Clinical Utility of Serum Homocysteine for Predicting Insulin Resistance in Women with Polycystic Ovary Syndrome

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Abstract: ***Objectives**: Polycystic ovary syndrome (PCOS) is a common disease among young women that causes hyperinsulinemia. Patients of PCOS are at increased risk of dyslipidemia and insulin resistance and much of their complications. Also, Hyperhomocysteinemia is shown to be correlated positively with the hyperinsulinemia. This study aimed to evaluate INSULIN RESISTANCE as a function of HOMAIR and its correlation with other serum metabolic and biochemical parameters amongst PCOS women. **Methods**: Fifty women with PCOS and 40 healthy subjects were studied. Hormonal assays, lipid profile, Hcy and fasting insulin levels, insulin resistance (IR) indices (HOMA) determinations and ultrasound evaluation were performed. **Results**: Mean of BMI was significantly higher in PCOS subjects as compared with the controls (26.82 ±3.62 vs 24.65 ± 2.62 kg/m2; p = 0.002). The mean fasting insulin levels were also significantly higher (20.77 ± 9.69 vs 7.54 ± 3.55 lU/dl; p = 0.000) where we found no difference in fasting glucose concentrations between groups. IR indices in PCOS women were significantly higher than control group (p < 0.000). We observed significantly higher mean serum Hcy levels in PCOS patients (11.87 ± 5.55 vs 7.81 ± 2.29 lmol/l; p = 0.000). When patients were stratified by BMI, the Hcy concentration was not significantly difference in both obese and normal-weight PCOS women. Fasting insulin concentrations and IR indices were significantly higher in obese PCOS patients as opposed to normal-weight PCOS women. IR indices showed increasing trends with Hcy levels. **Conclusions**: Our study shows the tendency toward hyperhomocysteinaemia, hyperinsulinemia and higher BMI in PCOS patients. These factors are critical predictors of PCOS; however, BMI and IR are an independent risk factor to increase plasma Hcy levels in PCOS women.***

Keywords: Polycystic ovary syndrome, Insulin resistance, HOMA-IR, Hyperhomocysteinemia, Lipid profile

1. Introduction

Also known as stein Leventhal syndrome. It holds prevalence of 5-10 % in adults. It's a complex disorder owing to contribution of numerous genetic and environmental factors. Important implicated genes are DENND 1A, THADAA gene.

Excessive production of **androgen** forms the pathological basis of PCOS. It sources from ovaries (due to hyperinsulinemia; increase in LH) and adrenals (due to increase in DHEA, DHEAS). Increase in serum levels of androgens is called hyperandrogenemia and clinical manifestations of increased androgens is identified as hyperandrogenism. Common manifestations include hirsutism, acne, androgenic alopecia, dyslipidemia.

Androgens gets converted into estrogen in **adipose tissue**. Increased serum estrogen ultimately causes hyperthecosis, anovulation, increased chances of cancers (breast, ovarian), NASH. Anovulation further leads to infertility, abnormal menses.

Obesity plays an important role in course of PCOS through lipid alterations and BMI. Approximately half of PCOS patients are obese and development of insulin resistance is common. Women with PCOS are 2 to 4 times more likely to have metabolic syndrome than women without PCOS (18). Triglyceride levels are seen to be high in PCOS. BMI holds a positive correlation with TGs but inverse correlation with HDL, low LDL/HDL ratio. (17)

Statistically analysis

Data was analyzed by Statistical Package for Social Sciences (SPSS-19) version 23. Normalcy of the data was measured by Shapiro-Wilk test and continuous variables which were normally distributed were presented as mean ± standard deviation. Not- normal data was presented as Median and interquartile range. Mean standard (mean ± S.D.) of all parameters was analyzed by descriptive statistics. An independent t-test was used to compare the scores of each of the measures and some of the parameters data between the two groups. Mann – Whitney U test was used for non parametric continuous variables. Pearson's correlation coefficients (Spearman rho coefficients for not-normal data) were used to calculate correlation between paired data sets. A p-value of 0.05 was considered statistically significant. Also, model multivariate logistic regression and ROC curve analysis was used to assess the ability of different parameters to predict INSULIN RESISTANCE according to HOMA IR in pcos patients.

