A study of insulin resistance and pancreatic beta cell function in diabetics and non-diabetics

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ABSTRACT

Introduction and Aim: The most common non-communicable disease affecting large population is type 2 diabetes mellitus. This metabolic disorder is characterized by hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. The causes of diabetes mellitus can vary greatly but always include either defects in insulin secretion of the pancreas or the cells of the body not responding properly to the insulin produced or in both at some point in the course of the disease.

Materials and Methods: 200 participants who were divided into two groups, non-diabetics with and without family history of diabetes were involved in this study. The outcomes of fasting plasma glucose, postprandial plasma glucose, glycated hemoglobin, fasting plasma insulin, serum c-peptide, HOMA -IR, HOMA-B were compared between both the groups.

Results: All these parameters were significantly correlated between the groups with the level of significance $p<0.05\%$. Non-diabetic off-springs of type 2 diabetes were found to have hyperinsulinemia, increased level of serum c-peptide level, moderate insulin resistance and pancreatic beta cell dysfunction than non-diabetics without the family history of diabetes. The fasting hyperinsulinemia, known to reflect decreased insulin sensitivity constitute the strongest independent predictor of type 2 diabetes.

Conclusion: The above findings show that insulin resistance is the primary abnormality in type 2 Diabetes Mellitus.

Keywords: Type 2 diabetes mellitus; insulin resistance; pancreatic function.

INTRODUCTION

The most common non-communicable disease affecting a large group of population throughout the world is diabetes mellitus, which occurs due to defective carbohydrate metabolism. It results in high blood sugar levels (1) caused due to defects in insulin secretion, insulin action, or both. Among the types of diabetes mellitus, type 2 diabetes mellitus is the most common form of diabetes mellitus characterized by hyperglycemia, insulin resistance, and relative insulin deficiency (2).

Early symptoms of diabetes mellitus include polydipsia, polyphagia, polyuria, weight loss and blurred vision. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or non-ketotic hyperosmolar coma. Long term complications of diabetes mellitus includes microvascular and macro vascular complications (3) such as diabetic nephropathy, neuropathy (peripheral and autonomic) and retinopathy. Retinopathy leads to defective or loss of vision, nephropathy causes renal failure, neuropathy causes foot ulcers, loss of peripheral sensation and sexual dysfunction. Also diabetic patients are more prone to develop cardiovascular, peripheral arterial and cerebrovascular disease. Complications can be delayed or prevented with adequate glycemic control.

The estimated prevalence of diabetes worldwide has increased from 382 million in 2013 (4) to 422 million people in 2016 (5) and is expected to be around 522 million by 2030 (6). In 2015, 416 million people were found to be affected by diabetes worldwide (7) among which 90% were with type 2 diabetes mellitus (8).

The pathophysiology behind development of type 1 diabetes mellitus range from autoimmune destruction of the β-cells of the pancreas also with insulin deficiency to abnormalities that result in resistance to insulin action. Among the various pathophysiology, type 2 diabetes mellitus mainly involves two endocrine dysfunctions: insulin resistance and insulin deficiency which plays a major role in the development of the disease (9). But defective insulin secretion and insulin resistance frequently coexist in the same individual. This leads to a confusion on finding the primary cause of the hyperglycemia in diabetic patients. To circumvent these controversies healthy people who are at 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 the development of type 2 diabetes.

Studies on subjects with high risk of developing diabetes would help in identifying the primary cause of development of the disease and would pave the way for novel primary intervention measurements to prevent or delay the onset and progression of diabetes. This study was carried out to correlate insulin resistance and pancreatic beta cell function in diabetics and non-diabetics. The objectives of the study were to evaluate insulin resistance, pancreatic beta cell function in non-diabetic subjects without family history of type 2 diabetes mellitus, to analyze insulin resistance, pancreatic beta cell function in non-diabetic subjects with family history of type 2 diabetes mellitus (first degree relatives with type 2 diabetes mellitus) and to compare insulin resistance, pancreatic beta cell function in diabetic patients and non-diabetic subjects to find their significance of the association.

