

Prevalence of Insulin Receptor Substrate-1 Gene (G972R) Polymorphism, Insulin Resistance, and Determination of β -Cell Function among Overweight and Obese Persons with Type 2 Diabetes Mellitus

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Abstract

Background. Type 2 diabetes mellitus (T2DM) is the most common metabolic disorder and its pathogenesis is characterized by a combination of peripheral insulin resistance and impaired insulin secretory capacity of pancreatic β cell. Genetic predisposition interacts with environmental factors including diet, physical activity, and age leading to the development of diabetes.

Objective. To determine the proportion of overweight and obese persons with type 2 diabetes and to compare the fasting blood sugar, fasting serum insulin, insulin resistance and β -cell function in G972R carrier and non-carrier overweight and obese persons with type 2 diabetes.

Methodology. One hundred overweight and obese patients with T2DM were recruited from persons with diabetes attending the Diabetes Outpatient Department of Yangon General Hospital. History taking and physical examination were done and blood samples were collected. Plasma glucose level was determined by the glucose oxidase method and fasting serum insulin was measured by enzyme linked immunoassay (ELISA) kit method. Polymerase chain reaction and Restriction Fragment Length Polymorphism were done for genetic polymorphism.

Results. Among 100 overweight and obese subjects with 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. There was no statistically significant difference in the proportion of genotypes between overweight and obese subjects with T2DM.

There was no significant difference in fasting blood sugar (FBS), fasting serum insulin, HOMA-IR, β -cell function, lipid parameters between IRS-1 (G972R) carriers and non-carriers. There is significant negative correlation between insulin resistance and TG level ($r^2=0.0529$, $p=0.01$).

Conclusion. It was concluded that IRS-1 G972R polymorphism was not important in insulin resistance, β -cell function and lipid parameters in overweight and obese T2DM. There could be a number of candidate genes in the pathophysiology of diabetes mellitus, 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 T2DM.

Key words: IRS-1, insulin resistance, β -cell function, lipid profile

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 at codon 972 (G972R).⁶

The prevalence of IRS-1 (G972R) polymorphism was higher in type 2 diabetes mellitus (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. There are also conflicting reports regarding the relationship between the IRS-1 (G972R) polymorphism and insulin resistance, fasting plasma insulin and blood glucose control. The insulin sensitivity or pancreatic β -cell function did not differ between carrier and wild type subjects in non-obese patients but showed significant difference in obese patients.⁷ Jellema et al., showed that differences in fasting insulin and homeostatic model assessment-insulin resistance (HOMA-IR) between carriers and non-carriers were more pronounced and significant in obese subjects, but not in the non-obese subjects.⁵ Analysis of variance also showed a significant interaction between the heterozygous forms of the codon 972 variant and obesity. In addition, the IRS-1 (G972R) polymorphism is associated with insulin resistance. The proportion of carriers was higher in T2DM patients with either insulin resistance or dyslipidemia.⁸

Genetic data of IRS-1 (G972R) polymorphism in Myanmar is not available yet, so this is a preliminary study. Since the prevalence of IRS-1 (G972R) polymorphism in Myanmar might differ from other regions and the effect of polymorphism on insulin resistance (IR) in T2DM subjects is not yet reported, the prevalence of polymorphism and the association of polymorphism with insulin resistance, β -cell function, and lipid parameters 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 and lipid parameters particularly in overweight and obese individuals.

METHODOLOGY

Hundred overweight and obese patients with T2DM were recruited from persons with diabetes attending the Diabetes Outpatient Clinic of Yangon General Hospital according to inclusion and exclusion criteria. Inclusion criteria were patients diagnosed with T2DM who are taking Metformin only, age over 40 years and BMI ≥ 25 kg/m². Excluded in the study were T2DM patients taking oral hypoglycemic drugs other than Metformin and T2DM patients with pregnancy.

