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PROTEIN GLYCATION, INSULIN SENSITIVITY AND PANCREATIC BETA CELL FUNCTION IN HIGH-RISK, NON-DIABETIC, FIRST DEGREE RELATIVES OF PATIENTS WITH TYPE 2 DIABETES

V. SATHIYAPRIYA[1], ZACHARIAH BOBBY[1]*,
APARNA AGRAWAL[2] AND N. SELVARAJ[1]

Departments of [1]Biochemistry and [2]Medicine,
Jawaharlal Institute of Postgraduate Medical Education and Research,
Pondicherry – 605 006

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Abstract : Insulin resistance and impaired beta cell function are widely recognized as features of type 2 diabetes. But it is still debated whether insulin resistance or beta cell dysfunction constitutes the primary abnormality. This study was done to evaluate the impact of family history of type 2 diabetes on insulin resistance, beta cell function and glycation of proteins. A total of 30 healthy subjects with a positive family history of type 2 diabetes and thirty two healthy age-matched subjects without any family history of type 2 diabetes were enrolled in this study. Fasting glucose, post prandial glucose, fasting plasma insulin, fructosamine and glycated hemoglobin were evaluated in both the study groups. The mean fasting glucose, fasting Insulin and HOMA-IR were significantly higher among the first-degree relatives of type 2 diabetics, but there was no alteration in HOMA-B. The levels of both glycated hemoglobin and fructosamine were significantly increased in the test group when compared with controls. In conclusion the results from the present study suggest that Indian subjects with family history of type 2 diabetes are associated with insulin resistance and enhanced glycation of proteins, but with no evidence of beta cell defect.

Key words : first-degree relatives type 2 diabetes hyperinsulinemia insulin resistance glycation

INTRODUCTION

Type 2 diabetes is a chronic progressive disorder with increasing world wide prevalence (1). India and other countries in Asia are experiencing rapidly escalating incidence of diabetes. The prevalence of type 2 diabetes among adults in urban India has increased from < 3% in the 1970s to 12% in 2000 (2). It is predicted that by 2025 India will have > 60 million diabetic patients; in other words, one in five diabetic patients in the world will be an Indian and this would be the leading cause of death in adults (1).

*Corresponding Author : Dr. Zachariah Bobby, Department of Biochemistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry – 605 006. E-mail: zacbobby@yahoo.com; Tel. : +91–413–2273078, Fax : +91–413–2372067.

Extensive investigations of the pathophysiology of type 2 diabetes have identified two endocrine dysfunctions: insulin resistance and insulin deficiency (3). Despite general agreement that both defects are present in most patients with established type 2 diabetes, many authorities have debated for some time the question of which defect is the primary cause of type 2 diabetes and which can be detected earlier in the course of the disease (4, 5). To circumvent these controversies healthy people who are at a considerable increased risk of developing diabetes, e.g. first-degree relatives of type 2 diabetic patients have often been studied to assess early metabolic abnormalities preceding development of overt type 2 diabetes.

There are many reports of both quantitative and qualitative changes in tissue insulin resistance and diminished insulin secretion as metabolic predictors of diabetes in different ethnic groups (6). There are only limited studies on insulin resistance among Indian subjects with family history of diabetes (7) with no information on the secretory defect of β-cell (7). The levels of glycated proteins reflect the glycemic status of an individual and are also implicated in the development of the complications of diabetes.

Studies on subjects with high risk of developing diabetes would cast light on mechanisms behind development of the disease and would pave the way for novel primary intervention programs aimed at preventing or delaying the onset of diabetes.

MATERIALS AND METHODS

Subjects

The study population consisted of thirty subjects who were non-diabetic first degree relatives of Type 2 diabetic patients as described by National diabetic data group criteria (8). Thirty two healthy subjects with no family history of diabetes served as controls. Only subjects with body mass index (BMI) less than 25 kg/m^2 were enrolled in order to avoid the influence of obesity towards insulin resistance. Subjects with diabetes, known or new, were excluded. Subjects with any known disease, infection, as well as smokers and alcoholics were excluded from the study. Informed consent was obtained from all individuals after explaining the purpose and nature of the study. This study was approved by the human ethics committee of our institute (Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry.

Biochemical analyses

After an overnight fast of about twelve hours, blood was drawn and collected in EDTA bottles. Whole blood was used for the estimation of glycated hemoglobin. Plasma was separated from the rest of the sample by centrifuging at 5000g for 5 minutes at 4°C. Postprandial blood sample was collected from the study subjects 2 hours after a carbohydrate rich breakfast. Plasma glucose levels were estimated immediately and the rest of the samples were stored at –70°C for the estimation of insulin and fructosamine. Fasting plasma glucose was measured by

glucose oxidase method (9). Plasma insulin was estimated by immunoradiometric assay (7) using the kit from BARC, Mumbai, India. Glycated hemoglobin (HbA1c) was estimated by ion-exchange chromatography method using kits supplied by Biocon Diagnostik, Germany. Plasma fructosamine was measured by p-iodonitrotetrazolium violet kinetic method (10) using Raichem kits (Haemagen Diagnostics, San Diego, CA) adapted to 550 express plus analyzer (Ciba Corning Diagnostics, Oberlin, OH).

