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Correlation of HbA_{1C} levels with body mass index in newly diagnosed polycystic ovary syndrome

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ABSTRACT

Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous, multisystem endocrinopathy in women of reproductive age manifested with various metabolic disturbances and a wide spectrum of clinical features such as obesity, menstrual abnormalities and hyperandrogenism. Hyperinsulinemia was noted in 50% to 70% of PCOS patients and plays a central role in the development of further complications. The prevalence of obesity in PCOS ranges from 38% to 87%. It has been reported in previous studies that the prevalence of insulin resistance (IR) is higher in obese PCOS women than obesity without PCOS.

Objective

To correlate HbA_{1c} levels with body mass index (BMI) in newly diagnosed polycystic ovary syndrom (PCOS) women.

Material and methods

This case-control study performed at Pt. B. D. Sharma PGIMS, Rohtak includes 30 newly diagnosed PCOS patients and 30 age matched healthy controls of any age. Fasting venous blood samples were obtained for

analysis of fasting blood glucose, haemoglobin and HbA_{1c} after obtaining written consent.

Results

HbA_{1c} concentration was higher in cases (6.03±0.19%) than in controls (5.35± 0.08%) with p = 0.002. 33.3% were in pre diabetic range (5.7- 6.4%), 36.7% in diabetic range (≥6.5%) and 30% in non diabetic range (<5.7%). BMI of cases (26.09±4.2 kg/m^2) was significantly higher than controls (22.9±3.3 kg/m^2) with p=0.002. HbA_{1c} showed positive correlation with BMI (p= 0.001).

Conclusion

In conclusion, BMI is associated with glycemic control in PCOS.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous, multisystem endocrinopathy in women of reproductive age manifested with various metabolic disturbances and a wide spectrum of clinical features such as obesity, menstrual abnormalities and hyperandrogenism. Current incidence of PCOS (5-6%) in women is related to change in lifestyle and stress [1].

According to the World Health Organization, it was estimated that 116 million women (3.4%) have PCOS with prevalence ranging from 2.2% to 26% globally [2]. Hyperinsulinemia was noted in 50% to 70% of PCOS patients and played a central role in the development of further complications. In spite of hyperinsulinemia, there is an increased prevalence of insulin resistance (IR) in PCOS patients which can lead to increased glucose tolerance and type 2 diabetic mellitus (T2DM). Increased insulin leads to increased androgen production from the ovarian thecal cells and this hyperandrogenemia is responsible for androgenic obesity.

The prevalence of obesity in PCOS ranges from 38% to 87%. It was reported that in obese PCOS women, the prevalence of IR was higher than obese women without PCOS in the control group [3].

MATERIAL AND METHODS

The present case control study was conducted in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynaecology, Pt. B. D. Sharma PGIMS, Rohtak. After getting written consent from the cases and controls, detailed history were obtained and recorded in their respective proforma. They were subjected to physical examination and anthropometric measurements as per protocol followed by systemic examination, urine pregnancy test was done to rule out pregnancy, ultrasonography of abdomen and pelvis were performed in all subjects of this study.

Inclusion criteria

Newly diagnosed cases of PCOS as per Rotterdam definition - the existence of the following three criteria to make the diagnosis of PCOS:

1. oligo-ovulation or anovulation;
2. clinical or biochemical signs of hyperandrogenism; and
3. polycystic ovaries by Ultrasonography [4].

Exclusion criteria

Any history suggestive of other potential causes of hyperandrogenism/oligo/amenorrhea [congenital adrenal hyperplasia, androgen secreting tumor], hypothyroidism, cushing's syndrome, hyperprolactinemia, other pituitary/adrenal disorders, other insulin resistance conditions (acromegaly), history of any drug intake and pregnancy [3].

Thirty age matched healthy females with regular menstrual cycle and not on any treatment were enrolled as controls.

