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Original Research Article

Association of triglycerides/high density lipoprotein cholesterol ratio with insulin resistance in polycystic ovary syndrome

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ABSTRACT

Background: Insulin resistance (IR) is frequently observed in women with polycystic ovary syndrome (PCOS). Recent studies advocated that triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL-C) can be used as a simple clinical indicator of IR. Hence, the present study was performed to investigate the use of TG/HDL-C and its association with IR in PCOS.
Methods: Forty-one patients with PCOS and 40 healthy age matched women were randomly enrolled. Demographic and clinical characteristics were obtained. Insulin resistance was defined by the homeostasis model assessment for insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI).
Results: In PCOS group, the insulin, HOMA-IR and TG/HDL-C ratio were significantly higher (p=0.001) than controls while, QUICKI was lower (p=0.001). Insulin, HOMA-IR were positively correlated with TG/HDL-C (ρ=0.303, p=0.006 and ρ=0.312, p=0.005 respectively) while, QUICKI was negatively correlated (ρ=-0.698, p=0.001). In receiver operating characteristic (ROC) analysis, area under the curve (AUC) for model based on QUICKI levels was better 0.898 (95% CI: 0.811-0.955, p=0.001) than HOMA-IR 0.636 (95% CI: 0.522-0.740, p=0.03). A cut-off value 3.23 for TG/HDL-C is proposed from the model based on QUICKI with best combination of sensitivity 83.3% and specificity 86.7%.
Conclusions: Results of present study support that TG/HDL-C ratio may be a simple indicator of IR in PCOS patients which helps clinicians to identify IR in small centers, where the assays for insulin measurement are not available.

Keywords: HOMA-IR; insulin resistance, QUICKI, PCOS, TG/HDL-C ratio

INTRODUCTION

Poly Cystic Ovary Syndrome (PCOS) is the most common form of chronic anovulation associated with androgen excess, occurring in 5-10% of reproductive age women.[1] PCOS is associated with increased cardiometabolic risk factors.[2] Insulin resistance (IR) has been shown to be the determinant of cardiovascular risk independent of obesity in PCOS women and is also seen in non obese women with PCOS.[2,3] However, obesity is known to exacerbate the underlying insulin resistance in PCOS women.[4]

The gold standard methods designed to measure insulin sensitivity are the hyperinsulinemic euglycemic clamp and intravenous glucose tolerance test (FSIVGTT). These are impractical in the clinical setting since it requires intravenous infusion and can only be performed in specialized centers.[5] Other surrogate markers based on fasting insulin and glucose levels have been proposed

such as the fasting insulin levels (FIL), the whole body insulin sensitivity index (WBISI), the homeostasis model assessment of IR (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), Matsuda index, Avignon index, Stumvoll index, and the new simple index assessing insulin sensitivity using oral glucose tolerance test (SIisOGTT).[6] These indices present important limitations related to their poor reproducibility and reliability.[7] In addition, no clear guidelines and no universally accepted cutoffs are available for most of the main surrogate markers used.[5]

Hypertriglyceridemia and low HDL-cholesterol are two key metabolic abnormalities associated with IR states.[8,9] Similar to fasting serum insulin levels, the triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio was found to be correlated with IR and hence is considered to indicate the concomitant presence of IR and dyslipidemia.[10] TG/HDL-C has been shown to predict IR.[11] In developing countries, the availability and cost of insulin assay can be a major limiting factor for the assessment of insulin resistance. Hence, use of alternate markers like the TG/HDL-C ratio which is feasible even in small centres and is cost-effective can be a useful alternate. The present study was thus taken up to study IR and its correlation with TG/HDL-C ratio in PCOS women and to assess the diagnostic utility of TG/HDL-C ratio in identifying IR in PCOS women.

METHODS

Forty-one PCOS patients attending the Endocrinology outpatient Department of Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, aged 20-38 years and diagnosed with PCOS based on National Institutes of Health (NIH) consensus 1990 criteria were included after informed consent.[12] The diagnostic criteria according to the NIH consensus 1990 criteria were oligomenorrhoea (≤9 menses/year) or amenorrhea (no menstrual periods for 3 or more months), hyperandrogenism and/or hyperandrogenaemia and after exclusion of related disorders with similar presentation like hypothyroidism [thyroid stimulating hormone (TSH) >5mIU/mL)], hyperprolactinaemia (serum prolactin >100ng/mL), Cushing's syndrome (cortisol >2µg/dL), adrenal hyperplasia and androgen secreting tumours (testosterone levels greater than 3 times the upper reference limit associated with relevant clinical features).Women with virilization, pregnancy, those on oral contraceptives, glucocorticoids, anti- androgens, ovulation inducing agents, antidiabetic drugs or antiobesity drugs or other hormonal drugs during the previous 6 months were excluded from the study. Forty age-matched healthy females from among the hospital staff were taken as controls. The criteria for healthy control group were absence of menstrual irregularities, hirsutism and major medical illness. Sample size calculation was done based on data from previous studies. The study was approved by Institutional ethics committee.

