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Insulin Receptor Substrate-1 Gene (G972R) Polymorphism and Insulin Resistance in Overweight and Obese Type 2 Diabetes Mellitus Patients

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The insulin receptor substrate-1 gene (IRS-1) gene has been considered a candidate for insulin resistance in type-2 diabetes and coronary artery disease. The most common IRS-1 variant, a glycine to arginine change at codon 972(G972R) is more prevalent among subjects who have features of insulin resistance syndrome associated with type 2 diabetes patients. The aim of the present study was to determine the insulin receptor substrate-1 gene (G972R) polymorphism and insulin resistance in overweight and obese type 2 diabetes mellitus patients. The genomic DNA of the subjects was amplified by polymerase chain reaction (PCR) and digested by restriction fragment length polymorphism (RFLP) with BstN1 used for codon 972. Fasting insulin and fasting glucose were determined and insulin resistance was evaluated using homeostasis model assessment index for insulin resistance (HOMA-IR). The prevalence of IRS-1(G972R) polymorphism was 19% in the study population, 18 patients were of heterozygous (A/G) genotype and only one patient was of homozygous (A/A) genotype. Remaining 81 patients were of wild type, (G/G) genotype. The percentage of patients with family history of diabetes mellitus was significantly higher in the G972R carrier group than in the non-carrier group. There was a significant association between family history of diabetes mellitus and IRS-1(G972R) polymorphism in the type 2 diabetic patients (p=0.02). Between IRS-1(G972R) carriers and non-carriers, there was no significant difference in insulin resistance. It was concluded that IRS-1(G972R) polymorphism might have a role in the genetic susceptibility of development of type 2 diabetes mellitus in those with family history. But, IRS-1(G972R) polymorphism does not significantly increase insulin resistance compared to the wild type individuals.

Key words: IRS-1gene, G972R, Polymorphism, T2DM

INTRODUCTION

Diabetes mellitus is now declared as a global epidemic.[1] World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes, according to 2005 figures.[2] This number is likely to be more than double by 2030 without intervention. According to WHO estimation, the prevalence of diabetes mellitus in Myanmar was 2.4% in 1995 and it will be 3.2% in the year 2025.[3]

Insulin receptor substrate-1 (IRS-1) occupies a key position in the insulin signaling pathway.[4] As IRS-1 is the first substrate in this cascade, an impaired IRS-1 function may result in a defect in insulin signaling.[5] Thus, genetic changes in IRS-1 may potentially contribute toward the development of insulin resistance, the most common of these being a glycine to arginine change

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at codon 972(G972R).[6] The prevalence of IRS-1(G972R) polymorphism was higher in T2DM especially in obese patients, and the prevalence of polymorphism is reported to be varied in various studies probably due to differences in genetics, race and ethnicity, etc.

There are also conflicting reports regarding the relationship between the IRS-1(G972R) polymorphism and insulin resistance, fasting plasma insulin and blood glucose control. Since, the prevalence of IRS-1(G972R) polymorphism in Myanmar might differ from other regions and the effect of polymorphism on insulin resistance in T2DM subjects is not yet reported, the prevalence of polymorphism and the association of polymorphism with insulin resistance were investigated in the present study.

The findings of the present study would highlight the genetic variation of polymorphism in T2DM among populations and show whether the IRS-1 variant has effect on the insulin resistance particularly in obese individuals.

MATERIALS AND METHODS

History taking and physical examination including anthropometric measurement were done and blood samples were collected from diabetic patients attending Diabetes Outpatients Department of YGH.

Fasting blood sugar, serum insulin were measured and polymerase chain reaction (PCR) and restriction fragment length plymorphism (RFLP) for IRS-1 gene were also done at Pathology Research Division, Department of Medical Research (Lower Myanmar). Data were analyzed by SPSS (version 16.0) statistical software. Overweight and obesity were defined according to WHO guideline (2006), overweight as BMI $\geq$25 kg/m^2, obesity as BMI $\geq$30 kg/m^2.

Data were presented as mean value± standard deviation (SD). Comparison between two means was done using Student's *'t'* test (unpaired) and the difference was considered significant when p value is <0.05. The disease association with proportions of sample variables was tested by 'Chi' square test with 95% confidence interval.

RESULTS

Among 100 overweight and obese T2DM, 81 patients were of homozygous (G/G) genotype, 18 patients were of heterozygous (G/A) and only one patient of homozygous (A/A) genotype. The allele frequencies of 'G' was 90% and that of 'A' was 10% (Table 1).

Table 1. Genotype distributions and allele frequencies for G972R mutation in IRS-1 gene in patients with overweight and obese type 2 diabetes mellitus

Variables	Obese	Overweight	Total (%)	Remark
Genotypes				NS
G/G	23	58	81(81)	
G/A	7	11	18(18)	
A/A	-	1	1(1)	
No. of patients	30	70	100	
Allele				NS
G	53	127	180(90)	
A	7	13	20(10)	
No. of patients	60	140	200	

NS=Not significant

Family history of diabetes mellitus between the G972R carrier and non-carrier groups

It was found that 10 out of 19 G972R carriers and 19 out of 81 non-carriers had family history of diabetes mellitus.