Anthropometric measurements

The weight and standing height of all study subjects were measured twice by using calibrated weighing scale and stadiometer with a fixed vertical backboard and an adjustable head piece respectively by two different examiners to avoid subjective error. BMI is expressed in the units of kg/m2. BMI can be calculated by the present weight in kg divided by square of height in metres (Quetelet index). According to the World Health Organisation, BMI can be graded into the categories listed in Table 1.

SAMPLE COLLECTION

Six mL of venous blood sample after fasting (10 - 12 hours) was taken from the antecubital vein aseptically on the second day of menstruation, out of which 2 mL of blood in EDTA anticoagulant vacutainer, 2 mL in sodium fluoride vacutainer and 2 mL in plain vacutainer. Serum from sodium fluoride and plain vacutainer were separated by centrifugation at 2000 rpm for 5 minutes and fasting plasma glucose (FBG) and hormones were determined. EDTA anticoagulant sample was used for hemoglobin and HbA_{1c} determination.

The FBG was performed by enzymatic method (Glucose oxidase method) [6], HbA_{1c} by latex agglutination inhibition assay [7] on the RANDOX autoanalyser. Prolactin, total testosterone, luteinising hormone (LH) & follicular stimulating hormone (FSH) were estimated on the Advia Centaur' CP immunoassay system by chemiluminescence method [8]. Thyroid stimulating hormone (TSH) was quantitated by immunoradiometric assay by IRMA kit (IRMAK - 9) [9]. Hemoglobin estimation was done by acid haematin method using Sahli's hemoglobinometer [10].

STATISTICAL ANALYSIS

Unpaired 't' test and two-tailed Pearson's correlation were done between variables of PCOS cases and controls using the IBM SPSS version 20 statistical package. Data were considered to be significant if $p < 0.05$ and highly significant with $p < 0.001$.

RESULTS AND OBSERVATIONS

In the present study, we found that 8 newly diagnosed PCOS cases (26.7%) had history of sudden weight gain. Based on categories of BMI, 16

Table 1 WHO classification of BMI grading [5]

BMI (kg/m^2)	Classification
< 18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Overweight
30.0–34.9	Class I obesity
35.0–39.9	Class II obesity
≥ 40.0	Class III obesity

Table 2 Descriptive data of the study groups

S.No.	Descriptive data	Cases (n = 30) Mean ± SD	Controls (n = 30) Mean ± SD	't' value	p value
1.	Age (years)	23.26 ± 5.65	23.6 ± 6.45	-0.213	0.832
2.	BMI (kg/m^2)	26.1 ± 4.2	22.9 ± 3.3	3.245	**0.002***

** - significant difference, ** - highly significant difference*

cases and 7 controls were overweight and obese (≥ 25 kg/m^2) with statistically significant difference between cases and controls (p=0.002). Thirteen cases were within the normal range of BMI and 1 case was underweight.

We observed that 7 cases (89.2 ± 13.9 mg/dL) and 2 controls (88.5 ± 2.7 mg/dL) had FBG ≥ 100 mg/dL with non significant p value > 0.05. We found that HbA_{1c} showed statistical significant difference (p=0.002) between cases (mean 6.03 ± 1.03%) and controls (mean 5.35 ± 0.43%) (Tables 2 & 3).

We noted from our HbA_{1c} data, 10 cases (33.3%) were in pre diabetic range (5.7 - 6.4%), 11 cases (36.7%) were in diabetic range (≥ 6.5%) and 9 cases (30%) were in non diabetic range (< 5.7%) according to ADA criteria (Figure 1).

We observed the prevalence of T2DM and pre-diabetes were 13.3% (4 cases) and 6.6% (2 cases) in obese PCOS women, respectively. But the prevalence of T2DM and prediabetes were 10% (3 cases) and 16.6% (5 cases) in overweight PCOS women, respectively.

We found that 7 cases (23.3%) were prediabetes with FG 100-126 mg/dL. But among controls, we noticed only one case (3.3%) had prediabetes (Figure 2).

TWO-TAILED PEARSON'S CORRELATION BETWEEN PARAMETERS

In the present study, it was found that HbA_{1c} had positive correlation with BMI (r=0.439, p=.001) (Figure 3).