Sample collection

Around 5ml of venous blood was collected from both controls and PCOS women, following 12hr of fasting. The plain samples were allowed to stand for half-an-hour and centrifuged at 3000rpm for 15min whereas the samples from anticoagulant bottle were centrifuged immediately and the plasma was separated. The serum and plasma samples obtained were stored at -80^{0}C until biochemical analysis.

Biochemical analysis

The plasma glucose was determined by glucose oxidase-peroxidase (GOD-POD) method (Autospan, Gujarat, India). Lipid profile was estimated using commercial kits from Aspen laboratories (Delhi, India) for TC, Accurex (Thane, India) for TG by enzymatic methods. HDL-C kits were obtained from Beckman Coulter (Galway, Ireland). All the parameters were analyzed on Beckman CX-9 fully automated analyzer (Galway, Ireland). LDL-C levels were calculated using Friedewald's formula.[13] Serum Insulin was determined by ELISA method using commercial kit (Dia source kit, Belgium). Insulin Resistance was calculated as Homeostasis Model Assessment-Insulin Resistance (HOMA−IR) using the formula: HOMA-IR = fasting insulin (µU/mL) x fasting glucose (mmol/L)/22.5.[14] Insulin sensitivity is calculated as quantitative insulin sensitivity check index (QUICKI) formula: 1/[log (insulin µU/mL)+log (glucose mg/dL)].[15]

Statistical analysis

Kolmogorov-Smirnov test was used to evaluate the distribution of continuous variables. Data was expressed as mean and standard deviation or median (IQR, interquartile range), depending on the data distribution. Statistical comparisons of the groups were made using an unpaired t-test or Mann Whitney U test, as appropriate. Spearman rank correlation was used to explore the associations among the variables (TG/HDL-C ratio with insulin, HOMA-IR and QUICKI). Logistic regression analysis was performed to determine the association of TG/HDL-C ratio with, HOMA-IR and QUICKI as dependant variable. Statistical analysis was performed using Microsoft excel spread sheet and SPSS for windows version 11.5. A 'p' value of <0.05 was considered statistically significant.

RESULTS

Table 1 shows the baseline and biochemical characteristics of the PCOS women and controls. There was no significant difference in age, BMI and fasting glucose between PCOS women and controls. The TG/HDL-C ratio was found to be significantly elevated in PCOS women compared to controls. Table 2 shows the insulin resistance markers i.e., insulin, HOMA-IR and QUICKI. Among these, insulin, HOMA-IR levels were significantly elevated in PCOS women. Concomitantly,

insulin sensitivity marker QUICKI was significantly decreased. TG/HDL-C ratio showed a significant positive correlation with insulin and HOMA-IR with (ρ=0.303, p=0.006; ρ=0.312, p=0.005). On the other hand, QUICKI showed a significant negative correlation with TG/HDL-C ratio (ρ= -0.698, p=0.001) (Table 3).

Table 1: Baseline and biochemical characteristics of the study subjects.

Characteristic	Controls	Cases	p-value
Age (years)	24.33±5.06	22.75±5.60	0.210
BMI (kg/m^2)	24.80±2.64	24.05±2.16	0.195
FBG (mg/dL)	92.50±5.63	93.02±11.39	0.794
Total cholesterol (mg/dL)	148.73±26.43	171.66±37.15	0.002*
Triglycerides (mg/dL)	107.25±38.29	151.24±63.97	0.001*
HDL-C (mg/dL)	45.15±4.16	36.73±3.65	0.001*
LDL-C (mg/dL)	82.13±26.89	105.76±37.91	0.001*
VLDL-C (mg/dL)	21.45±7.65	30.25±12.79	0.001*
TG/HDL-C	2.41±0.97	4.15±1.71	0.001*

Data was expressed as mean and standard deviation; BMI-body mass index; FBG - fasting blood glucose; HDL-C-high density lipoprotein cholesterol; LDL-C - low density lipoprotein cholesterol; VLDL-C - very low density lipoprotein cholesterol; TG/HDL-C - ratio between triglycerides and HDL cholesterol; HOMA-IR-homeostatic model assessment of insulin resistance; QUICKI - quantitative insulin sensitivity check index; *-statistically significant

Table 2: Insulin resistance markers in the study subjects.

Variable	Controls	Cases	p-value
Insulin (µIU/mL)**	7.17 (4.99-14.91)	15.30 (10.82-25.81)	0.001*
HOMA-IR**	1.57 (1.06-3.74)	3.64 (2.51-5.54)	0.001*
QUICKI	0.34 ± 0.03	0.28 ± 0.04	0.001*

Data was expressed as mean and standard deviation; ** median (IQR, inter quartile range); HOMA-IR - homeostatic model assessment of insulin resistance; QUICKI - quantitative insulin sensitivity check index; *-statistically significant

Table 3: Correlation between TG/HDL-C ratio and insulin resistance markers in PCOS patients.