MATERIALS AND METHODS

This case control study was conducted in the Department of Biochemistry, SreeBalaji Medical College and Hospital, Chrompet, Chennai during the period of January 2016 – June 2017 among 200 healthy non diabetic individuals who came for a routine check-up. The ground work for the study was started after getting clearance from the research committee and the Institutional human ethical committee of the institution. Age, gender, height, weight, BMI, general history, family history, medications and blood pressure were recorded. Routine clinical examination was done. The study was explained to the participants and informed consent obtained from them before taking the blood sample.

Sample Size: Total sample number (n) -200

Group I (100 subjects) - subjects without family history of type 2 diabetes mellitus.

Inclusion criteria

- Age group 20-50 years
- Clinically healthy individuals
- BMI <25kg/m2 were enrolled in the study to avoid the influence of obesity towards insulin resistance.

Exclusion criteria

- Age group less than 20 years and greater than 50 years
- Pre-existing type 2 diabetes mellitus
- Family history of type 2 diabetes mellitus (first degree relatives with type 2 diabetes mellitus)

Group II (100 subjects) - subjects with family history of type 2 diabetes mellitus (first degree relatives [parents] with type 2 diabetes mellitus).

Inclusion criteria

- Age group 20-50 years
- Clinically healthy individuals
- Family history of type 2 diabetes mellitus (first degree relatives[parents] with type 2 diabetes mellitus)
- BMI <25kg/m2 were enrolled in the study to avoid the influence of obesity towards insulin resistance.

Exclusion criteria

- Age group less than 20 years and greater than 50 years
- Pre-existing type 2 diabetes mellitus

Sample Collection

The blood samples were collected from subjects by venipuncture under aseptic precautions in specific vacutainers. Fluoride tube for blood glucose, a plain tube for insulin and c-peptide, EDTA sample for HbA1C were used. Both fasting (12 hours overnight fasting) and post prandial samples were collected.

Blood samples were withdrawn from the healthy individuals for estimation of serum fasting and post prandial glucose levels by enzymatic photometric method (GOD/POD), HbA1c by Immunoturbidimetry, fasting plasma Insulin by Chemiluminescence Immunosorbent Assay (CLIA) and serum C-peptide by Enzyme Linked Immunosorbent Assay (ELISA) methods.

Insulin resistance and pancreatic beta cell function and insulin secretion were calculated by homeostatic model assessment (HOMA) indices as follows:

Index for insulin resistance

HOMA-IR=FPI x FPG/405

Index for beta cell function and insulin secretion

HOMA-B % =360 x FPI/(FPG-63) %

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 (μIU/ml) and FPG is fasting plasma glucose levels (mmol/L) (11).

The blood samples were processed within half an hour. Blood glucose was estimated immediately. Serum was separated and stored under -20°C for estimation of serum insulin and serum c-peptide.

Statistical Analysis

The characteristics of study participants are summarized using mean and standard deviation. The outcome variables namely, fasting plasma glucose, postprandial plasma glucose, glycated hemoglobin

(HbA1c), fasting plasma insulin, serum c-peptide, HOMA -IR, and HOMA-B were compared between the two groups using a paired t-test. A two-sided p-value less than 0.05 was considered to be statistically significant. SPSS version 20 was used for statistical analysis.

RESULTS

Table 1: Characteristics of study participants

	Group I		Group II	
N	100		100	
Parameters	Mean	SD	Mean	SD
Age (years)	35.2	8.8	33.6	8.1
Weight (kg)	48.8	2.8	49.1	2.8
Height (cm)	148.6	4.3	148.8	4.3
BMI (kg/m2)	22.1	0.5	22.1	0.5
Systolic BP (mmHg)	116.9	15.6	116.8	15.6
Diastolic BP (mmHg)	77.0	6.7	77.2	6.9
FPG (mg/dL)	90.1	7.1	91.3	6.8
PPPG (mg/dL)	111.7	13.9	117.0	12.9
HbA1c (%)	5.4	0.4	5.4	0.4
Fasting plasma Insulin(µIU/ml)	12.2	5.2	22.9	10.3
C- peptide (ng/ml)	1.7	0.7	2.4	1.2
HOMA IR	3.2	1.2	4.9	2.5
HOMA B	195.8	74.2	283.3	115.5

SD- standard deviation; BMI- Body mass index; BP- Blood pressure; FPG- fasting plasma glucose; PPPG- postprandial plasma glucose; HOMA IR- Homeostatic model assessment of Insulin resistance; HOMA B- Homeostatic model assessment of beta cell function.

