## Letter to the Editor

# Evaluation of Insulin Resistance Markers and Their Relationship with *ADIPO Q* Gene Polymorphism in Clinically Euthyroid Type 2 Diabetic Patients

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Insulin resistance (IR) has a nexus with metabolic disorders. The homeostatic model assessment for insulin resistance (HOMA-IR), HOMA-β, quantitative insulin sensitivity check index (QUICKI), glucose-insulin ratio, and triglyceride (TAG) / high density lipoprotein (HDL) have been increasingly recognized as insulin sensitivity/resistance markers[1]. Hyperinsulinemic euglycemic clamp (HEC), the gold standard, has inherent practical demerits and thus paves the way for a simplified but objectivized approach to quantifying insulin sensitivity/ resistance.

Indices of insulin sensitivity/resistance based on the oral glucose tolerance test (OGTT) have been proposed. However, using simple ratios to identify IR would be useful as surrogate markers for IR[2]. Such indicators are sensitive and economically viable.

Insulin acts through specific extracellular receptors, following its postprandial release into the circulation and facilitating glucose utilization in peripheral tissues. Insulin suppresses hepatic glucose output. During the early stages of IR, an increase in insulin output maintains normoglycemia. Eventually, when insulin secretion is no longer adequate, hyperglycemia leads to frank diabetes mellitus[1]. IR is typified by suboptimal glucose utilization in tissues in response to insulin and many cellular events that significantly increase the risk of cardiovascular and other organ diseases[3].

Plasma TAG levels are independently associated with IR, and hyperinsulinemia is a predictor of cardiovascular diseases. The association of IR with lipoprotein ratios has also been observed in patients with type 2 diabetes mellitus (T2DM)[4].

Gene polymorphisms of insulin and other biochemical mediators influence IR. Adiponectin (ADIPO Q), elaborated by the adipose tissue, regulates TAG levels and is a regulator of IR, and SNPs of *ADIPO Q* genes have been studied. Despite such studies, ambiguity still lies in *ADIPO Q* gene polymorphism related to IR in anthropometry-T2DM patients[5].

Moreover, very few studies on the *ADIPO Q* gene polymorphism link the *ADIPO Q* gene to IR related to thyroid status. Also, trace elements are implicated in glucose homeostasis since they enhance insulin sensitivity by several mechanisms, including the effects as antioxidants[6].

Relatively simple, rational, economical, sensitive, and specific methods are needed to investigate IR in special reference populations, namely clinically euthyroid T2DM patients. The association of the genetic polymorphism, namely SNP + 45 (ADIPOQ rs2241766) of *ADIPO Q*, with IR has been studied in the Indian population[7]. Also, the same study proposed that a significant association was observed between anthropometric indices and T2DM[5]. This study focused on gene polymorphism about SNP + 45 of *ADIPO Q*, as linked to IR based on a previous report from South India[7]. A focal point that emerged in recent years is that ADIPO Q levels are associated with thyroid status and IR[5].

Our primary interest in this study stems from a thorough insight into IR, as demonstrated previously by the indices that would enable us to plan suitable and personalized therapeutic modalities for clinically euthyroid T2DM patients. Moreover, we started this study because of the available reports on adipokine, IR, and thyroid status. Also, the studies performed to date have not understood the special population, namely clinically euthyroid T2DM patients.

This study was undertaken at a tertiary care hospital in Puducherry, South India (March 2018 to January 2019). The study included 65 T2DM subjects [fasting blood sugar (FBS) ≥ 126] of both genders, aged 35–60 years, with a diabetes mellitus duration of five years or more. The research project was scrutinized by the appointed Research Advisory

*doi: 10.3967/bes2022.072*

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Committee and approved by the Institutional Ethics Committee, as per the signified protocol. The purpose of the study was comprehensively explained to the participants in the local language, and informed consent was obtained. The study included only individuals with clinically and biochemically confirmed T2DM with no known obvious manifestations of thyroid diseases. A thorough medical history was elicited and documented. After obtaining consent from subjects, 5 mL of peripheral blood was collected in EDTA-coated containers, and samples were stored at −70 °C until analysis.

Isolation of DNA: DNA was isolated from whole blood using the established procedure utilizing the QIAamp DNA Mini Kit (50) protocol. Polymerase chain reaction (PCR) analysis was based on an established protocol. Exon 2 of the *ADIPO Q* gene featuring the SNP of interest was amplified with specifically designed flanking primer sequences and others supplied by M/s Genei, Bangalore, India. The wild type (TT), heterozygous type (TG), and homozygous variant type (GG) were studied by resolving them on 2.5% agarose gel. The bands were visualized under UV transilluminator light.