Parameter	ρ-value	p-value
Insulin	0.303	0.006*
HOMA-IR	0.312	0.005*
QUICKI	-0.698	0.001*

ρ = spearman's correlation coefficient, TG/HDL-C-ratio between triglycerides and HDL cholesterol; HOMA-IR - homeostatic model assessment of insulin resistance; QUICKI - quantitative insulin sensitivity check index; *statistically significant

Receiver operating characteristic curve analysis was performed for TG/HDL-C ratio to discriminate those who were insulin resistant from those who were insulin sensitive using control cutoff values for HOMA-IR and QUICKI (i.e. 2.84 and 0.34 respectively). The AUC for the model based on QUICKI was superior 0.898 (95% CI: 0.811-0.955, p=0.001) than HOMA-IR 0.636 (95% CI: 0.522-0.740, p=0.03). Cutoff value with the best combination of sensitivity and specificity was obtained from the model based on QUICKI levels. A cutoff value of 3.23 for TG/HDL-C ratio was proposed with sensitivity 83.3% and specificity 86.7% (Figure 1, 2).

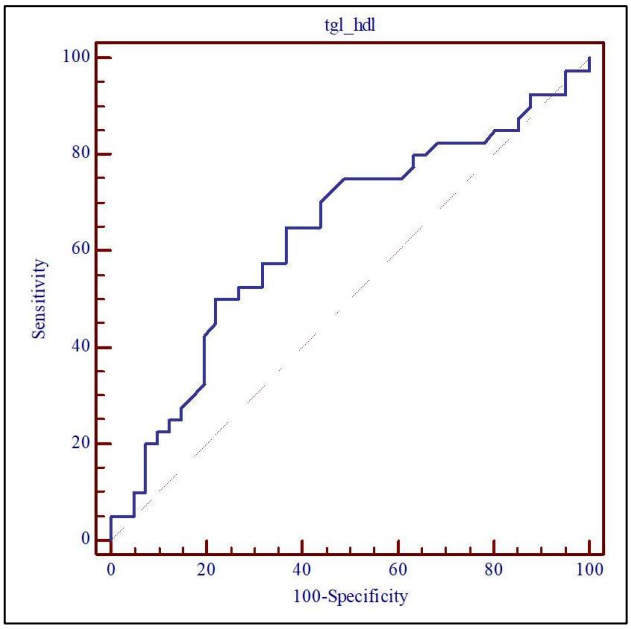

Figure 1: Receiver operating characteristics (ROC) curve analysis for TG/HDL-C ratio cutoff value based on the HOMA-IR model.

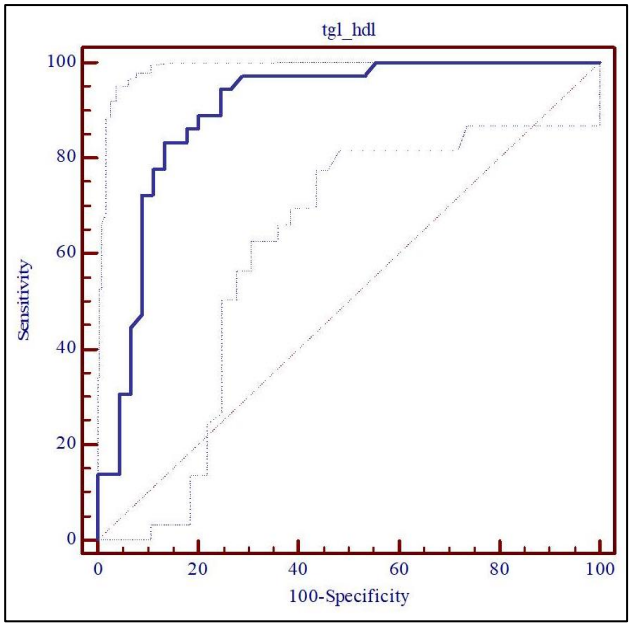

Figure 2: Receiver operating characteristics (ROC) curve analysis for TG/HDL-C ratio cutoff value based on the QUICKI model.

Logistic regression analysis using TG/HDL-C ratio (with cutoff value of 3.23) as independent variable and insulin resistance markers i.e., HOMA-IR and QUICKI as dependent variables showed significant negative association of QUICKI (standard coefficient β= -0.895, p<0.001), while no association was found with insulin and HOMA-IR (Table 4).

Table 4: Logistic Regression analysis.