The indices of basal insulin secretion and sensitivity were evaluated by HOMA and calculated as follows : HOMA-IR = FPI X FPG/22.5 and HOMA-B = 20 X FPI/(FPG – 3.5), where FPI is fasting plasma insulin level (μU/ml) and FPG is fasting plasma glucose levels (mmol/L) (11).

Statistical analysis

Results for Gaussian-distributed continuous variables are expressed as the mean±S.D. Due to the non-Gaussian distribution of HOMA–B the median of an interquartile interval was used for the same. For the difference analyses, Student's unpaired t-test for Gaussian variables and a Mann-Whitney U test for non-Gaussian variables were used. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The general characteristics of the study groups are shown in Table I. There was no significant difference in age and BMI between the two groups. The mean fasting glucose was significantly higher in the test group as compared with controls but there was no significant difference in the postprandial glucose levels between the groups. Fasting Insulin and HOMA-IR were significantly higher among the first-degree relatives of type 2 diabetics. There was no significant difference in the level of HOMA-B between the test groups. As shown in Table I, the levels of both glycated hemoglobin and fructosamine were significantly increased in the test group when compared with controls.

TABLE I : Mean±SD for age, BMI, plasma glucose, insulin, HOMA-IR, HOMA-B and glycated proteins in first degree relative of patients with type 2 diabetes and control subjects.

Parameters	*Control (n=32)*	*Test (n=30)*
Age (years)	33.3±8.5	36.3±8.9
BMI (kg/m^2)	23.4±2.9	24.1±3.3
Fasting glucose (mmol/l)	4.6±1.0	5.4±0.9**
Postprandial glucose (mmol/l)	5.9±0.7	6.3±1.3
Fasting insulin (mU/ml)	24.1±10.0	33.1±13.0**
HbA_{1C} (%)	5.2±1.0	6.3±1.5**
Fructosamine (mmol/l)	2.0±0.5	2.3±0.6*
HOMA-IR	5.2±2.9	7.9±3.3**
HOMA-B	102±44	126±63

*P<0.05; **P<0.01.

DISCUSSION

In our study we found that the non-diabetic offsprings of type 2 diabetes patients had hyperinsulinemia, and had impaired insulin sensitivity but with no evidence of pancreatic beta cell dysfunction. Furthermore, these subjects had increased levels of fructosamine and glycated hemoglobin.

In our study though fasting plasma glucose was well within the normal range it was significantly higher than the controls. Subjects with a family history of type 2 diabetes also had higher fasting insulin

levels. This finding represents an important extension of previous findings that ethnic groups with high propensity for diabetes are markedly hyperinsulinemic with fasting glucose within the normal range (12, 13). There are several lines of evidence to suggest that fasting hyperinsulinemia itself may be a primary metabolic defect and not simply a secondary consequence of insulin resistance (13–15). Based on the above findings, it has been suggested that basal hypersecretion of insulin may be an independent abnormality in the pathogenesis of diabetes (20) and that in some population, hyperinsulinemia, rather than insulin resistance, may be the primary genetic defect (16). Indeed, fasting hyperinsulinemia, known to reflect decreased insulin sensitivity and decreased insulin secretion together constitute the strongest independent predictor of type 2 diabetes (17).

In this study, indices of insulin secretion (HOMA-B) and insulin resistance (HOMA-IR) were evaluated from a fasting sample by homeostasis model assessment (HOMA) (11). These models of estimations have been found to correlate well with the insulin secretion and insulin sensitivity indices of minimal model analysis (11). In our study, beta cell function was found to be unaltered as estimated by means of the homeostasis model assessment for insulin secretion (HOMA-B). We also found that first-degree relatives of type 2 diabetics were more insulin resistant when compared to controls. Previous studies on the offsprings of type 2 diabetic have given controversial results like some have suggested that insulin resistance is the primary cause of type 2 diabetes (3, 4) whereas others have emphasized the role of an insulin secretory defect (18). In our study we have found insulin resistance to be the primary cause.

Most of the complications of diabetes are reported to be mediated through glycation reactions (19). Glycation is the nonenzymatic reaction of glucose with susceptible amino groups in amino acid residues (usually lysine) of proteins (20). This nonenzymatic modification of proteins alters not only the structure, but also their biological properties. In the present study we found increased levels of both fructosamine and glycated hemoglobin. The important aspect of this increase was that it occurred within ranges of glucose concentrations hitherto considered normal. Similar findings have been reported by Osei *et al*, who have found that an increased glycated hemoglobin levels in non-diabetic African-Americans compared to white Americans (21). The mechanisms for the increased fructosamine and HbA_{1C} among the first-degree relatives of type 2 diabetic patients are not clear. Jain *et al* and we have identified that lipid peroxides and reduced glutathione can *per se* influence the glycation of proteins (21, 22). Thus estimation of these parameters in first-degree relatives of type 2 diabetes would throw more light on the mechanism of increase in glycated protein levels.

It must be noted that our study has limitations. First, the study involved a small number of subjects and the results must be confirmed in a large sample. Insulin resistance has been measured indirectly only, although good correlation between HOMA insulin resistance and the values obtained by euglycemic clamp have been reported (11). Similarly, a mathematical derivation of the β-cell function is indirect. Again this method

has been also validated as a reliable procedure to measure insulin secretory function (23).

In conclusion, this study shows metabolic alterations in non-obese first-degree relatives of type 2 diabetes with significant changes in insulin resistance but with normal beta cell function. An increased glycation levels in these subjects with normal glucose levels may also have important pathophysiological implications.

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