History taking and physical examination including anthropometric measurement were done and blood samples were collected. Fasting blood sugar (FBS), fasting serum insulin (FSI) and lipid profile were measured at the Pathology Department, Department of Medical Research. Plasma glucose level was determined by the glucose oxidase method and fasting serum insulin was measured by enzyme linked immunoassay (ELISA) kit method. DNA was purified from FTA card for PCR amplification. Polymerase chain reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) for IRS-1 gene was also done at Pathology Department, Department of Medical Research, Lower Myanmar, Yangon. HOMA IR and HOMA β -cell function were calculated using the formula by Matthews et al., in 1985.⁹

$$\text{HOMA IR} = \frac{\text{FPI}(\mu\text{IU/mL}) \times \text{FPG}(\text{mmol/L})}{22.5}$$

$$\text{HOMA } \beta\text{-cell function} = \frac{20 \times \text{FPI}(\mu\text{IU/mL})}{\text{FPG}(\text{mmol/L}) - 3.5}$$

Data were analysed using SPSS (version 16.0) statistical software. Overweight and obesity were defined according to WHO guideline (2006), overweight as BMI ≥ 25 kg/m², obese as BMI ≥ 30 kg/m². Data were presented as mean value \pm standard deviation (SD). Comparison between two means was done using Student's 't' test (unpaired) and the difference was considered significant when the two-tailed p-value is < 0.05 . The differences in proportions of specific genotypes and alleles by BMI category (i.e., overweight or obese) were tested using Fisher's exact test or chi-squared test, as appropriate. The correlation between insulin resistance and triglyceride was determined using Pearson's correlation.

Ethical Consideration

The thesis including pro forma and written informed consent form was submitted to the ethical review committee to obtain ethical approval to conduct the research work. History taking, physical examination and 5 ml of blood sample were taken after getting informed consent. The study was approved by the Academic Board, University of Medicine 1, Yangon.

RESULTS

Among 100 overweight and obese patients with 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). There was no statistically significant difference in the proportion of genotypes between overweight and obese T2DM patients.

Out of 100 patients, 70 were overweight (male: female=25:45) and 30 were obese (male: female =12:18). The mean age and BMI of the present study were 56.93 \pm 10.96 year and 28.35 \pm 4.36 kg/m².

The mean value of insulin resistance (HOMA-IR) calculated from FBS and FSI was 6.28 \pm 6.29 and β -cell function was 117.78 \pm 121.27 % in the present study.

There was no significant difference in FBS, FSI, HOMA-IR, β -cell function (Table 2), TC, TG, HDL and LDL between IRS-1 (G972R) carriers and non-carriers (Table 3).

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				
G/G	23	58	81 (81%)	p=0.583
G/A	7	11	18 (18%)	
A/A	–	1	1 (1%)	
n	30	70	100	
Allele				
G	53	127	180 (90%)	p=0.607
A	7	13	20 (10%)	
n	60	140	200	
Chi-squared test				

Table 2. Fasting blood sugar, fasting serum insulin, HOMA-IR and β -cell function between IRS-1 (G972R) carrier and non-carrier

Parameters	Carrier (n=19)	Non-carrier (n=81)	Remark
Fasting blood sugar (mmol/L)	8.27 \pm 2.10	8.24 \pm 3.63	$p=0.9725$
Fasting serum insulin (μ U/mL)	17.39 \pm 11.34	17.47 \pm 15.38	$p=0.9830$
(Log fasting serum insulin)	(1.15 \pm 0.29)	(1.09 \pm 0.34)	$p=0.4792$
HOMA-IR	6.43 \pm 4.63	6.25 \pm 6.65	$p=0.9114$
(Log HOMA-IR)	(0.70 \pm 0.31)	(0.62 \pm 0.36)	$p=0.3739$
β -cell function(%)	84.90 \pm 64.29	125.49 \pm 130.2	$p=0.1906$
{Log β -cell function (%)}	(1.80 \pm 0.34)	(1.83 \pm 0.51)	$p=0.8081$

Table 3. Lipid parameters of IRS-1 (G972R) carrier and non-carrier

Lipid Parameters	Carrier (n=19)	Non-carrier (n=81)	Remark
Total cholesterol (mg/dL)	171.00 \pm 27.21	164.43 \pm 36.15	$p=0.4592$
Triglyceride (mg/dL)	186.84 \pm 35.11	190 \pm 38	$p=0.7416$
HDL (mg/dL)	37.94 \pm 5.89	37.32 \pm 8.12	$p=0.7546$
LDL (mg/dL)	95.84 \pm 28.16	89.59 \pm 37.75	$p=0.4996$

The mean level of TG and HDL in IRS-1 carrier group was 186.84 \pm 35.11 mg/dl, 37.94 \pm 5.89 mg/dl and that of IRS-1 non-carrier group was 190 \pm 38 mg/dl, 37.32 \pm 8.12 mg/dl, respectively. There was no significant difference between these two groups.

Figure 1 shows significant negative correlation between insulin resistance and TG level ($r^2 = 0.0529, p=0.01$).