All other biochemical parameters were based on established procedures. Utmost care was taken to ensure quality. Fasting blood glucose was determined by the glucose oxidase-peroxidase method (GOD/POD), and fasting insulin was determined by automated chemiluminescence. Insulin resistance and indices were measured by the formula[8] HOMA-IR [fasting plasma glucose (mmol/L) x plasma fasting insulin (mIU/L)/22.5]. The lipid profile-triacylglycerols in serum were measured by the glycerol kinase method. Total cholesterol was determined by the enzymatic method. HDL cholesterol was measured by polyanion precipitation, and LDL cholesterol was computed by the Friedwald Equation: - low density lipoprotein (LDL) cholesterol = Total cholesterol — (HDL cholesterol + very low-density lipoprotein [VLDL]) where VLDL = TAG/5. Small dense LDL particles were quantitated using the surrogate marker (TAG/HDL), whereas triiodothyronine (T3), tetraiodothyronine (T4), and thyroid stimulating hormone (TSH) were estimated by the automated electrochemiluminescence method. The data are described as mean ± standard deviation. The correlation between biochemical parameters was studied using Spearman's rank correlation test. Multivariate regression analysis was performed to determine the association between the parameters. The analysis was performed at the 5% significance level, and $P < 0.05$ was considered statistically significant. Statistical analysis was performed using IBM SPSS statistics version 20 for Windows.

Since all parameters in Table 1 had a normal distribution, Pearson's correlation test was applied to find the strength of the association between the biochemical parameters. Both groups showed a significant, high negative correlation between HOMA-IR and QUICKI index, HOMA-β, and

**Table 1.** Correlation coefficients of insulin resistance (IR) indices with different biochemical parameters in two groups (TG/TT)

| Parameters | TG | | TT | |
|---|---|---|---|---|
| | *r*-value | *P*-value | *r*-value | *P*-value |
| HOMA-IR *vs.* QUICKI | −0.887** | 0.000 | −0.928** | 0.000 |
| HOMA-IR *vs.* Mg | −0.005 | 0.987 | −0.287* | 0.039 |
| HOMA-IR *vs.* TAG/HDL | 0.772** | 0.002 | 0.174 | 0.217 |
| QUICKI *vs.* Glu/Ins | 0.573* | 0.041 | 0.298* | 0.032 |
| HOMA-β *vs.* Glu/Ins | −0.924** | 0.000 | −0.726** | 0.000 |
| HOMA-β *vs.* Zn | −0.338 | 0.259 | −0.282* | 0.043 |
| T3 *vs.* Glu/Ins | 0.355 | 0.234 | −0.388** | 0.004 |
| T3 *vs.* TSH | −0.666** | 0.013 | −0.019 | 0.893 |
| T4 *vs.* Zn | 0.649* | 0.016 | −0.112 | 0.429 |

***Note.*** HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; TAG/HDL, triacyl glycerol/High-density lipoprotein; HOMA-β, homeostatic model assessment β cell; Glu/Ins, glucose/insulin; Zn, zinc; Mg, Magnesium; T3, triiodothyronine; T4, thyroxine; TSH, thyrotropin. **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level.

glucose/insulin. HOMA-IR had a negative correlation with magnesium (Mg) in the TT wild-type group, whereas the two independent variables, HOMA-IR and TAG/HDL, had a significant positive correlation in the TG heterozygous group. In contrast, zinc (Zn) showed a negative association between HOMA-β and T3 with glucose/insulin in the TT groups. However, Zn exhibited a positive association with T4 in the TG group.

A multiple linear regression analysis was applied to find the influencing factors among the independent variables towards the dependent variable in Tables 2 and 3. While constructing the regression model, only correlated factors from Table 1 were considered.

Table 2 presents the genotypes and alleles holding rs2241766 that had significant associations with HOMA-IR as a dependent variable signifying QUICKI in TT and TG. In contrast, TAG/HDL was highly significant with TT and Mg with TG.

Table 3 shows that HOMA-IR was highly significant in TT and T4 associated with TG when TAG/HDL was considered the dependent variable.

Adiponectin is an essential hormone among the adipokines because of its insulin-enhancing actions in the liver and muscle, significant anti-inflammatory properties, and TAG lowering actions[9,10]. This study was primarily conducted to assess the potential association between rs2241766 (*ADIPO Q*), an SNP of the *ADIPO Q* gene, and IR in the T2DM group. Earlier studies on T2DM patients had underlined SNP + 45 T/G[7]. A common point of interest is that