The proportion of patients with family history of diabetes was greater in the G972R carrier than in the non-carrier group (52% *vs.* 23%). The G972R polymorphism is significantly associated with family history of diabetes in the study groups (p=0.02) (Fig.1).

Insulin status of the study population

Mean value of insulin resistance (HOMA-IR) calculated from fasting blood sugar and fasting serum insulin was 6.28 (0.93-34.59) and β-cell function was 117.78 (7.12-540.91)% in the present study (Table 2).

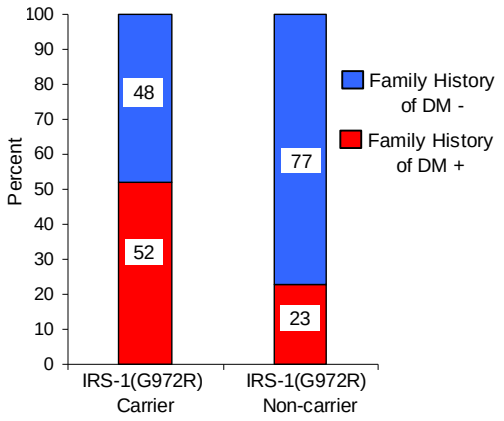

Fig. 1. Family history of diabetes mellitus in the study groups

Table 2. Insulin status of the study population

Clinical characteristics	Study group (n=100), Mean±SD
Fasting blood sugar (mmol/L)	8.25±3.38
Fasting serum insulin (µU/mL)	17.45±14.65
HOMA-IR	6.28±6.29
Log HOMA-IR	0.64±0.35
β-cell function (%)	117.78±121.27
Log β-cell function	1.83±0.48

Table 3. Insulin status of IRS-1 (G972R) carriers and non-carriers

Insulin status	Carrier (n=19)	Non-carrier (n=81)	Remark
Fasting blood sugar (mmol/L)	8.27±2.10	8.24±3.63	NS
Fasting serum insulin (µU/mL)	17.39±11.34	17.47±15.38	NS
Log fasting serum insulin	1.15±0.29	1.09±0.34	
HOMA-IR	6.43±4.63	6.25±6.65	
Log HOMA-IR	0.70±0.31	0.62±0.36	NS
β-cell function (%)	84.9±64.29	125.49±130.2	NS
Log β-cell function (%)	1.80±0.34	1.83±0.51	

NS=Not significant

Insulin status of the IRS-1 (G972R) carriers and non-carriers

There was no significant difference in FBS, FSI, HOMA-IR and β-cell function between IRS-1(G972R) carriers and non-carriers (Table 3).

DISCUSSION

Demographic risk factors and IRS-1 gene (G972R) polymorphism

In the present study, the G972R polymorphism was observed in 19% of T2DM. The prevalence of the G972R polymorphism appears to be higher than other Western (Danish, Finnish, African, Turkish and American) and Asian (Japanese, Taiwanese and Indian) studies.[7-10]

IRS-1(G972R) polymorphism was detected in various age groups in different study population. G972R polymorphism was reported in younger T2DM cases (age range 18-32 years) in one study.[11] The polymorphism was also detected in later ages (54.5± 8.2 years) in another study.[12] In the present study, the mean age of G972R carrier was 56.89±11.4 years which was similar to that of two other studies (59±6 years).[12, 13]

The prevalence of polymorphism does not seem to vary in different age groups. One study[14] showed that the prevalence was 12.4% and age of the study population was 54.81±6 years whereas in another study,[15] the prevalence was 15.8% and age of the study population was 37.5±12.2 years. The prevalence was 1.8% and age of the study population was 31.38±11.7 years in a study[16] whereas the prevalence (4.2%) and age of the study population (53.7±15 years) were found in Japanese patients.[17]

The prevalence of G972R polymorphism does not seem to be related to BMI since the prevalence was quite high 15.8% in the study[15] in which BMI was only 22.14± 3.98 kg/m^2; yet it was only 4.2% in another study[17] with more or less similar BMI, 22.9±3.8 kg/m^2. In a meta-analysis study,[5] there was no association between BMI and G972R was reported among individuals with BMI less than 27 kg/m^2. However, it was found that a stronger association between the G972R and T2DM was reported among participants with BMI less than 23.1 kg/m^2 than among participants with BMI of at least 23.1 kg/m^2.[18]

The prevalence of G972R polymorphism in the present study was much higher than that reported in the Asian region. The BMI of other studies was found to be much lower than the present study (25.1±3.1 kg/m^2, 25.69±5.27 kg/m^2 *vs.* 28.35±4.36 kg/m^2).[16, 19]

However, the prevalence of G972R polymorphism in those studies was 1.1% and 1.8%, respectively and that of the present study was many folds higher, 19%. Thus, it seems that factors other than regional and ethnic difference might have a role in the prevalence of polymorphism since the percentage of polymorphism differs between studies carried out in two places on the population with comparable BMI range.

