 ± 4.4 (4-22)
Salivary Fructosamine	99.8 ± 50.1	130 ± 71.6	202.1 ± 103.4*†
Salivary Total Proteins	139.4 ± 39.3	120.4 ± 47	147 ± 65.3

[Table/Fig-2]: Overview of serum & salivary parameter comparison between groups (Values are Mean + SD)

FPG: Fasting Plasma Glucose; HbA_{1C}: Glycosylated hemoglobin; FA: Fructosamine; TP: Total Proteins; * p ≤ 0.05 (Group 1 V/s Group 2 & 3); † p ≤ 0.05 (Group 2 V/s Group 3).

Parameters	Group 1 n= 23	Group 2 n = 20	Group 3 n = 23
FPG v/s HbA_{1c}	0.586*	0.583*	0.569*
FPG v/s Ser FA	–0.074	0.256	0.596*
FPG v/s Salivary FA	0.061	0.171	–0.078
FPG v/s SG	0.063	0.170	–0.039

[Table/Fig-3]: Comparison of FPG with other glycaemic indices

FPG- Fasting plasma glucose; HbA1c- glycosylated Hemoglobin; FA- fructosamine; SG – salivary glucose; * r ≥0.4 is significant.

Parameters	Group 1 n= 23	Group 2 n = 20	Group 3 n = 23
FPG v/s WC	0.347	0.613*	0.174
FPG v/s BMI	0.196	0.400*	0.189
FA v/s WC	0.221	-0.087	-0.326
FA v/s BMI	0.091	-0.261	-0.311
HbA1c v/s WC	0.123	0.129	0.029
HbA1c v/s BMI	0.077	-0.124	-0.043

[Table/Fig-4]: Glycemia Vs metabolic status

FPG- Fasting plasma glucose; HbA1c- glycosylated Hemoglobin; FA- fructosamine
* r ≥0.4 is significant

was indicative of a risk for diabetes at a similar FPG range [85–106 mg/dl]. Wiener and Roberts (1998) [16] suggested that the HbA_{1c} values which were > 6.2% had a 100% specificity for the diagnosis of diabetes. The first degree relatives in the present study had HbA_{1c} in the range of 5-7% with near normal FPG [85-111 mg/dl], but with significantly high serum FA values.

FA depicts the glycaemic index over a shorter period of time and it has been used to distinguish between the normal and well controlled from the poorly controlled diabetes [17] and in the screening of the "at-risk" subjects [18]. Studies in which the FA results were converted to HbA_{1c} equivalents and then compared, found that the values were comparable in some cases [17, 19, 20] and discordant in some [21], leading to a glycosylation gap. The first degree relatives presently showed significantly higher serum FA values with FPG and HbA_{1c} around normal cut offs, suggesting the glycation of proteins at relatively lower levels of FPG in the "at-risk" people. In contrast, the diabetic group had near normal serum FA values and high FPG and HbA_{1c} values [Table/Fig-2].

An important consideration in the clinical interpretation of the FA concentration is the effect of the variations in the serum total protein concentrations. Baker and co-workers (1983) [20] found no demonstrable correlation between FA and serum total protein in normal subjects and in those with uraemia. Allgrove and Cockrill (1998) [22] and Johnson and co-workers (1987) [5] also reported similar findings. In the present study, the serum total protein levels did not differ significantly between the groups. On the correction of serum FA by taking a ratio between FA and TP [serum FA/serum TP], the significance which was observed earlier in Group 2 persisted because of evident reasons.

The estimation of saliva has been studied as a non-invasive method to measure the glycaemic status in diabetes. Studies which were done by Twetman *et al.,* (2002) [23] in type 1 diabetics have shown the salivary glucose levels to vary in relation to the HbA_{1c} levels. In contrast, studies have also shown that the salivary glucose levels did not reflect the blood glucose levels and that no significant correlation was found between the two measures in healthy individuals [24] and in diabetics [23,25,26]. Presently, we found the salivary glucose levels of the diabetic group to be similar to those of the first degree relatives and the controls. There was no significant correlation between the salivary and the serum indices of glycaemia between any of the groups [Table/Fig-3].

The comparison of FPG with other measured glycaemic indices in serum [Table/Fig-3] reinforced the positive correlation of FPG with HbA_{1c} across the groups. A significant positive correlation of FPG with the serum FA levels was seen only in the diabetic group, while no remarkable correlation was seen in the first degree relatives, though this group had significantly high serum FA values. Takahashi *et al.,* (2007) [27] have shown glycated albumin to correlate significantly with HbA1c in Type 2 diabetics, when the HbA1c level was < 7.5%.

BMI and WC were assessed as the indicators of the metabolic status and they were compared with the glycaemic parameters [Table/Fig-4]. Indian studies have shown that the waist girth strongly correlated with the cluster of findings which were associated with the metabolic syndrome [7-10]. Follow up studies which were done by Agostino *et al.,* (2004) [28] in normal and IGT subjects found that the people who developed diabetes were older, they had larger WCs, they were more dyslipidaemic, they had higher SBP and that they were insulin resistant. There was also a doubling of the risk for conversion to diabetes in people who had these risk factors. The Group 2 subjects with a family history of diabetes were younger, but they had larger WCs. A significant positive correlation of FPG with WC and BMI was also seen typically in this group. On the contrary, the diabetic group had a larger WC which did not correlate with the FPG. Other indices of glycaemia viz. FA and HbA1c did not correlate with WC or BMI across the groups. Hence, the FPG of the high risk individuals remained the only glycaemic marker that correlated with the metabolic indicators which were studied.

CONCLUSION

In non diabetic subjects, a positive family history (as in Group 2) was associated with an increased risk of developing diabetes, as was indicated by the elevated serum FA levels. This group also had a larger waist girth which significantly correlated with the FPG. This proves that, the presence of one risk factor i.e. a family history of diabetes predisposes these people to the earlier development of diabetes or the metabolic syndrome. Hence, monitoring of the serum FA, FPG, HbA1c levels and the lipid profile along with the WC should be taken up in a large group of an 'at-risk' population. The salivary parameters, though non- invasive, did not reflect the serum parameters or the disease state. Thus, their use appears doubtful.

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