**Table 2.** Multiple linear regression coefficients of genotyping TT & TG with HOMA-IR as the dependent variable

| Parameters | TT | | | | | TG | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unstandardized Coefficients | | Standardized Coefficients | *t* | *P* | Unstandardized Coefficients | | Standardized Coefficients | *t* | *P* |
| | *B* | Std.Error | Beta | | | *B* | Std.Error | Beta | | |
| (Constant) | 62.592 | 17.166 | | 3.646 | 0.011 | 67.098 | 3.937 | | 17.044 | < 0.0001 |
| QUICKI | −221.854 | 42.587 | −0.676 | −5.209 | 0.002 | −211.645 | 12.204 | −0.951 | −17.342 | < 0.001 |
| HOMA β | 1.702 | 1.796 | 0.222 | 0.948 | 0.38 | −0.429 | 0.280 | −0.111 | −1.531 | 0.133 |
| TAG/HDL | 1.126 | 0.282 | 0.425 | 4 | 0.007 | −0.062 | 0.154 | −0.021 | −0.399 | 0.692 |
| T4 | −3.417 | 3.993 | −0.074 | −0.856 | 0.425 | 0.969 | 1.484 | 0.034 | 0.653 | 0.517 |
| Mg | 2.505 | 2.391 | 0.099 | 1.047 | 0.335 | 1.459 | 0.655 | 0.113 | 2.229 | 0.031 |
| Glu/Ins | 0.254 | 0.342 | 0.182 | 0.743 | 0.486 | 0.016 | 0.035 | 0.034 | 0.457 | 0.650 |

***Note.*** QUICKI, quantitative insulin sensitivity check index; HOMA-β, homeostatic model assessment β cell; TAG/HDL,triacyl glycerol/High-density lipoprotein; T4, Thyroxine; Mg, magnesium; Glu/Ins, glucose/insulin. Dependent Variable: Homa IR.

**Table 3.** Multiple Linear Regression Coefficients of Genotyping TT & TG with TAG/HDL as the dependent variable

| Parameters | TT | | | | | TG | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unstandardized Coefficients | | Standardized Coefficients | *t* | *P* | Unstandardized Coefficients | | Standardized Coefficients | *t* | *P* |
| | *B* | Std.Error | Beta | | | *B* | Std.Error | Beta | | |
| (Constant) | −29.211 | 16.185 | | −1.805 | 0.114 | 2.338 | 9.595 | | 0.244 | 0.809 |
| QUICKI | 59.632 | 49.972 | 0.481 | 1.193 | 0.272 | −9.51 | 29.006 | −0.124 | −0.328 | 0.745 |
| HOMA-IR | 0.489 | 0.136 | 1.297 | 3.605 | 0.009 | 0.035 | 0.128 | 0.101 | 0.273 | 0.786 |
| T4 | 1.02 | 2.562 | 0.059 | 0.398 | 0.702 | 3.185 | 1.411 | 0.329 | 2.257 | 0.029 |
| T3 | 3.61 | 1.835 | 0.397 | 1.968 | 0.09 | −0.293 | 0.538 | −0.078 | −0.545 | 0.588 |
| TSH | 0.039 | 0.271 | 0.026 | 0.143 | 0.891 | 0.145 | 0.102 | 0.196 | 1.42 | 0.162 |

***Note.*** QUICKI, quantitative insulin sensitivity check index; HOMA-IR, homeostatic model assessment for insulin resistance; T4, thyroxine; T3, triiodothyronine; TSH, thyrotropin. Dependent Variable: TAG/HDL.

thyroid disorders are linked to IR and cardiovascular morbidity[5].

In the present study, the TG genotype (rs2241766) was associated with HOMA-IR, QUICKI, TAG/HDL, and T3 suggesting a role for the *ADIPO Q* gene polymorphism in perceiving the key IR indicators. Furthermore, the frequency of the TG genotype was higher in IR participants. Our study unequivocally indicates that even the wild-type SNP + 45 in exon 2 (TT) could be a molecular indicator of apparently altered thyroid status in IR T2DM patients. Earlier studies had implicated serum Mg in IR associated with T2DM.

Magnesium might possess a central role in insulin action and ADIPO Q activity[6]. Thyroid dysfunction (latent) could potentially affect IR and imminent complications. This study was undertaken to evaluate the utility of simple IR indicators in clinically euthyroid T2DM in the light of *ADIPO Q* gene polymorphism. Hence, routine biochemical investigations performed as standard care and thyroid assessment can be used to identify patients at different stages of IR. HOMA-β, QUICKI, Glu/Ins, TAG/HDL ratio, and Mg and thyroid levels could serve as surrogate IR markers. We recommend appropriate lifestyle modifications and additional therapeutic measures to delay IR complications.

More studies need to be undertaken in different populations to convincingly implicate the role of additional biochemical IR indicators and *ADIPO Q* gene polymorphism and, most importantly, relate them to thyroid status, which acquires newer dimensions in biochemical pharmacology and pharmacogenomics.

In conclusion, our study has found that nucleotide changes in rs2241766 of the *ADIPO Q* gene could play a pivotal role in IR. HOMA-IR surrogate markers could assume relevance in revealing thyroid status in a special population, namely clinically euthyroid T2DM patients.

The authors declare that there is no conflict of interest in this study.

The authors thank Prof. Seetesh Ghose, Dean, Mahatma Gandhi Medical College and Research Institute, a constituent teaching unit of Sri Balaji Vidyapeeth, Dr. S. R. Rao, Vice-President (Research, Innovation & Development), Sri Balaji Vidyapeeth and Prof. S. C. Parija, Vice-Chancellor, Sri Balaji Vidyapeeth for enabling this communication.

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Received: December 9, 2021;

Accepted: April 11, 2022

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