2. Results

The clinical, biochemical and hormonal characteristics of the study population is depicted in the below table. The groups were similar for age. BMI was significantly higher (p value =0.002) in PCOS women as compared to controls (26.82 vs 24.65). Hormonal analysis showed lower mean LH in controls (5.58 vs 9.78 p value - <0.001), lower mean LH/FSH ratio (0.982 vs 2.29, p value - <0.001), and lower mean testesterone levels (33.92 vs 58.53, p value - <0.001), whereas, serum prolactin levels were comparable in both groups. Fasting insulin levels were significantly higher in study pcos subjects than controls (20.77 vs 7.57, p value - <0.001). we also found significantly higher homocystiene values in PCOS subjects as compared to controls (11.87 vs 7.81 p value - <0.001).

Table 1: Comparison of clinical and biochemical parameters in PCOS and control groups

Group Statistics					
	Group	N	Mean (median)	Std. Deviation (interquartile range)	P value
AGE	Control	40	27.03	4.282	0.299
	Case	50	26.10	4.082	
S.LH	Control	40	4.39*	4.54*	0.000
	Case	50	9.7826	4.15828	
S.LH/S.FSH	Control	40	0.83*	0.6*	0.000
	Case	50	2.294	.9637	
S.TSH	Control	40	3.03*	2.59*	0.513
	Case	50	2.77*	2.01*	
TESTOSTERONE	Control	40	29.6*	17.12*	0.000
	Case	50	58.5368	24.00674	
INSULIN LEVEL	Control	40	6.45*	1.6*	0.000
	Case	50	20.769	9.6931	
HOMOCYSTEINE	Control	40	7.09*	2.89*	0.000
	Case	50	9.95*	7.95*	
HOMA-IR	Control	40	1.13*	0.20*	0.000
	Case	50	4.4236	2.23609	
W/H	Control	40	.80*	.04*	0.001
	Case	50	.8416	.05132	
BMI	Control	40	23.97*	3.19*	0.002
	Case	50	26.8232	3.62695	
CHOLESTEROL	Control	40	144.34*	77.4*	0.006
	Case	50	187.4676	58.72197	
LDL	Control	40	91.65*	49.64*	0.057
	Case	50	114.3975	35.98355	
TGS	Control	40	87.98*	51.97*	0.001
	Case	50	134.1*	80.76*	
HDL	Control	40	78.87*	24.6*	0.001
	Case	50	52.5058	25.42376	
S. PROLACTIN	Control	40	8.238	4.1830	0.968
	Case	50	8.204	3.8340	
TGYL/HDL ratio	Control	40	1.03*	0.68	0.019
	Case	50	2.69*	3.42*	
TCHOL/HDL ratio	Control	40	1.78*	0.99*	0.025
	Case	50	4.34*	4.10*	

- data presented as median value + interquartile range

Results

1) Comparison of biochemical parameters in IR and IS patients of PCOS

Comparison of BMI and biochemical parameters in PCOS patients as stratified based on insulin resistance is shown below. Overall, 36% and 64% of PCOS patients were IS and IR respectively. Biochemical parameters including fasting insulin, trig/HDL ratio, chol/HDL ratio depicted statistically significant mean value, which was higher in IR PCOS group against NIR PCOS group. Albeit, mean value of BMI was higher in IR PCOS group against NIR PCOS group but it was not statistically significant.

2) Hcy stratification and other variables in PCOS

Following table shows parameters stratified on the basis of serum homocysteine levels. The cut-off value of hcy was 11 mcmol/L. Hcy value of of 11 or less was considered to be in normal range, on the other hand value more than 11 was considered to be abnormal. Number of Patients landed in normal value range group and abnormal value range group was 32 and 18 respectively. Mean BMI was significantly higher ($p<0.001$) in PCOS patients with hcy greater than 11. indicators of peripheral insulin resistance such as fasting insulin and HOMA-IR were also significantly higher($p<0.001$) in PCOS patients with hcy greater than 11.