Group I – Non diabetics without the family history of T2DM
Group II - Non diabetics with family history of T2DM (only parents)

The mean age of participants was 34.4± 8.4years. The mean weight was 48.9±2.8kg and the mean BMI was 22.1±0.5kg/m^2. The mean FPG, PPPG HbA1c, fasting plasma insulin, serum c-peptide levels, HOMA IR and HOMA B were higher in group II compared to group I.

Table 2: Comparison of fasting plasma glucose between the two groups

Group	Mean(mg/dL)	Standard Deviation	n	p value
I	90.1	7.1	100	P = 0.2401
II	91.3	6.8	100	

The fasting plasma glucose level of group II was higher than that of group I (p = 0.24). FPG was not statistically significantly different between the two groups (table2)

Table 3: Comparison of postprandial plasma glucose between the two groups

Group	Mean(mg/dL)	Standard Deviation	n	p value
I	111.7	13.9	100	P = 0.001
II	117.0	12.9	100	

The post prandial plasma glucose level of group II was higher than that of group I. The PPPG was significantly different between the two groups (table 3).

Table 4: Comparison of glycated hemoglobin (HbA1c) between the two groups

Group	Mean%	Standard Deviation	n	p value
I	5.4	0.4	100	<0.001
II	5.4	0.4	100	

HbA1c levels of groups I and II were the same (5.40±0.4) with a p value of <0.05. The glycated hemoglobin (HbA1c) was significantly different between the two groups (table 4).

Table 5: Comparison of fasting plasma insulin between the two groups

Group	Mean	Standard Deviation	n	p value
I	12.2	5.2	100	<0.0001
II	22.9	10.3	100	

The fasting plasma insulin of group II was higher than that of group I with p value < 0.05. The fasting plasma insulin was significantly different between the two groups (table 5).

Table 6: Comparison of serum C-peptide between the two groups

Group	Mean	Standard Deviation	n	p value
I	1.7	0.7	100	<0.0001
II	2.4	1.2	100	

The serum c-peptide levels of group II was higher than group I with p value of < 0.05. The serum C-peptide was significantly different between the two groups (table 6).

Table 7: Comparison of HOMA-IR between the two groups

Group	Mean	Standard Deviation	n	p value
I	3.2	1.2	100	<0.001
II	4.9	2.5	100	

The HOMA – IR of group II was higher than that of group I, with level p value < 0.05.The HOMA IR was significantly different between the two groups (table 7).

Table 8: Comparison of HOMA-B between the two groups

Group	Mean	Standard Deviation	N	p value
I	195.8	74.2	100	<0.001
II	283.3	115.5	100	

HOMA – B of group II was higher than that of group I, with the level p value < 0.05. HOMA B was significantly different between the two groups (table 8).

DISCUSSION

The main pathophysiology of type 2 diabetes mellitus includes insulin resistance and pancreatic beta cell dysfunction affecting insulin secretion (3). Evaluating insulin resistance and beta- cell function is important to understand the pathophysiology of the disease and to decide the mode of treatment. The golden standard method to evaluate insulin sensitivity is glucose clamp test (10), but it is limited to research use since it is difficult to perform in medical institutional level. This study was done involving 200 participants who were divided into two groups of subjects consisting of non-diabetics with and without the family history of diabetes. Indices of beta cell function and insulin secretion (HOMA-B) and insulin resistance (HOMA-IR) were evaluated from fasting sample by homeostasis model assessment method (HOMA). This study shows that the mean of insulin resistance of non-diabetics with the family history of diabetes is higher than non-diabetics without the family history of diabetes. Apart from this, insulin resistance is present in most of the non-diabetics without family history of diabetes proving the other causes also play a role in development of insulin resistance. It suggests that insulin resistance is predominant before the development of pancreatic beta cell dysfunction in people prone to developing diabetes thus confirming that insulin resistance plays the main role in the development of type 2 diabetes mellitus. Ferrannini *et al.,* has also reported that non diabetics with the family history of diabetes (first degree relative) have a higher insulin resistance than individuals without family history of diabetes mellitus (11). This was again confirmed in a study by Srinivasan *et al.,* (12). Sathiyapriya *et al.,* have also suggested the same in her study that insulin resistance acts as the primary cause of type 2 diabetes mellitus (13).