Independent variable	B	Std. Error	β	t-value	p-value
Model 1 TG/HDL-C	-1.201	0.913	-0.206	-1.314	0.196
Model 2 TG/HDL-C	-0.007	0.001	-0.895	-12.39	0.001*

Model 1; HOMA-IR as a dependant variable, Model 2; QUICKI as a dependant variable, B-unstandardized coefficient; β-standardized coefficient; *-statistically significant

DISCUSSION

In the present study PCOS women were found to be insulin resistant as evident from increased levels of serum insulin and increase in HOMA-IR in PCOS women compared to controls (p=0.001). On the other hand, a significant decrease in insulin sensitivity marker QUICKI was seen in PCOS women compared to controls (p=0.001). This is in agreement with previous reports.[16] Mechanisms underlying insulin resistance in these women are unclear. Multiple factors have been implicated; of which hyperandrogenemia and obesity which are commonly seen in these women along with alteration of adipose tissue morphology seems to play an important role.[17] Hyperandrogenism produces its effect through increased lipolysis.[17] Another mechanism put forth is the central/visceral distribution pattern of body fat favored by hyperandrogenemia.[18] The increased lipolytic activity of visceral fat results in increased free fatty acid flux leading to skeletal muscle insulin resistance. Androgens also seem to have an inhibitory effect on lipoprotien lipase activity and women with PCOS have been shown to have lower lipoprotein lipase activity.[19] Insulin resistance is an important cause of dyslipidemia in PCOS women.[20]

In the present study, PCOS women had significantly increased triglycerides, and lower HDL-C when compared to controls (p = 0.001). TG/HDL-C in PCOS women was found to be significantly higher than those of the age-matched healthy women (p = 0.001). This is in agreement with previous reports.[21] The dyslipidemia in the setting of polycystic ovary syndrome can occur due to multiple causes. The increased prevalence of obesity, insulin resistance and hyperandrogenemia have all been proposed to be involved in the lipoprotein disturbances observed in PCOS women.[22] Increased lipogenesis, decreased clearance, reduced oxidation of fatty acids and their increased availability and an increased secretion of very low density lipoprotein (VLDL) particles by the hepatocytes contribute to the increased triglyceride levels in the presence of insulin resistance.[22]

A significant positive correlation was observed between insulin, HOMA-IR with TG/HDL-C ratio (ρ=0.303, p=0.006; ρ=0.312, p=0.005). On the other hand, QUICKI showed a significant negative correlation with TG/HDL-C ratio (ρ= -0.698, p=0.001) (Table 3).

The relationship between the TG/HDL-C ratio and a direct measure of IR was first reported by Mc Laughlin T et al and they proposed a cutoff value of 3.0 for TG/HDL-C ratio which showed 57% sensitivity and 71% specificity for detecting IR.[23] However, different cut-off values have been proposed in different ethnic groups.[10,24-26] Racial differences in lipoprotein lipase (LPL) activity may be responsible for racial differences in TG. African-Americans have been reported to have higher LPL levels than the caucasians.[27] Insulin resistance causing impairment in lipoprotein lipase activity thereby leading to higher TG levels has been reported in caucasians, while no such impairement has been seen in African-Americans.[28,29] Indian studies evaluating surrogate markers of IR found a good correlation of TG/HDL-C ratio with fasting glucose-to-insulin ratio (G:I ratio) in adolescent girls with PCOS.[30] In the present study, the IR was measured using HOMA-IR and QUICKI and included women in the range of 20-38 yrs.

To assess the diagnostic ability of TG/HDL-C ratio to discriminate those who were insulin resistant from those who were insulin sensitive, receiver operating characteristic curve analysis using control cutoff values for HOMA-IR and QUICKI (i.e. 2.84 and 0.34 respectively). The AUC for the model based on QUICKI

was superior 0.898 (95% CI: 0.811-0.955, p=0.001) than HOMA-IR 0.636 (95% CI: 0.522-0.740, p=0.03). Cutoff value with the best combination of sensitivity and specificity was obtained from the model based on QUICKI levels. A cutoff value of 3.23 for TG/HDL-C ratio is proposed with a sensitivity 83.3% and specificity 86.7% (Figure 1 and Figure 2). Further, logistic regression analysis using TG/HDL-C ratio (with cutoff value of 3.23) as independent variable and insulin resistance markers i.e., HOMA-IR and QUICKI as the dependent variable showed significant negative association of QUICKI (standard coefficient β= -0.895, p<0.001) with TG/HDL-C; while no association was seen with insulin and HOMA-IR (Table 4).

CONCLUSION

To conclude, the present study shows a significant association between TG/HDL-C ratio and the IR markers i.e., HOMA-IR, QUICKI in the PCOS group and thus TG/HDL-C ratio can be used as a marker of IR. The TG/HDL ratio can thus serve as a simple, convenient and inexpensive surrogate marker which can be used to screen PCOS women for IR in Indians.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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