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# Hypoadiponectinemia and high TG/HDLc ratio as risk markers of insulin resistance in obese PCOS women

Meera S[1], Arul Senghor K A[*1], Vinodhini V M[1], Sunita Samal[2]

[1]Department of Biochemistry, SRM Medical College Hospital & Research Centre, SRM Institute of Science & Technology, SRM Nagar, Kattankulathur – 603203, Chengalpattu, Chennai, Tamil Nadu, India
[2]Department of Obstetrics & Gynecology, SRM Medical College Hospital & Research Centre, SRM Institute of Science & Technology, SRM Nagar, Kattankulathur – 603203, Chengalpattu, Chennai, Tamil Nadu, India

ABSTRACT

PCOS is characterized by reduced insulin sensitivity and cardiovascular risk. Adiponectin, plays a major role that favours insulin sensitivity and possess vaso-protective function. TG:HDLc ratio, an inexpensive tool predicts insulin resistance. Study aims to relate adiponectin and TG:HDLc ratio with insulin resistance in obese infertile women with PCOS. In this cross-sectional observational study, as per Rotterdam's criteria 129 obese PCOS women with BMI $\geq$ 23 were compared with 110 aged matched controls with BMI < 23. Cardiac risk ratio-1 (TC:HDLc) and 2 (LDLc:HDLc) were calculated. Insulin resistance represented as HOMA-IR, HOMA-adiponectin and triglyceride HDLc ratio (TG:HDLc) were calculated. Adiponectin levels was significantly lowered and pearson correlation showed negative correlation with TC, TGL, LDL, TC:HDLc, LDL:HDLc and TGL:HDLc; whereas strongly positive correlation with HDLc. TG:HDL ratio was significantly elevated (4.23 $\pm$ 0.81) in 64% of PCOS women and were insulin resistant as divulged by the elevation of HOMA-IR and HOMA-adiponectin. Simple linear regression analysis of adiponectin with TGL:HDLc (R2 = 0.68) LDLc (R2 = 0.84) HDLc (R2 = 0.86) revealed the association between variables. Adiponectin and TGL:HDLc ratio are useful markers of insulin resistance that relates to the threat of cardiovascular diseases in obese women with PCOS.

*Corresponding Author

Name: Arul Senghor K A
Phone:
Email: arulsenk@srmist.edu.in

## INTRODUCTION

Polycystic ovary syndrome a multifaceted disorder affecting women in child bearing age. It adversely influences reproductive health of the women and are associated with significant cardiovascular and metabolic morbidity. Multiple hypothesis have put forth to explain the etiopathogenesis of PCOS ranging from genetic susceptibility, lifestyle modification, environmental influences and epigenetic modifications (De Leo *et al.*, 2016).

Prevalence of PCOS ranges between 3.7 – 22.5% in Indian women depending upon the population and diagnostic criteria studied (Nidhi *et al.*, 2011). Adiposity especially upper abdominal obesity is

the major predisposing factor that provokes insulin resistant state which leads to metabolic phenotypic characterization in PCOS. Moreover about 30 – 60% of PCOS women are obese with greater risk to develop cardiovascular co-morbidity due to metabolically unhealthy condition.

In the current scenario, adipose specific cytokine namely Adiponectin is found to be linked in the pathogenesis of PCOS. Adiponectin is a 30kda protein produced by the white adipose tissue, other tissues such as cardiomyocytes, ovaries, uterus, placenta also (Mirza, 2014).

Genetic impact by the APM1 gene transcript of chromosome 3q27 is dysregulated in obese individuals which inturn is potentially linked to insulin resistant state (Ganie *et al.*, 2019). The presenting clinical features in obese PCOS are associated with altered biochemical profiles and reduced insulin sensitivity which is reflected by altered lipid levels. Upper body obesity is observed in 50 to 70% women with PCOS and dyslipidemia represented by increased LDLc and decreased HDLc which may increase the cardiovascular risk (Ramanand *et al.*, 2014; Vionnet *et al.*, 2004).