Table 3 Analytical data of the study groups

S.No.	Analytical data	Cases (n = 30) Mean ± SE	Controls (n = 30) Mean ± SE	't' value	p value
1.	FG (mg/dL)	89.23 ± 2.5	88.47 ± 1.4	0.264	0.792
2.	Hb (g/dL)	14.16 ± 3.24	11.5 ± 0.17	0.929	0.357
3.	HbA_{1c} (%)	6.03 ± 0.19	5.35 ± 0.08	3.364	**0.002***

** - significant difference, ** - highly significant difference*

Figure 1 The mean value ± SE of HbA_{1c} of the cases and controls

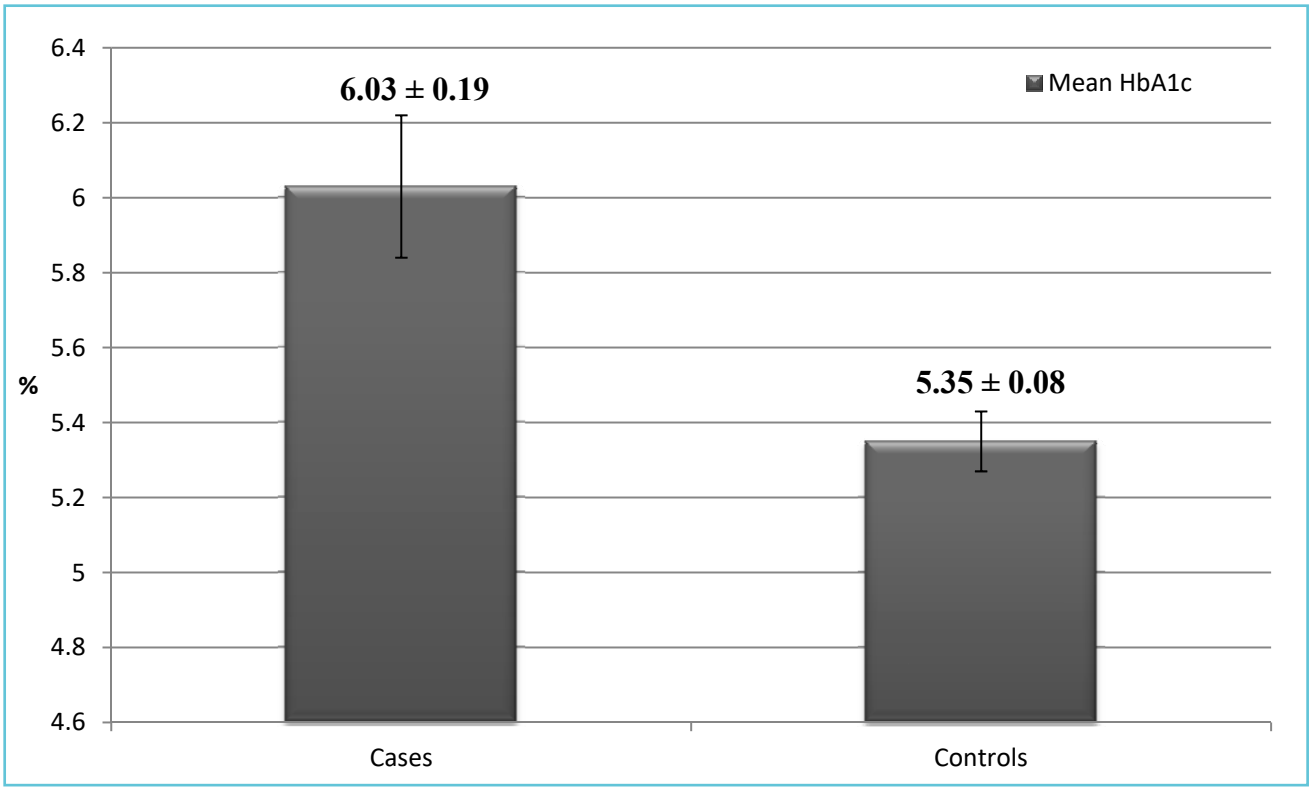


Figure 2 Bar diagram representing various categories based on HbA_{1c} level in the cases and controls