Biochemical parameters of dyslipidemia including trig/hdl ratio, Chol/hdl ratio, showed mean values on higher side in PCOS patients with hcy greater than 11.

Table 2: Comparison of BMI and biochemical parameters in PCOS patients

Parameters	INR	IR	P-Value
Numbers	18	32	-
Age (years)	26.56±3.53	25.84 ± 4.39	0.559
BMI (kg/m2)	25.73 ± 2.60	27.44 ± 3.99	0.110
Fasting insulin (IU/dl)	9.25 ± 1.39	27.24 ± 5.20	0.000
waist-hip ratio	0.82 ± 0.04	0.85± 0.05	0.067
trig/hdl ratio	1.79 ± 1.29	7.08 ± 7.38	0.004
Chol/hdl ratio	2.51±1.71	7.35 ± 5.82	0.001
HOMA-IR > 2.5	1.86 ± 0.28	5.86 ± 1.38	<0.0001
Hcy (lmol/L)	8.24 ± 2.77	13.92 ± 5.69	<0.0001

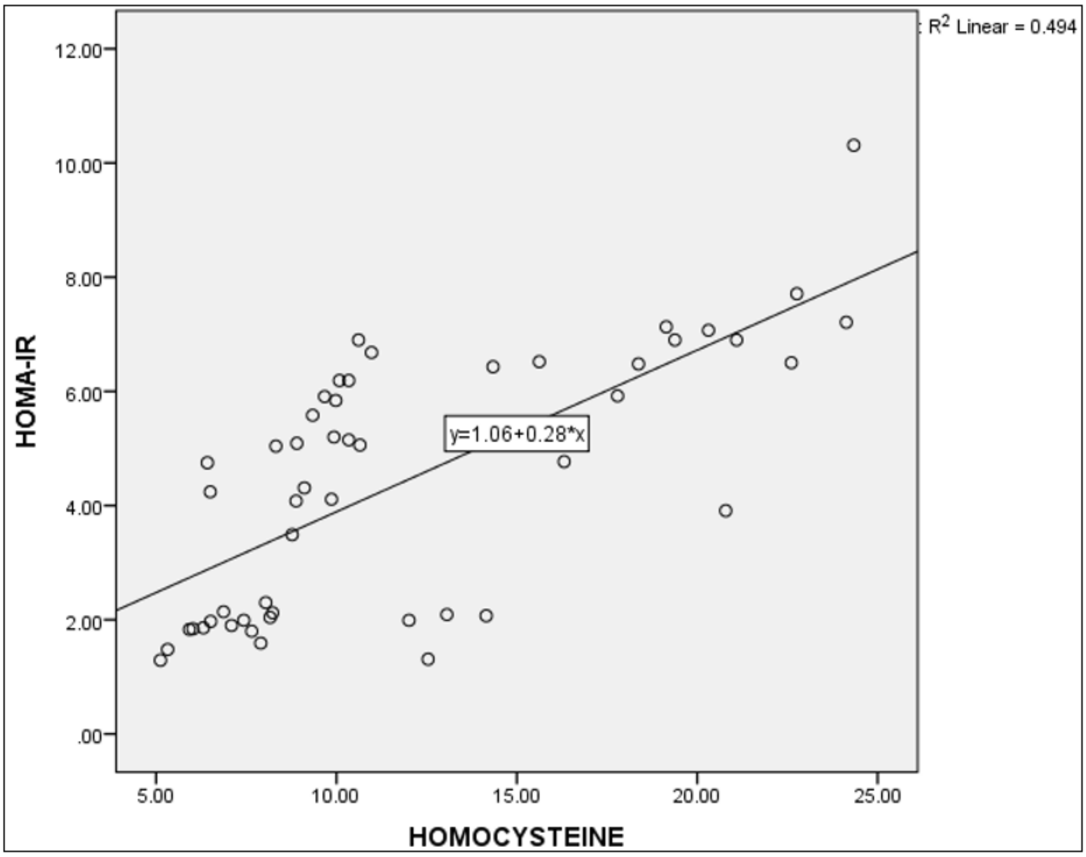
Parameters	INR	IR	P-Value
Numbers	32	18	-
Age (years)	25.75±3.63	26.72 ± 4.82	0.425
BMI (kg/m2)	25.52 ± 3.39	29.14 ± 2.81	0.000
Fasting insulin (IU/dl)	17.72 ± 8.15	26.17 ± 10.05	0.002
Waist- Hip Ratio	0.83 ± 0.44	0.86 ± 0.54	0.010
Trig/hdl ratio	1.93 ± 1.19	10.95 ± 7.89	0.000
Chol/hdl ratio	2.77±1.57	10.67 ± 5.82	0.000
HOMA-IR > 2.5	3.75 ± 1.84	5.62 ± 2.42	0.003
Hcy (lmol/L)	8.28 ± 1.71	18.26 ± 4.00	0.000