The percentage of G972R polymorphism in general population was 13% in a study[20] and 4% in the other.[6] In the above studies, the prevalence of G972R polymorphism in T2DM was 23% and 11%, respectively. In one combined analysis, it was reported that G972R substitution was present in 15% of 117 patients with T2DM and 7% of 94 normal subjects, indicating that the prevalence of G972R polymorphism was twice higher in diabetic patients than the normal subjects. These observations are consistent with the hypothesis that mutations in the IRS-1(G972R) gene contribute to the pathogenesis of T2DM in 10-20% of the population.[20]

Moreover, family history of diabetes played an important role in T2DM patients. In the present study, 29% of T2DM patients had family history of diabetes mellitus. Among them, 34% of the subjects were G972R carrier patients. It was reported that among 153 non-diabetic offspring with only one parent affected by T2DM, 17% of patients had G972R polymorphism.[21] Comparing with this study, the prevalence of G972R polymorphism was half less in non-diabetic with family history than diabetic with family history.

In the present study, the proportion of patients with family history of diabetes was greater in the G972R carrier than in the non-carrier group (52% *vs.* 23%) which was similar to the Mexican study (36% *vs.* 13%).[18] These findings suggested that diabetes risk might be higher in the G972R carrier with family history of diabetes.

IRS-1 genotypes and insulin status

In the present study, out of 100 overweight and obese individual, 18 patients were heterozygous (G/A) carrier and only one overweight patient was homozygous (A/A) carrier. In two studies,[12, 14] it was highlighted that the majority of the G972R polymorphism was of heterozygous (G/A) but there was no case of homozygous (A/A) carrier in T2DM patient in the Japanese study.[12]

The insulin and glucose status, and the severity of diabetes mellitus was found to be higher in the population with higher G972R polymorphism prevalence. In this study and also in African-Americans study,[14] although the age and BMI are comparable, FBS and FSI levels were found to be higher. That might also imply to normal subjects because it was reported in a study that two people who were of homozygous G972R(A/A) substitution showed impaired glucose tolerance and a moderate degree of insulin resistance.[12]

However, no significant differences in BMI, FBS and FSI level were observed between G972R carrier and non-carrier in the present study. It thus suggested that G972R polymorphism alone may not impair the insulin and glucose status but other genetic, environmental and life style factors would play a role in the development and progression of the disease. Therefore, analysis of polymorphism at site other than 972 in IRS-1 gene and finding out other genetic alterations and consideration of the risk factors seem to be required when attempt is made to determine the role of genetic polymorphism in the disease pathophysiology.

The G972R polymorphism has 2 forms: heterozygous (G/A) and homozygous (A/A). Although it is not known that whether genotypic difference has an effect on the insulin and glucose status, in the present study, one and only homozygous case was found to be insulin resistant whereas 13 out of 18 heterozygous cases were insulin

resistance. The allele frequencies of ˙G' was 90% and that of ˙A' was 10%. Neither the allelic frequency nor the genotypic frequency seems to be significantly different between the overweight and obese T2DM patients, further disapproving the notion of any relationship between BMI and gene polymorphism.

It was reported in a study that among the common polymorphisms of the IGF-1R, IRS-1 and IRS-2 genes, IRS-1 and IRS-2 genes did not show the conversion from IGT to T2DM, whereas IGF-1R may regulate the risk of developing T2DM.[22] Moreover, a study have analyzed the same two polymorphisms in diabetes subjects participating in the United Kingdom Prospective Diabetes Study (UKPDS) and found only an association between obesity and the beta-3-adrenergic receptor (beta-3-AR) polymorphism but not obesity and the IRS-1 polymorphism[23] but when both polymorphisms were present, there was a huge increase in the frequency of T2DM in Caucasian obese subjects.[24]

Conclusion

The prevalence of IRS-1G972R polymorphism was 19% in overweight and obese type 2 diabetes mellitus patients in the present study. In the presence of family history of diabetes mellitus, IRS-1(G972R) polymorphism is associated with the development of type 2 diabetes mellitus.

It was concluded that IRS-1(G972R) polymorphism might have a role in the genetic susceptibility of development of type 2 diabetes mellitus in those with family history. But IRS-1(G972R) polymorphism does not significantly increase insulin resistance compared to the wild type individuals. Genetic sequencing of IRS-1 and other genes in the insulin signaling pathway, and finding out the alteration in their genetic patterns would provide clues for the association of the site-specific polymorphisms of these genes with insulin resistance in type 2 diabetes mellitus.

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