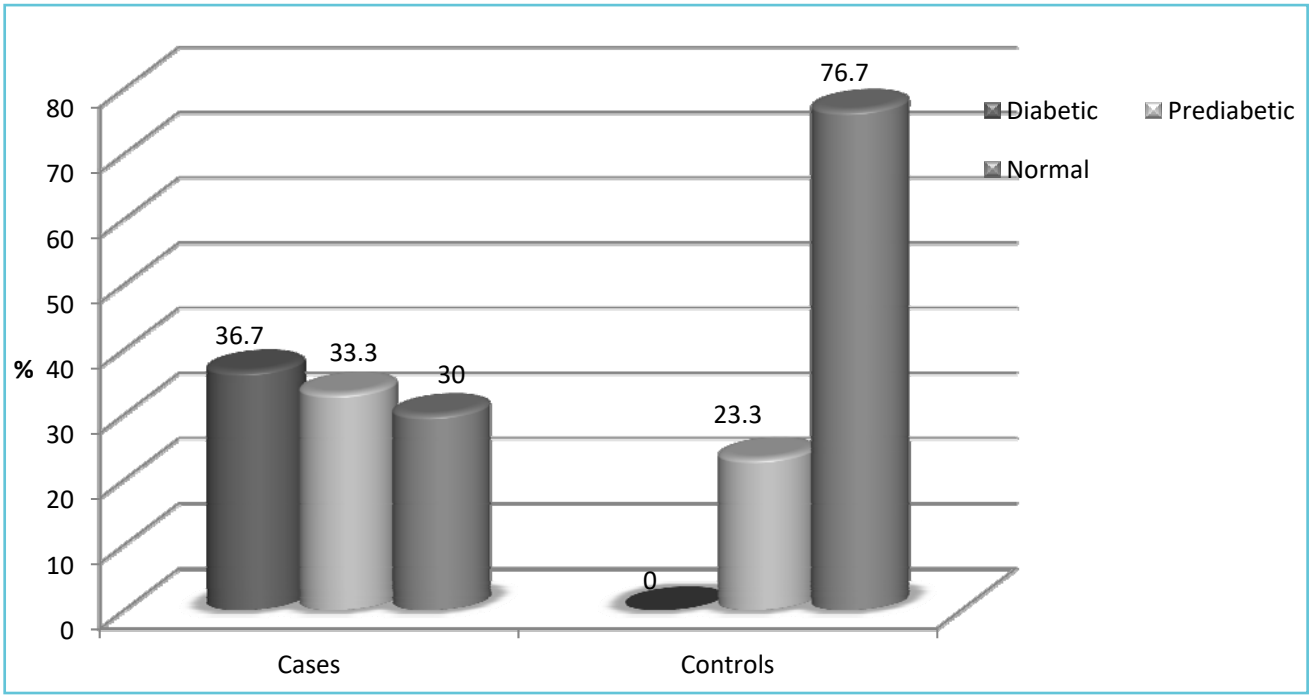
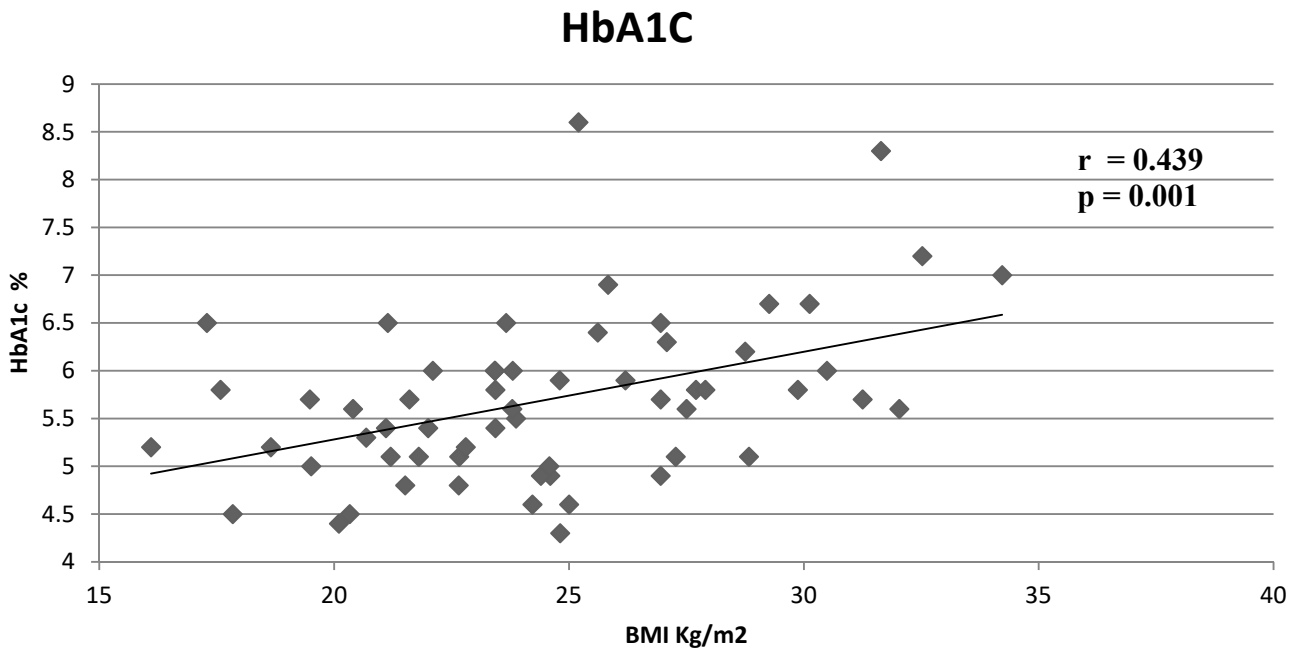