Below table (3) shows correlation of HOMA-IR and Hcy with various clinical and biochemical parameters. HOMA-IR correlation is being adjusted to age and AMI in PCOS patients. Lipid profile markers including TC, LDL-C, TG/HDL-C, TC/HDL-C showed positive correlation with HOMA-IR (<0.001). On the other hand, an important lipid biomarker HDL-C showed negative correlation with HOMA-IR(p<0.001). Circulating homocystein levels showed positive correlation with HOMA-IR(p<0.001). Similar results were seen in correlation of TC, LDL-C (positive; p<0.001) and HDL-C (negative; p<0.001) with Hcy.

Table 3: The correlation between HOMA-IR indices and HOMOCYSTIENE with clinical and biochemical parameter in the women with PCOS

Variable	
Age	0.101 (0.483)
BMI	0.368 (0.008)
Insulin	0.976 (0.000)
HOMOCYS	0.706 (0.000)
LH	0.652 (0.000)
FSH	0.385 (0.007)

* Signifies The Spearman Rho Correlation Coefficient.



Correlation between HOMOCYSTIENE and HOMA-IR. A positive significant correlation was observed between IR and HOMOCYSTIENE ($p < 0.001$).

A multivariable logistic-regression analysis was performed to ascertain the effects of level of homocystiene (homocysteine categorised at a cut off of <11 and > 11), BMI (obese or non-obese with BMI cutoff of 25) and CHOL/HDL ratio on the likelihood of INSULIN RESISTANCE. Less variables were taken due to lower no. of samples for each group and due to problems of collinearity. Linearity of the continuous variables with respect to the logit of the dependent variable was confirmed. Odds ratios and corresponding 95% confidence intervals were calculated.

The below table shows the results of a multivariate regression model. Patients with Homocysteine levels <8.5 (0.050, 95% CI [0.006–0.386]), or in other sense patients with serum homocystiene increase >8.5 had 20 times the odds of being insulin resistance, according to our model. However, other parameters were not statistically significant related with the HOMA-IR index.

		Logistic coeff	S.E.	Wald	df	Sig.	ODDS R	95% C.I for OR	
								Lower	Upper
	tcholbyhdl	.116	.149	.597	1	.440	1.122	.837	1.505
	BMI	-.027	.129	.044	1	.834	.973	.755	1.254
	Homocystienebinned (1)	-2.998	1.044	8.248	1	**.004**	**.050**	.006	.386
	Constant	1.955	3.459	.319	1	.572	7.063		

