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TYPE-2 DIABETES RELATED INTERMEDIATE PHENOTYPIC TRAITS IN NORTH INDIAN DIABETICS

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ABSTRACT

Asian Indians are known to be at a higher risk of developing T2DM, but the underlying genetic factor in this population is still not well understood.T2DM is a complex genetic trait and assessment of disease related intermediate phenotypic traits is an important initial step towards any systematic genomic study. Therefore, in the present study we have assessed diabetes related intermediate phenotypic traits of insulin secretion and insulin resistance in the patients belonging to this population. The study included157 T2DM patients of either sex ranging in age from 45-80 years and 84 non-diabetic subjects with no family history of diabetes, ranging in age from 45 to 75 years served as controls. Intermediate phenotypic traits studied were BMI, W: H ratio, fasting free fatty acid level and Insulin resistance and secretion. Diabetics were found to have significantly higher W: H ratio ($p< 0.001$), FFA ($p<0.001$) and HOMA-R ($p<0.001$) as compared to non-diabetics. However, there was no significant difference in their BMI and HOMA-β. There was a positive correlation between FFA level and HOMA-R among diabetics, but not among controls. These findings suggest that in abdominal obesity FFA mediated insulin resistance is an important causative factor underlying T2DM in this population. Moreover, comparable HOMA-β in diabetics reflects compensatory insulin hyper secretion in these subjects. There is a need to examine relative contribution and precise nature of genetic factor in their tendency for central obesity, free fatty acidemia and insulin resistance.

KEY WORDS

Type-2 Diabetes Mellitus, intermediate phenotypic traits, North Indian, Free Fatty Acid, Insulin Resistance, HOMA-R, HOMA-β

INTRODUCTION

Asian Indians were found to be at higher risk of developing type 2 diabetes mellitus (1-4). But, the underlying genetic predisposition in this population is unknown. Type 2 Diabetes (T2DM) is a complex genetic trait and there is need of systematic investigations in accordance with established standard strategies of finding genes underlying such traits (5). Like most other diseases having multifactorial inheritance, the genes underlying complex traits like T2DM pose special challenges that make gene discovery more difficult and it is because of locus heterogenecity, epistasis, low penetrance, variable expressivities and pleiotrophy, and limited statistical power (6-7). Disease related intermediate phenotypes are proximate to genetic factor than the disease state itself. Therefore study of these intermediate phenotypic traits rather than disease state itself is a more powerful approach. In fact, identification of the intermediate phenotypic traits is first step towards systematic genomic study of T2DM in any population. In the present study, we have assessed status of some of the known parameters of diabetes related intermediate phenotypic traits of body fat (content and distribution), insulin resistance and secretion in Asian IndianT2DM patients.

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MATERIALS AND METHODS

This is a case control study of diabetics and age and sex matched non-diabetic controls. The patients were selected from the Endocrinology clinic of S.M.S. Medical College,

Jaipur. There were 157 diabetic subjects ranging in age from 45 to 80 years with mean age 57.42 ± 13.65 years. M: F ratio was103:54. Subjects on drugs likely to alter the measured intermediate phenotypic traits (i.e., metformin, thiazolidinedione, insulin etc) and diabetes with complications like renal failure, heart failure etc influencing these phenotypic traits were excluded from the study. Age and sex matched 84 non-diabetic subjects with no family history of diabetes and ranging in age from 45 to 75 years with mean age 54.94± 10.11 years and M:F ratio 56:28 served as controls.

All the subjects were assessed for following diabetes related intermediate phenotypic traits- Body Mass Index (BMI), Waist :Hip ratio (W: H), Fasting free fatty acids level (FFA), Homeostasis Model Assessment of Insulin Resistance (HOMA-R) and Beta- Cell Function (HOMA-β). BMI was calculated as weigh (Kg)/ height (meter). Waist to hip ratio was measured by standard method(8).FFA was estimated by calorimetric method using kits supplied by Randox, UK. Insulin resistance and secretion (HOMA-R and HOMA-β) were assessed by homeostatic method using standard formulas for calculation of these parameters (9). Fasting plasma glucose was estimated by glucose oxidase method (Merck Vitalab selectra autoanalyser, with kits supplied by CDR diagnostics Ltd., Hyderabad). Insulin was estimated by ELISA using ORTO-BRIO ELISA processor and kits supplied by UBI - MAGIWEL, USA)

Student's 't' test was used for comparison of parameters in two groups. Pearson's correlation coefficient was used for analysis of association between two parameters.

RESULTS AND DISCUSSION

General characteristics and diabetes related intermediate phenotype traits in diabetics and the control groups are given in Table1. Both the groups were matched for age and sex. Among 157 diabetic subjects, 102 were recently diagnosed and were on diet control and exercise. The remaining 55 patients were receiving sulfonylureas in addition to diet control and exercise.

The diabetic subjects despite receiving appropriate treatment had significantly higher plasma glucose and comparable insulin secretion. Association of diabetes with impaired insulin secretion is a universal fact, but the comparable HOMA-β among them reflects compensatory insulin hyper-secretion. However this compensation is inadequate for the given degree of hyperglycemia. As it is not possible to differentiate from controls on the basis of HOMA-β, therefore this cannot be taken as intermediate phenotypic trait of insulin secretion defect among diabetics. More sensitive markers of insulin secretion like disposition index are not practical for large scale studies and also they become abnormal when insulin secretion dysfunction is severe enough to cause impaired glucose tolerance (11). Therefore, the best way of finding genetic basis of insulin secretion defect (in other words susceptibility of beta cells to FFA and other mediated induced damage) is to use IGT or diabetic state itself as a qualitative trait in genome wide association studies with non diabetic individuals age > 60 years and comparable insulin resistance should be taken as controls.