Figure 3 Graph showing the correlation between BMI & HbA_{1c}



DISCUSSION

When the hemoglobin is exposed to plasma glucose, there is a spontaneous non-enzymatic glycation of hemoglobin resulting in HbA_{1c} formation. The rate of formation of HbA_{1c} is directly proportional to the concentration of the glucose in the blood and represents integrated values for the glucose over the preceding 8 to 12 weeks. HbA_{1c} is formed by the condensation of glucose with the N-end of each beta chain to form an unstable schiff base. The schiff base may dissociate or may undergo an amadori rearrangement to form a stable ketomine called as HbA_{1c}. It has been established that HbA_{1c} is an index of long term blood glucose concentrations and as a measure of the risk for the development of microvascular complications in patients with diabetes mellitus. The formation of HbA_{1c} is an irreversible process and depends on lifespan of RBC and glucose concentration in the plasma. Severe anemia can show false high HbA_{1c} value as the old red blood cell (RBC) population is higher than non anaemic conditions. In haemolytic anaemia, HbA_{1c} value is falsely low as life span of RBC is less. Therefore, it is mandatory to rule out anaemia in our study to avoid false results [11].

Lerchbaum et al reported that the prevalence of obesity was 24.8% and overweight was 21.8% among PCOS women [12]. Gomathi et al found that 54% of the women with PCOS were overweight or obese with respect to WHO classification of BMI grading [13]. Randeva et al reviewed that excess triglycerides enter into cells and activate proteins kinase C-ε and C-θ, ultimately reducing the glucose uptake. This leads to compensatory hyperinsulinemia which can stimulate excess fat deposition by hypertrophy and hyperplasia of adipose cells in the excess calorie environment. This is further aggravating IR by increasing obesity as a vicious cycle. This abnormal fat accumulation increases IR causing glucose intolerance and T2DM [14].