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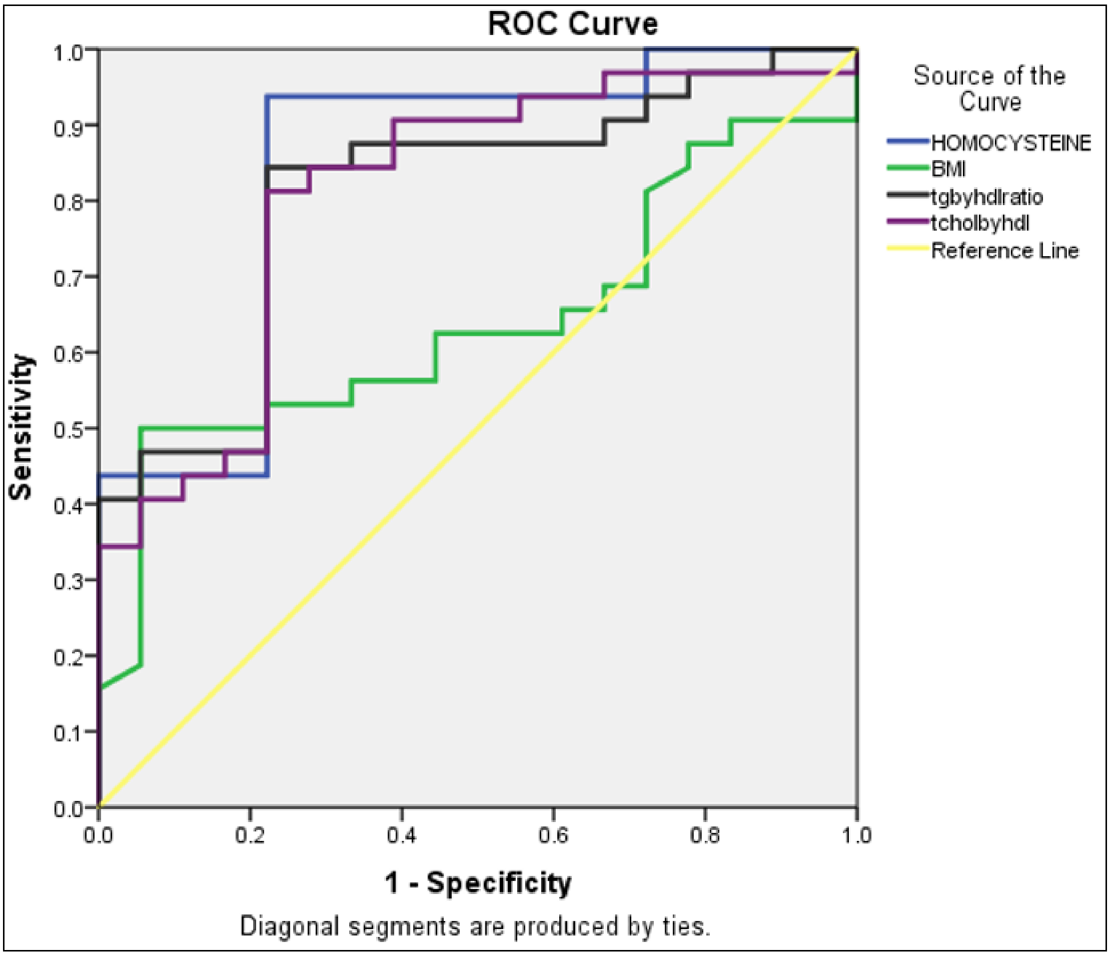

FIG. The results of ROC curve analysis regarding the predictability of TC/HDL-C, and TG/HDL-C, HOMOCYSTIENE AND BMI indices in classifying the IR in patients of PCOS is shown below. TC/HDL and TG/HDL cholesterol ratio strongly predicted IR with of AUC value of 0.81 ($p<0.001$). Serum Hcy levels more than 8.26 predicted insulin resistance with sensitivity of 94 % and specificity of 78 % ($p<0.001$). However, BMI did not predict insulin resistance ($p>0.001$).

Table: The areas under ROC curve (AUC), sensitivity, specificity by the optimized cut-off points for TyG, TC/HDL-C, and TG/HDL-C indices in predicting the HOMA-IR INDICES

Predictors					
HOMA-IR	HOMOCYSTIENE	0.84 (0.72–0.96)	0.000	≥ 8.27	0.94
0.78	TG/HDL		0.000	≥ 1.56	0.84
0.78	TC/HDL		0.000	≥ 2.58	0.81
0.78	BMI		0.106	≥ 25.31	0.63
0.57					

3. Discussion

Insulin resistance plays an important role in ascertaining the onset, pathogenesis, complications and prognosis of pcos patients. Patients of pcos ar at increased risk of hyperinsulinemia, dyslipidemia and thus, develop disorders like type 2 DM, hypertension. [1,2]. Many studeis have shown association between obesity, insulin resistance and serum homocysteine levels [3,4].