DISCUSSION

The prevalence of IRS-1 (G972R) polymorphism was higher in obese patients with T2DM, and the prevalence of

polymorphism is reported to be varied in various studies probably due to differences in genetics, race and ethnicity. The findings of the present study would highlight the genetic variation of polymorphism in T2DM in Myanmar and show whether the IRS-1 variant has effect on the insulin resistance, β -cell function and lipid parameters particularly in overweight and obese individuals.

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.⁷⁻¹³

In the present study, out of 100 overweight and obese individuals, 18 patients were heterozygous (G/A) carriers and only one overweight patient was a homozygous (A/A) carrier. In Lei et al., and Yamada et al., the majority of the G972R polymorphism were of hetero-zygous (G/A) and there was no case of homozygous (A/A) carrier in patients with T2DM in Yamada’s study.¹⁴⁻¹⁶

The insulin and glucose status, and the severity of diabetes mellitus were found to be higher in the population with higher G972R polymorphism prevalence. In the present study and also in the study of Lei et al., although the age and BMI were comparable, FBS and FSI levels were found to be higher. That might also apply to normal subjects because in the study of Yamada et al., it was reported that 2 people who were of homozygous G972R (A/A) substitution showed impaired glucose tolerance and a moderate degree of insulin resistance.¹⁴⁻¹⁶

The prevalence of G972R polymorphism does not seem to be related to BMI since the prevalence was quite high at 15.8% in Orkunoglusuer’s study in which BMI was only 22.14 \pm 3.98 kg/m²; yet it was only 4.2% in Ura’s study with more or less similar BMI, 22.9 \pm 3.8 kg/m².^{17,18}

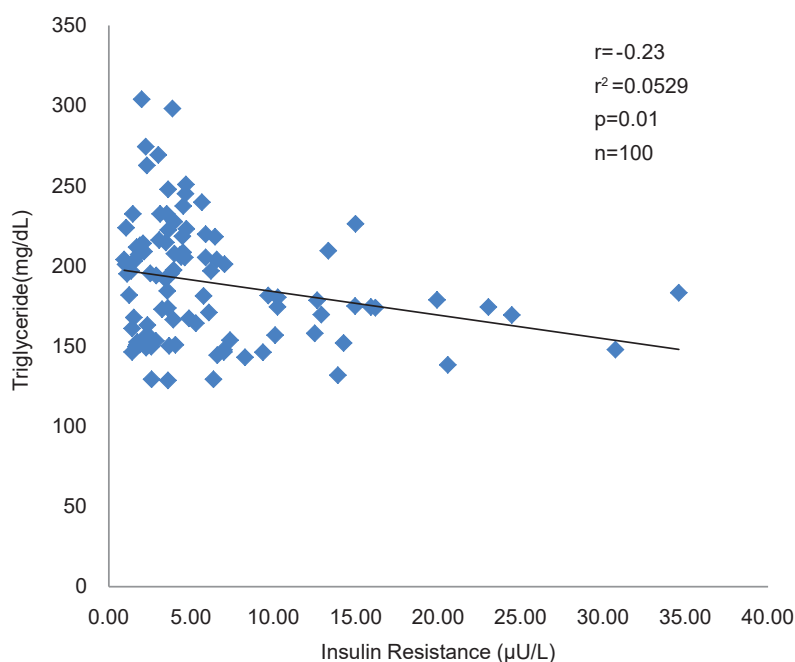


Figure 1. Negative correlation between insulin resistance and TG.

r = Pearson correlation coefficient; n = total numbers of subjects

In a meta-analysis by Jellema et al., no association between BMI and G972R was reported among individuals with BMI less than 27 kg/m².⁵ However, in the study of Burguete-Garcia in 2010, a stronger association between the G972R and T2DM was reported among participants with BMI less than 23.1 kg/m² than among participants with BMI of at least 23.1 kg/m².²⁰

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/m², 25.69±5.27 kg/m² versus 28.35±4.36 kg/m²}.^{19,21} However, the prevalence of G972R polymorphism in them was 1.1% and 1.8% respectively and that of the present study was many folds higher at 19%. Thus, it seems that factors other than regional and ethnic differences 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 the general population was 13% in the study of Imai et al., and 4% in that of Almind et al.^{6,21} 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 persons with diabetes than in 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.²¹ Although the present study reported that G972R substitution was present in 19% of patients with T2DM, the study could not conclude G972R polymorphism was higher in persons with diabetes than the normal subjects because the present study did not determine G972R polymorphism in normal subjects.