The mean pancreatic beta cell function and insulin secretion (HOMA – B) among non-diabetics with the family history of diabetes is higher non diabetics

without the family history of diabetes. The pancreatic beta cell function and insulin secretion of individuals with diabetic first degree relatives' increase progressively with increase in insulin resistance. These findings are consistent with the study conducted by Festa *et al.,* (14). There are some studies with controversial results, as Sathiyapriya *et al.,* in their study have found that beta cell function remained unaltered as estimated by means of the homeostasis model assessment for insulin secretion (HOMA-B; 13). Fasting plasma glucose and serum insulin concentration are mainly regulated by the feedback mechanism between liver and β cells of the pancreas (15). Increased insulin resistance in the liver increases insulin secretion to overcome the hepatic glucose efflux. The plasma glucose level is maintained at normal level till the ability of β cells to secrete insulin is appropriate against insulin tolerance. But the defective β cell function results in increased hepatic glucose efflux and finally leads to hyperglycemia. A rise in FPG from 80 to 140 mg/dl results in an increase in fasting plasma insulin, and increases in FPG beyond 140 mg/dl are associated with reduced insulin secretion and increased hepatic glucose output (16).

However, there is no much change in fasting plasma glucose, post prandial plasma glucose and glycated hemoglobin levels between non diabetic off springs of T2DM and non-diabetics without family history of T2DM. But there is an increase in insulin resistance, insulin secretion and c-peptide level in off springs of diabetes rather than non-diabetics without the family history of T2DM. It has been shown that during the development of type 2 diabetes, fasting plasma insulin increases and then decreases as insulin resistance develops (the Starling's curve of the pancreas). The increase in fasting plasma insulin is basically a compensation mechanism aiming to reverse the effect of insulin resistance, and the subsequent decrease as a decompensation mechanism reflecting beta cells exhaustion. Basal insulin levels were higher in non-diabetic first-degree relatives of type 2 diabetic patients than the corresponding values in controls (17). Similarly, in this present study, the mean fasting insulin level was higher in non-diabetic first-degree relatives (21.6±10.0 U/ml) than the corresponding values in non-diabetics without the family history of diabetes (14.2± 5.2 U/ml). Bonora *et al.,* (18) in his study has found a negative correlation between insulin sensitivity and fasting plasma insulin in mild glucose intolerance and suggested that insulin resistance as the cause for overproduction of insulin. Olefsky *et al.,* (19) found a similar correlation in normal subjects, subjects with impaired glucose tolerance and type 2 diabetes mellitus and has stated that raise in fasting plasma insulin as a result of an attempt to overcome insulin resistance. C-peptide is a by-product of insulin which is cleaved off during secretion of insulin was found to be more in non-diabetic first-degree relatives (2.4± 1.2 ng/ml) than the corresponding values in non-diabetics without the family history of diabetes (1.7 ± 0.8 ng/ml). As fasting plasma insulin level increase c-peptide level also increases. Thus, it was observed that gradual worsening of glucose tolerance in first degree relatives of T2DM leads to elevation of mean fasting insulin level, c-peptide level and insulin resistance, thus following the natural pathway of the disease.

Glycation is the non-enzymatic reaction of glucose, modifying the structure and biological properties of the protein. This study shows that glycated hemoglobin level remains similar in non-diabetics with and without the family history of diabetes. Pieme *et al,* (20) and Selvaraj *et al.,* (21), in their study have found that HbA1C has increased among the first-degree relatives of type 2 diabetic patients which he attributes to lipid peroxidation and reduced glutathione causing glycation of proteins.

CONCLUSION

The study results clearly show that non diabetics with a family history of diabetes are more prone to develop diabetes mellitus with significant alterations in insulin resistance and pancreatic beta cell insulin secretion. Even though the fasting plasma glucose, postprandial plasma glucose, glycated hemoglobin is not much indicative of impaired glucose tolerance status, on the contrary the levels of fasting insulin, IR shows the trend towards altered glycemic status. This trend is indicative of the pathogenesis of type 2 diabetes mellitus where the insulin resistance has just started and on the path towards altered glycemic balance.

Limitations of the study

The study involved a small number of subjects and the results must be confirmed in a large sample size.

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