Dyslipidemia is a common metabolic disorder which affects pcos patients per se and also imposes major health hazard owing to its various complications. stats show about 70 % of pcos patients present with dyslipidemia [5]. Many concitions including diabetes, hereditary syndromes are known to affect lipid levels in our body. Women with pcos have increased levels of triglycerides, total cholesterol and LDL-C and low levels of HDL-C. [6]. We can use these lipid ratios for estimating insulin resistance in patients of pcos

In our study we found significant differences in HOMA-IR and Hcy levels between PCOS patients and controls, as evidenced by higher mean of respective values in PCOS patients. This is also evident from previously conducted studies which also showed significant differences in Hcy levels with respect to PCOS patients. [7,8,9].

Entire PCOS study group was stratified only on the basis of HOMA-IR and categorised as insulin resistant and non-insulin resistant group. The number of pateints being insulin resistant took a major stake of 32 out of 50 patients and rest were non-insulin resistant. Various parameters including BMI, lipid biomarker ratios, HOMA-IR were studied and compared among the groups. HOMA-IR, lipid marker ratios (Chol/Hdl; Trig/Hdl) showed mean value on higher side in IR group($p<0.05$).

Various studies have shown that the TG/HDL and TC/HDL ratios serve as independent predictor of diabetes incidence and have significant association with IR [10]. The positive correlation of TG/HDL-C and TC/HDL-C with the HOMA-IR index has been demonstrated among PCOS women in China [11] and diabetic patients in Thailand [12]. Also, several studies have advocated that TG/HDLC ratio could be used as an indicator of IR in some populations, including the middle-aged and elderly populations in Taiwan [13], euthyroid normal-weight healthy adults in Peru [14], PCOS females in India [15] and non-diabetic subjects in China [16].

In our study we found significant positive correlation of Tg/Hdl, TC/HDL and Hcy with HOMA-IR among PCOS patients. Assessment of diagnostic ability of lipid profile ratios (Tg/Hdl and TC/HDL) in classifying insulin resistance was also studied. ROC curve analysis was applied and showed a good predictability of TG/HDL and Hcy in classifying insulin resistance (AUC). In our study Hcy had the highest AUC with sensitivity of 94% and specificity of 78 % at cut-off value of 8.27. A positive significant relation was also found for Hcy levels with HOMA-IR ($r = 0.7$). This is in contrast to studies which show negative correlation of Hcy and HOMA-IR (17). TG/HDL-C and TC/HDL-C values had similar AUC values with regards to HOMA-IR index (0.81) with cut off values at 1.56 and 2.58 respectively. Thus, it seems that these ratio are effective indicators of HOMA-IR in PCOS patients. In a previous study with similar sample size in Iranian PCOS women, these lipid profile ratios predicted IR based on HOMA-IR levels with lower sensitivity and specificity at optimal cut off values (17). Insulin resistance is intrinsic to patients of PCOS. Lipid ratios and homocystiene levels can be easily accessible, cost- effective tools for assessing insulin resistance in patients of PCOS. Also homocystinemia might be additional independent risk factor for PCOS. Further epidimiological studies with large data is required to evaluate and refine the findings.

References

[1] M. Ashrafi, F. Sheikhan, A. Arabipoor, R. Hosseini, F. Nourbakhsh, Z. Zolfaghari, Gestational diabetes mellitus risk factors in women with polycystic ovary syndrome (PCOS), Eur. J. Obstet Gynecol. Reprod. Biol. 181 (2014) 195–199.