In fact high triglycerides and low HDLc is an atherogenic pattern and recently has been focused because it is measured as surrogate marker of small/dense LDL particles and insulin resistant status. The plausible mechanism provides the information that VLDL co-exists with atherogenic dyslipidemia that exhibit increased subclinical inflammation, disturbance in metabolic status and IR status (Yadav *et al.*, 2011).

Adiponectin (APN) is an adipokine exclusively expressed in white adipose tissue and shows insulin-sensitizing properties and plays a protective role against atherogenicity (Hu *et al.*, 1996). Adiponectin possess anti-inflammatory and vasoprotective function;and levels are found to be decreased in coronary artery disease. Moreover, atherogenic dyslipidemia, characterized by high triglyceride HDLc ratio is reflected as an ideal marker of small dense LDL particles and insulin resistant state that would contribute to cardiovascular diseases (Choudhury *et al.*, 2018; Dinizvilela, 2016).

The study evaluates the association of adiponectin levels with atherogenic lipid markers such as TGL:HDLc pattern, cardiac risk ratios 1, 2 and markers of insulin resistance (HOMA-adiponectin) in obese PCOS women of reproductive age group.

## MATERIALS AND METHODS

This cross-sectional study was conducted in women of reproductive age 20 to 40 yrs attending the infertility clinic of our hospital. The Institutional Ethics Committee has reviewed and approved this research protocol with IEC number: No: 963/IEC/2016. The participants were well explained the purpose of the work and written informed consent was obtained then enrolled for further proceeding as per the protocol.

### Inclusion criteria

Out of 363 women screened about 129 women were recruited. The proforma for each participants were filled as they satisfy the following inclusion criteria,

1. Primary infertile women

2. Body mass index greater than 25 (obese)

3. Rotterdam's criteria with a) irregular menstrual cycle or ovulatory dysfunction b) hyperandrogenism and c) polycystic ovaries (transvaginal ultrasound findings of more than 12 ovarian follicles).

### Exclusion criteria

Infertile women with anatomical defect of the uterus, blocked fallopian tubes, thyroid dysfunction, pregnant women, recent infection, inflammation were excluded from the study.

Age matched healthy women were registered as controls with the following inclusion criteria of (i) regular menstrual cycle,(ii) one live birth through natural conception, (iii) without any recent infection or systemic illness.

The participants details were documented in the proforma that includes general examination, medical, menstrual history were recorded. Gynecological examination and ultrasound findings to check for polycystic ovaries were done.

The following anthropometric measurements weight, height, waist circumference, hip circumference were also documented. The anthropometrics indices such as BMI and waist-hip circumference ratio were calculated. According to revised consensus statement for the Asian population the participants were categorized as obese (Ganie *et al.*, 2019).

### Sample collection and analysis

After 10 to 12 hours of overnight fasting on $2^{nd}$ or $3^{rd}$ day of menstrual cycle, blood samples were drawn from the enrolled individuals. Routine hormonal investigations were estimated using standard

automated hormone analyzer. Plasma fasting glucose, serum Total cholesterol, triglycerides, HDLc, LDLc was estimated in autoanalyzer Beckman Coulter AU480 using the company standard reagents. The special parameter of this study includes serum Adiponectin levels were evaluated in Bio-Rad ELISA equipment with BioVendor company 96 wells ELISA kit reagents. As per the adiponectin kit insert normal reference range of 8.2 – 19 $\mu$g/ml for individuals with BMI less than 25 was considered. The following insulin resistance markers were calculated: HOMA-IR , HOMA-adiponectin (Vilela *et al.*, 2016), Cardiac risk ratio 1, Cardiac risk ratio 2 and TG : HDLc

## Statistical analysis

Statistical analysis was performed with SPSS version 16.0. The data was analyzed and expressed as mean and SD and comparison between the group as p-value < 0.05 was considered statistically significant. Pearson correlation analysis was performed to correlate adiponectin with biochemical parameters. With Linear regression analysis, the strength of association between the variables was assessed statistically.

## RESULTS AND DISCUSSION

Out of 363 participants 129 women were diagnosed as obese PCOS and were observed in young obese women with mean age of $23.91 \pm 3.97$ yrs. PCOS women with higher BMI and WC had increased mean adiponectin levels. In Table 1 the anthropometric characteristics were evaluated and compared between obese PCOS and healthy controls.