The prevalence of obesity in the PCOS population ranges from 38% to 87%. It has been reported that in obese PCOS women, the prevalence of IR was higher than obese women with normal menstrual cycle in the control group [3].

Medeiros et al concluded from their study, among amazonian PCOS women, that HbA_{1c} was elevated in nearly 40% of PCOS patients and had positive correlation with several anthropometric, metabolic factors and androgen levels [15].

While evaluating PCOS women, along with FBG estimation, they are needed to have oral glucose tolerance test (OGTT) or HbA_{1c} also to assess their metabolic status and can be categorised as per the American Diabetic Association (ADA) guidelines 2015 by the following any one of the criteria listed in Table 4.

As OGTT is time consuming and cumbersome test, HbA_{1c} and FBG have been suggested as screening tools for prediabetes and T2DM. They also recommended HbA_{1c} as the superior screening tool for dysglycemia assessment in PCOS women [12].

Increased LH leads to increased stromal growth accounts for increased ovarian volume. Increased circulating LH stimulates ovarian thecal cells to produce more androgens leading to hyperandrogenemia in turn leads to increased male pattern (top / apple shaped upper abdomen) of fat accumulation both subcutaneously and around viscera which in turn leads to obesity, increased BMI.

High saturated fat, trans fat diet, less poly unsaturated fatty acid (PUFA) intake, high fructose/sucrose intake, low fat and high carbohydrate diet, sedentary life style, leptin resistance can contribute high triglycerides and VLDL-C level in the circulation which can alter plasma membrane composition resulting in decreased tyrosine acetylphosphorylation of insulin receptor [17-26].

Table 4 The ADA guidelines 2015 for diagnosis of diabetes & prediabetes [16]

	Criteria for diabetes diagnosis
1.	HbA_{1c} ≥ 6.5%
2.	FBG ≥ 126 mg/dL (7.0 mmol/L)
3.	2-hr plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during OGTT (75g)
4.	Random plasma glucose ≥ 200mg/dL (11.1mmol/L)
	Criteria for prediabetes diagnosis
1.	HbA_{1c} 5.7%-6.4%
2.	FBG 100-125 mg/dL (5.6-6.9 mmol/L)
3.	2-hr plasma glucose 140-199 mg/dL (7.8-11.0 mmol/L) during OGTT (75g)

Kumar et al also observed higher BMI in women with PCOS than in controls [27]. The present study observations showed that 14 cases (46%) had increased BMI (≥ 25 Kg/m^2) and increased HbA_{1c} and 16% of cases had normal BMI with increased HbA_{1c} levels. Among controls who had increased HbA_{1c}, we found that 6.7% of them had increased BMI and 3.3% had normal BMI. The prevalence of IR was found to be increased in obese PCOS women than in normally menstruating obese women. IR plays a central role in the pathogenesis of PCOS. Obesity is a co-morbid condition which can increase its risk. Though obesity is more prevalent among PCOS women, about 20 - 30% of PCOS women are not obese. Hyperinsulinemia causes direct hypothalamic effects which lead to abnormal appetite and gonadotropin secretion resulting in increased LH secretion in PCOS. This increased level of LH causes excessive androgen production in the ovaries [3].

CONCLUSION

In conclusion, BMI is associated with glycemic control in PCOS.

REFERENCES

1. Disorders of the ovary and benign tumours. In: Padubidri VG, Daftary SN, editors. Howkins & Bourne Shaw's textbook of gynaecology. 15thed. New Delhi: Elsevier; 2011. p. 369-71.

2. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the global burden of disease study 2010. Lancet 2012;380:2163-96.

3. Attaran M. Disease management project clinical decisions. Polycystic ovary syndrome [Internet]. 2010 [cited 2010 August 1]. Available from: http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/womens-health/polycystic-ovary-syndrome

4. The Rotterdam ESRE/ASRM sponsored PCOS consensus workshop group revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovarian syndrome. Fertil Steril 2004;81:19-25.