However, no significant differences in FBS, FSI, β -cell function, and lipid parameters were observed between G972R carrier and non-carrier in the present study. It is thus suggested that G972R polymorphism alone may not impair the insulin, glucose and lipid status but other genetic, environmental and life style factors play a role in the development and progression of the disease. Therefore, analysis of polymorphism at sites other than 972 in IRS-1 gene and finding other genetic alterations and consideration of the risk factors seem to be required when any 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 whether genotypic difference has an effect on the insulin, glucose and lipid status, in the present study, only one homozygous case was found to be insulin resistant whereas 13 out of 18 heterozygous cases were insulin resistant. 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 patients with T2DM, further disproving the notion of any relationship between BMI and gene polymorphism. Laukkanen et al., reported 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, Zhang and coworkers have analysed the same two polymorphisms in diabetic subjects participating in the United Kingdom Prospective Diabetes Study and found only an association between obesity and the β -3-adrenergic receptor polymorphism but not obesity and the IRS-1 polymorphism²³ but when both polymorphisms were present, there was a huge increase in the frequency of T2DM in Caucasian obese subjects.²⁴

In the present study, HOMA-IR and fasting insulin were not correlated with total cholesterol, HDL, and LDL levels. However, significant negative correlation was observed between HOMA-IR and TG level. Although it was reported that TG level lowered after blood glucose control²⁵ in the group with high insulin resistance, such lowering of triglyceride level was also observed despite high fasting blood glucose. It is possible that in such cases with high HOMA-IR, hepatic TG synthesis is blunted and reduced. In addition, since insulin stimulates lipoprotein lipase activity which increases entry of free fatty acids for TG synthesis in the liver, insulin resistance would cause decreased TG synthesis.

Insulin leads to decreased gluconeogenesis and increased synthesis of fatty acids and TG in normal persons. Selective insulin resistance in the liver of mice with type 2 diabetes, insulin fails to decrease gluconeogenesis, but it continues to stimulate synthesis of fatty acids and TG. This produces the deadly combination of hyperglycemia and hypertriglyceridemia. Insulin fails to decrease gluconeogenesis, and it also fails to stimulate synthesis of fatty acids and TG in total insulin resistance in the liver of LIRKO mice. This leads to hyperglycemia without hypertriglyceridemia, a state that may have consequences less severe than those observed with the combined elevation.²⁶

Zheng T et al.,²⁷ Zheng S et al.,²⁸ Ma M et al.,²⁹ reported that β -cell function was inversely related with TG level and TG promotes β -cell apoptosis. Therefore, lowering of TG level would reduce the β -cell inhibition effect by TG, and that seems to explain why in the present study fasting plasma insulin level was higher in the group with low TG level.

The present study reported that G972R substitution was present in 19% in patients with T2DM, the study could not conclude that G972R polymorphism was higher in persons with diabetes than the normal subjects because the present study did not determine G972R polymorphism in normal subjects. The present study determines only one genetic polymorphism in insulin signaling pathway and other genetic polymorphisms such as β -3-adrenergic receptor polymorphism could be considered to show correlation between genetic polymorphism and T2DM.

CONCLUSION

The prevalence of IRS-1 G972R polymorphism was 19% in overweight and obese T2DM patients in the present study. In this preliminary study, there are no differences in HOMA-IR, FBS, FSI and lipid parameters between the IRS-1 G972R carrier and non-carrier groups.

Insulin resistance was found in 72% and only 18% of the study group have good glucose control. Plasma TG level was higher and HDL level was lower than normal range and plasma TG level was significantly and inversely correlated with insulin resistance.

Triglyceride production falls at high insulin resistance in T2DM, suggesting that the fall in lipid parameters should not be taken only as good glycemic control. Measurement of FBS and FSI level is also recommended for formulating the efficient management strategies.

It was concluded that IRS-1 G972R polymorphism was not important in insulin resistance, β -cell function and lipid parameters in overweight and obese patients with T2DM. There could be a number of candidate genes in the pathophysiology of diabetes mellitus, 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 T2DM.

Limitation of the study

The present study could not conclude whether G972R polymorphism was higher in overweight and obese T2DM patients than in normal weight subjects because the present study did not determine G972R polymorphism in normal subjects. The present study determined only one genetic polymorphism in the insulin signaling pathway and other genetic polymorphisms such as Human leucocyte antigen (HLA), glycogen synthase, glucagon receptor, β -3-adrenergic receptor polymorphism could be considered to show correlation between genetic polymorphism and T2DM.

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Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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