[2] F. Foroozanfard, S.G. Moosavi, F. Mansouri, F. Bazarganipour, Obstetric and neonatal outcome in PCOS with gestational diabetes mellitus, J. Family Reprod. Health 8 (1) (2014) 7–12

[3] I. Tarkun, B. Cetinarslan, E. Turemen, T. Sahin, Z. Canturk, B. Komsuoglu, Effect of rosiglitazone on insulin resistance, C-reactive protein and endothelial function in non-obese young women with polycystic ovary syndrome, Eur. J. Endocrinol./ Eur. Federation Endocrine Soc. 153 (1) (2005) 115–121

[4] M. Schachter, A. Raziel, S. Friedler, D. Strassburger, O. Bern, R. Ron-El, Insulin resistance in patients with polycystic ovary syndrome is associated with elevated plasma homocysteine, Hum. Reprod. 18 (4) (2003) 721–727.

[5] Lath R, Shendye R, Jibhkate A. Insulin resistance and lipid profile in polycystic ovary syndrome. Asian J Biomed Pharm Sci. 2015; 5: 30.

[6] Marshall JC, Dunaif A. Should all women with PCOS be treated for insulin resistance? Fertil Steril. 2012;97:18–22

[7] Sachan Rekha, M.L. Patel, Gupta Pooja, Sachan Pushpalata, S.M. Natu, Y. Pradeep, Correlation of plasma homocysteine levels with BMI and insulin resistance, amongst obese, overweight and non obese infertile women, Int. J. Sci. Res. Publ. 2 (5) (2012) 1–6.

[8] Sachan Rekha, M.L. Patel, G. Pooja, D. Amita, S. Pushplata, S.M. Natu, Correlation between elevated homocysteine levels and insulin resistance in infertile women with or without polycystic ovary syndrome in North Indian population, Int. J. Med. Med. Sci. 5 (3) (2013) 116–123.

[9] S. Salehpour, O. Manzor-Al-Ajdad, E.N. Samani, A. Abadi, Evaluation of homocysteine levels in patients with polycystic ovarian syndrome, Int. J. Fertility Sterility 4 (4) (2011) 168–171

[10] Hadaegh F, Hatami M, Tohidi M, Sarbakhsh P, Saadat N, Azizi F. Lipid ratios and appropriate cut off values for prediction of diabetes: a cohort of Iranian men and women. Lipids Health Dis. 2010; 9: 85.

[11] Xiang S-K, Hua F, Tang Y, Jiang X-H, Zhuang Q, Qian F-J. Relationship between serum lipoprotein ratios and insulin resistance in polycystic ovary syndrome. Int J Endocrinol. 2012; 2012: 173281.

[12] Tangvarasittichai S, Poonsub P, Tangvarasittichai O. Association of serum lipoprotein ratios with insulin resistance in type 2 diabetes mellitus. Indian J Med Res. 2010; 131: 641.

[13] Yeh W-C, Tsao Y-C, Li W-C, Tzeng I-S, Chen L-S, Chen J-Y. Elevated triglyceride-to-HDL cholesterol ratio is an indicator for insulin resistance in middle-aged and elderly Taiwanese population: a cross-sectional study.

[14] Pantoja-Torres B, Toro-Huamanchumo CJ, Urrunaga-Pastor D, GuarnizoPoma M, Lazaro-Alcantara H, Paico-Palacios S, et al. High triglycerides to HDL-cholesterol ratio is associated with insulin resistance in normal-weight healthy adults. Diabetol Metab Syndr. 2019; 13: 382–8. Lipids Health Dis. 2019;18:176

[15] Kalra A, Nair S, Rai L. Association of obesity and insulin resistance with dyslipidemia in Indian women with polycystic ovarian syndrome. Indian J Med Sci. 2006; 60:447

[16] Zhang L, Chen S, Deng A, Liu X, Liang Y, Shao X, et al. Association between lipid ratios and insulin resistance in a Chinese population. PLoS One. 2015; 10: e0116110.

[17] Evaluation of lipid ratios and triglycerideglucose index as risk markers of insulin resistance in Iranian polycystic ovary syndrome women Kheirollahi et al. Lipids in Health and Disease (2020) 19:235 https://doi.org/10.1186/s12944-020-01410-8

[18] Hyperhomocysteinemia, insulin resistance and body mass index in Iranian young women with polycystic ovary syndrome --Sedigheh Esmaeilzadeh a, Eisa Tahmasbpour b, Maryam Gholinezhad-Chari