The mean of lipid profile parameters were evaluated in obese PCOS women. We observed elevated TC, triglycerides and LDL c with decreased HDLc in obese PCOS group. Also, atherogenic indices as compared revealed elevated cardiac risk ratio 1 (TC:HDLc $4.77 \pm 1.18$) and cardiac risk ratio 2 (LDL:HDLc $3.4 \pm 1.012$) in PCOS individuals; likewise insulin resistance markers such as HOMA-IR ($4.16 \pm 1.66$) and HOMA-adiponectin ($0.73 \pm 0.48$) were also elevated.
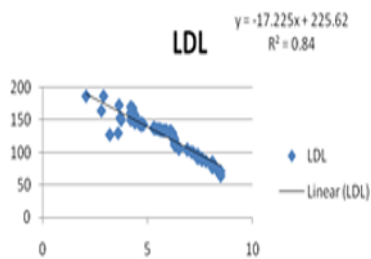


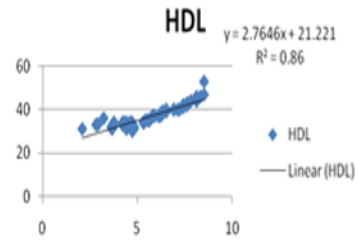**Figure 1: Regression analysis:Adiponectin & LDLc**



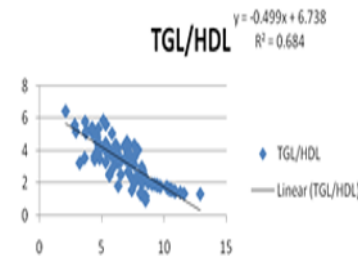**Figure 2: Regression analysis:Adiponectin & HDLc**



**Figure 3: Regression analysis:Adiponectin & TG/HDLc ratio**

As summarized in Table 2, adiponectin levels were correlated with anthropometric indicators that had significant negative correlation with BMI, waist circumference and waist hip ratio. Furthermore, pearson correlation of adiponectin revealed negative correlation with TC, TGL, LDLc, TC:HDLc, LDL:HDLc, HOMA-IR, HOMA-adiponectin and positive correlation with HDLc.

As per Table 3, in participants with PCOS the mean TGL:HDLc is significantly increased in obese PCOS as compared to controls and further classified based on cutoff of 3. In the group with TGL:HDL ratio $\geq 3$ had high LDLc and low HDLc; and insulin resistant markers HOMA-IR and HOMA-adiponectin were increased in the group with TG:HDL ratio $\geq 3$.

The linear regression analysis (Figures 1, 2 and 3) shows a strong association of adiponectin with the variables of risk markers such as LDLc ($R^2$ = 0.81) fig 1, HDLc ($R^2$ = 0.86)fig 2 and TG:HDLcratio ($R^2$ = 0.68) Figure 3.

Adiponectin is a favorable adipose tissue derived adipokine and considered as an excellent marker of adipose function. Adiponectin possess anti-inflammatory and anti-atherogenic effects thereby has protective role against initiation and progression of atherosclerosis. Dyslipidemia is an important contributor to develop cardiovascular events in obese individuals. In the present study the infertile PCOS women had hypoadiponectinemia which is associated with insulin resistant and dyslipidemic state.

In obese PCOS individuals the anthropometric indi-

**Table 1: Comparison of anthropometric indicators, lipid parameters and markers of insulin resistance between control and obese PCOS infertile women**