5. The world health report 2000 - Health systems: improving performance. p. 9.

6. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz textbook of clinical chemistry. 5th ed. New Delhi: Elsevier; 2012. p. 718-21.

7. Khan MAH, Rabeya MR, Saiedullah M. Measurements of HbA_{1c} by high performance liquid chromatography in D-10 analyzer and immunological method by Beckman Coulter AU480 System: A comparative study. J Enam Med Col 2012;2:62-6.

8. Mohseni MG, Hosseini SR, Alizadeh F, Rangzan N. Serum testosterone and gonadotropins levels in patients with premature ejaculation: A comparison with normal men. Adv Biomed Res 2014;3:6.

9. Samad MA, Haque MM, Shah MK, Islam MR, Mia MC. Evaluation of TSH, T4 and T3 in human serum: standardization on normal individuals. American Journal of Modern Physics 2013;2:202-7.

10. Balasubramiam P, Malathi A. Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. J Postgrad Med 1992;38:8-9.

11. Sacks DB. Diabetes Mellitus. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz textbook of clinical chemistry. 5th ed. New Delhi: Elsevier; 2012. p. 1441-3.

12. Lerchbaum E, Schwetz V, Giuliani A, Pietsch OB. Assessment of glucose metabolism in polycystic ovary syndrome. Hum Reprod 2013;28:2537-44.

13. Gomathi K, Shaafie IA, Mummigatti K, Shahid S, Sreedharan J. Biochemical parameters in women with polycystic ovary syndrome in Ajman, UAE. NJOG 2011;6:7-10.

14. Randeva HS, Tan BK, Weickert MO, Lois K, Nestler JE, Sattar N, et al. Cardiometabolic aspects of the polycystic ovary syndrome. Endocr Rev 2012;33:812-41.

15 Medeiros SFD, Yamamoto MMW, Bueno HB, Belizario D, Barbosa JS. Prevalence of elevated glycated hemoglobin concentrations in the polycystic ovary syndrome: anthropometrical and metabolic relationship in amazonian women. J clin med res 2014;6:278-86.

16. American Diabetes Association. Standards of medical care in diabetes-2015. Diabetes Care 2015;38(Suppl-1): S93.

17. Schinner S, Scherbaum WA, Bornstein SR, Barthel A. Molecular mechanisms of insulin resistance. Diabetic Med 2005;22:674-82.

18. Morino K, Petersen K, Shulman G. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes 2006;55:S9-15.

19. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Diabetes 1991;40:280-9.

20. Kazunori K, Chen G, Lee Y, Unger RH. Tissue triglycerides, insulin resistance and insulin production: implications for hyperinsulinemia of obesity. Am J Physiol 1997;273:708-13.

21. Michael R, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, et al. Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest 1996;97:2859-65.

22. Heather B, Lisa F, Khosrow A. Fructose, insulin resistance and metabolic dyslipidemia. Nutr Metab (Lond) 2005;2:5.

23. Huang YJ, Fang VS, Juan CC, Chou YC, Kwok CF, Ho LT. Amelioration of insulin resistance and hypertension in a fructose fed rat model with fish oil supplementation. Metab Clin Exp 1997;46:1252-8.

24. Wang J, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L. Overfeeding rapidly induces leptin and insulin resistance. Diabetes 2001;50:2786-91.

25. Davis MEJ, d'Agostino R, Karter AJ, Haffner SM, Rewers MJ, Saad M, et al. Intensity and amount of physical activity in relation to insulin sensitivity. JAMA 1998;279:669-74.

26. Haugaard SB, Sten M, Erik HC, Allan V. Dietary intervention increases n-3 long-chain polyunsaturated fatty acids in skeletal muscle membrane phospholipids of obese subjects. Implications for insulin sensitivity. Clin Endocrinol 2006;64:169-78.

27. Kumar A, Woods K S, Bartolucci A A and Azziz R. Prevalence of adrenal androgen excess in patients with the polycystic ovary syndrome (PCOS). Clin Endocrinol 2005;62:644-9.