Table 1: Clinical, Biochemical and Type-2 Diabetes Related Intermediate Phenotypic Traits in the Control and Diabetic Group

Parameter	Control Group	Diabetic Group
Age (years)	54.94 ± 10.11	57.42 ± 13.65
Male %	66.67 %	65.56 %
BMI (Kg/m2)	26.13 ± 3.44	25.42 ± 3.08
W:H ratio	0.87 ± 0.046	0.98 ± 0.088*
Hypertension %	13.09	32.05
Fasting Glucose (mmol/L)	4.96 ± 0.596	8.641 ± 2.497*
Fasting Insulin (ìU/ml)	12.23 ± 6.60	33.55 ± 10.22*
FFA level (mmol/L)	0.843 ± 0.216	1.37 ± 0.53*
HOMA-R	2.80 ± 1.726	12.938 ± 5.14*
HOMA-β	170.37 ± 83.26	172.48 ± 100.60

Statistical Comparison was done between control and diabetics: *p<0.001; rest not significant

The diabetic subjects in the present study were found to have higher W: H ratio, FFA and HOMA-R but there was no significant difference in their BMI. Association of visceral obesity with increased free fatty acid flux and high insulin resistance is well known (12). Moreover Asian Indians in previous studies were found to have not only predominantly visceral adiposity and high insulin resistance than other populations (1-4,13), but they also had increased FFA flux for the given degree of visceral fat (14). This could be because of their tendency of intramyocellular fat deposition (15-18). Our findings suggest that higher degree of these defects was associated with diabetes mellitus. Therefore, these parameters can be taken as intermediate phenotypic traits for genome-wide association studies. However it is worth mentioning here that there is both genetic and environmental component in these traits. Therefore heritability of these parameters should be considered before any genomic association study.

Pearson's correlation analyses of insulin resistance with

Table 2 Pearson's Correlation Analysis of Insulin Resistance Measured as HOMA-R with the Measured Variables in the study Population

	Control Group (n=84)		Diabetic Group (n=157)	
	r	Significance	r	Significance
Age	0.7141	<0.001	0.1611	<0.001
BMI	0.2339	<0.05	0.08049	NS
W:H Ratio	0.2984	<0.01	0.1854	<0.02
FFA Level	0.13733	NS	0.6479	<0.001

NS- Not Significant

various parameters studied is given in Table 2 and Figure 1. There was a significant positive correlation between HOMA-R and age, BMI and W: H ratio; but no significant correlation with FFA level in control group. However, among diabetics there was a strong positive correlation between HOMA-R and FFA level and also a weak correlation with age and W:H ratio. But no correlation was observed between HOMA-R and BMI. These findings suggest that diabetics and the controls represent two different populations. One in which generalized adiposity is associated with lower degree of FFA flux and insulin resistance. Moreover, this FFA flux doesn't play an important role in insulin resistance. The other population represented by diabetics has a tendency towards visceral obesity in which heightened FFA flux play an important role in high insulin resistance. This visceral adiposity-FFA flux-insulin resistance relationship possibly represents the basic metabolic defect in Asian Indian type-2 diabetes mellitus patients and finding its genetic basis is highly desired.

Free fatty acids were proposed by Randel to compete with glucose as fuel at cellular level (Randel cycle) and play an important role in insulin resistance(19,20) Insulin normally suppresses FFA flux from adipose tissue. But FFA on the other hand causes beta cell dysfunction (lipotoxicity) and contribute

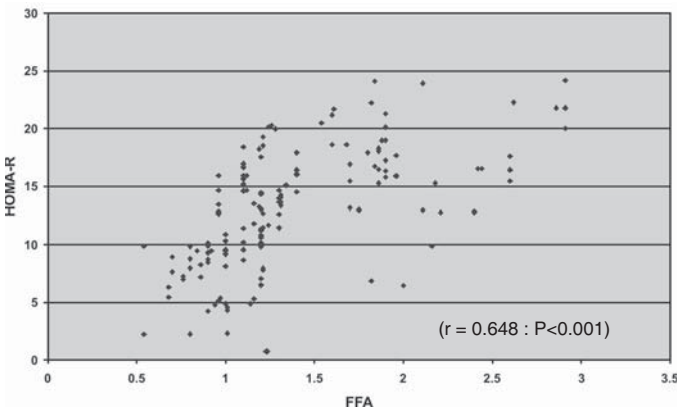

Fig 1 : Relationship between HOMA-R and FFA amongst Diabetics

to decline in insulin secretion(21-22). Our finding in the present study support the hypothesis that primary defect in Asian Indian diabetics is insulin resistance at adipose tissue in suppressing FFA flux. This heightened FFA flux further contributes to beta cell dysfunction and decrease in insulin secretion, which causes further increase in FFA flux. Thus FFA induced insulin resistance and beta cell dysfunction exists in individual predisposed to diabetes. However the contribution and precise nature of genetic factor for central obesity, Free Fatty Acidemia and Insulin Resistance in Type 2 Diabetes Mellitus needs to be explored. There is a need to examine relative contribution and precise nature of genetic factor in their tendency for central obesity, free fatty acidemia and insulin resistance.

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