| Anthropometric indicators | Control (n=110) | Obese PCOS (n=129) | P value |
|---|---|---|---|
| Age yrs | 25.7 ± 5.58 | 23.91± 3.97 | 0.047* |
| Weight (kg) | 50.26 ± 4.24 | 68.06 ± 6.28 | 0.000*** |
| Height (mt2) | 1.54 ± 0.04 | 1.54 ± 0.037 | 0.945(NS) |
| BMI | 21.08 ± 1.13 | 28.89 ± 2.76 | 0.000*** |
| WC (cm) | 78.41 ± 5.35 | 92 ± 4.49 | 0.000*** |
| HC (cm) | 95.43 ± 6.63 | 107.53 ± 4.42 | 0.000*** |
| WHR | 0.82 ± 0.025 | 0.86 ± 0.103 | 0.000*** |
| Total Cholesterol (mg/dl) | 120.98 ± 29.66 | 177.45 ± 25.23 | 0.000*** |
| Triglycerides (mg/dl) | 62.64± 16.5 | 121.53± 38.31 | 0.000*** |
| HDLc (mg/dl) | 48.48 ± 7.56 | 38.27 ± 4.71 | 0.000*** |
| LDLc (mg/dl) | 78.6± 26.34 | 125.7 ± 25.02 | 0.000*** |
| VLDL (mg/dl) | 12.74± 3.3 | 16.09 ± 2.42 | 0.000*** |
| CRR1(TC/HDLc) | 2.612 ± 0.98 | 4.77 ± 1.18 | 0.000*** |
| CRR2 (LDLc/HDLc) | 1.43 ± 0.806 | 3.4 ± 1.012 | 0.000*** |
| TGL/HDLc | 1.3 ± 0.49 | 3.29 ± 1.33 | 0.000*** |
| Adiponectin ($\mu$g/ml) | 15.14 ± 2.03 | 6.57 ± 1.86 | 0.000*** |
| Insulin ($\mu$IU/ml) | 5.32 ± 1.27 | 16.57 ± 6.48 | 0.000*** |
| FPG (mg/dl) | 90.14 ± 6.62 | 102.1 ± 14.37 | 0.031* |
| HOMA-IR | 1.18 ± 0.285 | 4.16 ± 1.66 | 0.000*** |
| HOMA-adiponectin | 0.081 ± 0.028 | 0.73 ± 0.48 | 0.000*** |

The values are expressed in mean and SD, * P value < 0.05 is considered significant
***very highly significant **highlysignificant * significant

cators reflect the severity of visceral adiposity as justified by the increased waist circumference and waist hip ratio. Hypoadiponectinemia is evident in individuals with increased visceral adiposity as supported by the evidence of inverse relationship of adiponectin with WC and negative correlation with visceral fat accumulation (Choudhury et al., 2018).

The current study exhibited direct positive correlation between adiponectin and HDLc. Many researchers have put forth evidences to show that adiponectin influences HDLc via apo-A1 and ATP binding cassette transporter expression. Furthermore, recently it was reported that adiponectin activates ceramidase activity which in turn affects sphingosine-1-phosphate present in HDLc that has vasoprotective effects (Holland et al., 2011).

TGL:HDL ratio is simple, cost-effective and useful tool to assess insulin resistance and cardiovascular disease in PCOS (Song et al., 2016). The highlight of the present study is the elevation of CRR-1, CRR-2 and markers of insulin resistance HOMA-IR and HOMA – adiponectin in PCOS women with TGL:HDL $\geq$ 3 that revealed the valuable effect of adiponectin against dyslipidemic environment.

As per the WISE study in myocardial ischemic women the evidences directs the importance of TG:HDL ratio in relation to cardiovascular diseases (Bittner et al., 2009). Evidences have proposed that adiponectin activates the ligand for peroxisome proliferator-activated receptor- $\alpha$ and tailed up by reduced release of apo B and apo E which reduces the release of triglyceride rich lipoproteins (Yamauchi et al., 2007). Clinical experimental reports have derived the fact that adiponectin is found to reduce triglyceride levels by increasing lipoprotein lipase activity, VLDL receptor expression and thus VLDL-TGL catabolism in the peripheral tissues (Qiao et al., 2008).

Elevated cardiac risk ratios in the study group of infertile PCOS women implicates the hypoadiponectinemic effect on cholesterol and studies have shown that the circulating adiponectin levels promotes apoA-I/HDL mediated cholesterol efflux (Hafiane et al., 2019).

Our study revealed a better association of adiponectin levels with LDLc and this is sup-

**Table 2: Correlation of Adiponectin with anthropometric and biochemical variables in PCOS infertile women**

| (PCOS Infertile) | r value | P value |
|---|---|---|
| Weight (kg) | - 0.247 a | 0.005 |
| Height (mt2) | 0.173 a | .051 |
| BMI | - 0.31 b | 0.000 |
| WC (cm) | - 0.315 b | 0.000 |
| HC (cm) | - 0.175 a | 0.047 |
| WHR | - 0.316 b | 0.000 |
| Total Cholesterol (mg/dl) | - 0.72 c | 0.000 |
| Triglycerides (mg/dl) | - 0.699 c | 0.000 |
| HDLc (mg/dl) | 0.907 c | 0.000 |
| LDLc (mg/dl) | - 0.68 c | 0.000 |
| VLDL (mg/dl) | - 0.69 c | 0.000 |
| TC/HDLc | - 0.83 c | 0.000 |
| LDLc/HDLc | - 0.788 c | 0.000 |
| TGL/HDLc | - 0.827 c | 0.000 |
| Insulin ($\mu$IU/ml) | - 0.40 b | 0.000 |
| FPG (mg/dl) | - 0.08 | NS |
| HOMA-IR | - 0.41 b | 0.000 |
| HOMA-adiponectin | - 0.68 c | 0.000 |

a Small correlation (0.3–0.1)
b Moderate correlation(0.5–0.3)
c Strong correlation (1.0–0.5)

**Table 3: Comparison of atherogenic indices and markers of insulin resistance in PCOS infertile women**

| (PCOS Infertile) | TGL/HDL ratio < 3 (n = 43) | TGL/HDL ratio $\geq$ 3 (n=86) | P value |
|---|---|---|---|
| HDLc (mg/dl) | 43.37 $\pm$ 3.87 | 35.5 $\pm$ 2.87 | 0.000*** |
| LDLc (mg/dl) | 94.54 $\pm$ 23.64 | 148.53 $\pm$ 22.01 | 0.000*** |
| TC/HDLc | 3.63 $\pm$ 0.80 | 5.42 $\pm$ 0.88 | 0.001** |
| LDLc/HDLc | 2.23 $\pm$ 0.73 | 3.57 $\pm$ 0.77 | 0.01* |
| TGL/HDLc | 1.91 $\pm$ 0.46 | 4.23 $\pm$ 0.81 | 0.001** |
| HOMA-IR | 2.51 $\pm$ 1.42 | 4.38 $\pm$ 1.65 | 0.000*** |
| HOMA-adiponectin | 0.37 $\pm$ 0.21 | 0.89 $\pm$ 0.53 | 0.000*** |
| Adiponectin ($\mu$g/ml) | 8.92 $\pm$ 1.59 | 5.54 $\pm$ 1.35 | 0.000*** |

The values are expressed in mean and SD, P value < 0.05 is considered significant
***very highly significant **highly significant * significant

ported by the evidences of anti-oxidant effect of adiponectin against oxidation of LDLc whereby oxidized LDL contributes to atherosclerotic plaque formation and 3-fold increased risk of cardiovascular disease (Coimbra, 2019; Kumar *et al.*, 2017).

In the current study we utilized HOMA-adiponectin that incorporates the part of adipocytokine to assess insulin resistance. Here the individuals with hypoadiponectinemia exhibit elevated HOMA-adiponectin and TGL:HDL ratio which points to the insulin resistant environment (Balsan *et al.*, 2015).

Our study strongly suggests that lowered adiponectin and elevated TGL:HDL ratio may be considered as risk markers of insulin resistance which forms the background of dyslipidemic circulating lipoprotein. Our findings leads for future study of lifestyle interventions in PCOS women that reduced visceral adiposity improves insulin sensitivity (Tamilselvi and Nalini, 2019) and promotes

augmenting effect of adiponectin favouring insulin signal transduction pathway.

**CONCLUSION**

Hypoadiponectinemia is evident in obese PCOS infertile women and is considered as valuable marker that represents dyslipidemia in PCOS women. TGL:HDLc ratio is a convenient and effective tool for the physicians to identify the individuals with insulin resistance and thereby lifestyle interventions can be advised that improves insulin sensitivity and fruitful reproductive process.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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