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Abnormal Glucose Metabolism in Patients with PCOS: Comparison between OGTT and HbA1c

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Abstract Introduction: Polycystic ovarian syndrome is one of the most common Endocrine disorders affecting women and is associated with a raised risk of impaired glucose tolerance, impaired fasting glucose, and type 2 diabetes mellitus. Insulin resistances, and the development of compensatory hyperinsulinemia, are usual findings in polycystic ovarian syndrome. Aims: To Compare the diagnosis of diabetes mellitus in patients with polycystic ovarian syndrome (PCOS) using glycated hemoglobin (HbA1c) and OGTT in a sample of Iraqi ladies with PCOS. Methods: This is a cross section study enrolled 40 patients female with PCOS, The patients were tested for OGTT, HbA1c and fasting blood glucose Was carried out at the endocrinological outpatient department of al Imamein Kadhimein medical city Teaching Hospital, and National diabetes center. Results: The present study included 40 diagnosed patients with PCOS. The mean age of the patients at presentation was 30.95±9.63years (range 16-44 years). According to 2h-OGTT, 28 (70%) women had normal glucose level. Among those, only 24 were also normal according to HbA1c, and 16 normal according to FPG. Impaired glucose tolerance was reported in 9 patients. 3 women (7.5%) were considered to have diabetes according to 2h-OGTT, all of whom were detected as normal in both HbA1c and FPG. Receiver operating characteristic was used to evaluate the predictive value of HbA1c in predicating IGT. The test's sensitivity and specificity were 77% and 65%, respectively. Conclusions: T2DM and prediabetes common in PCOS patient, and when compared with the 2-hr OGTT, HbA1c is insufficient screening tool for prediabetes and T2DM screening in female with PCOS

Keywords: *dysglycemia, PCOS, HBA1C, PRE DM, IR*

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1. Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common Endocrine disorders affecting women [1]. PCOS is having many facets, constantly changing disease and a challenging disorder for the treating physician due to the persistent need for treatment modifications and adjustments based on the patient's fluctuating needs and preferences during the course of her life [1,2]. The pathophysiology of PCOS involves a combination of factors such as adrenal and ovarian hyperandrogenism, adiposity, insulin resistance and gonadotropin secretion abnormalities [4]. The appearance of diabetes mellitus type 2(T2DM) in PCOS can be expected to some extent given that the two prerequisites for T2DM development, insulin resistance (IR) and ß-cell dysfunction, are commonly present in women with PCOS. Indeed, IR which is a key player in underlying PCOS pathophysiology which occurs to a greater extent compared to healthy body mass index-matched female. An additional effect of obesity on the degree of IR reported in these female must be also taken into concern [4]. An insulin resistance at least in part contributes to IGT and elevates risk of developing type2 diabetes mellitus [2]. PCOS is described by increased ovarian and adrenal androgen secretion, hyper androgenic symptoms, menstrual irregularity and polycystic ovaries [2]. The development of compensatory hyperinsulinemia, are usual findings in PCOS [4]. In particularly, evidence from large prospective cohorts has shown lead to either prediabetes or type 2 diabetes mellitus (T2D) over time [5]. A meta-analysis of cross-sectional studies including 4795 female from the general population found higher testosterone concentrations in patients with T2DM compared to control [7]. In addition bioavailable testosterone correlated significantly with insulin resistant, with higher concentrations predicting the development ofT2DM [8]. Same results were obtained from a systematic review and meta-analysis pooling data from the Rotterdam Study and other studies, which found that patients at the highest three

times of free androgen index had a 42% higher risk of developing T2DM, in a complex multivariate analysis controlling, among others, for age, BMI, glucose, and insulin concentrations [9]. Given that PCOS is characterized by markedly higher androgen concentrations compared to those in the unaffected women, an association between the PCOS and T2DM constitutes a rational hypothesis [10]. Ever since, several studies have confirmed this relationship in both lean and obese female with polycystic syndrome. However, IGT can be underdiagnosed, even in populations at high risk, for the reason that the diagnosis of IGT necessarily an oral glucose tolerance test (OGTT) [6]. A newly meta-analysis of genetic studies suggests that there is no inherent T2DM risk in PCOS and that T2DM instead of occurs as a consequence of either increased adiposity or hyperandrogenemia [5]. On the other side, PCOS constitutes a polygenic trait and elegant studies have shown that clusters of genes leading to metabolic disturbances are different from those associated with overt hyper androgenic signs in PCOS female [6]. Therefore, a genetic component of dysglycemia among PCOS female must be considered. in the opinion of American study, IGT and T2DM are present in 31–35% and 7.5–10%, respectively, of American women with PCOS [7]. correspondence over a definitive recommendation with regard to the optimal method of working for assessment of glycemic status has not yet been achieved to date [6]. Thus, we aimed to examine the usefulness of glycated hemoglobin (HbA1c) and fasting glucose (FG) in estimating the risk of prediabetes and Type2 diabetes mellitus in this study PCOS women from national diabetes center and Al imamein kadhimein medical city teaching hospital that diagnosed with the Rotterdam criteria. On the other hand, in daily practice, the performance of OGTT may be inconvenient and time consuming in view of the fact that the patient has to be assessed while fasting and, for that reason, needs to be present at the clinic usually on two different days [7]. Another screening method is fasting plasma glucose (FPG), the concentration of which depends on hepatic glucose secretion. Although cheap and easy to administer, like OGTT it requires patients to fast for at least eight hours prior to blood draw and can vary significantly relying on illness, medications, prolonged fasting, exercise, and stress. Thus, a strategy that could diminish or replace the need for an OGTT would be useful [8]. A third frequently utilized screening test is HbA1C, which quantifies the quantity of glycated hemoglobin in the blood and is a measure of long-term glycemic exposure [9]. HbA1c has been shown to correlate with the risk of development of microvascular disease such as diabetic retinopathy and has less within-person variation than FPG and 2h GTT values, which can fluctuate significantly from day to day [10,11]. Measuring a HbA1C value does not demand the patient to fast and only be in need of a single blood draw, to prepare it more comfortable for both the patient and health provider [12,13]. However, there are a number of hematologic conditions in which HbA1c may not give an accurate result; For example, hemoglobinopathies may interfere with the HbA1c explanation, as well as any condition in which red blood cell turnover is increased, such as hemolytic anemia, chronic malaria, and acute blood loss [14,15,16]. Hemoglobin A1c (HbA1c) is a commonly used marker of chronic glycemia, and it reveals the average blood glucose levels over a two- to three-months period [17]. The disturbance of the day-to-day variability of glucose values and the need for fasting and preceding dietary preparations can be avoided most of the time using HbA1c instead of OGTT [18]. Latterly, an international expert committee composed of members of the European Association for the Study of Diabetes, the International Diabetes Federation and the American Diabetes Association (ADA) stated that HbA1c levels between 5.7-6.4% should lead to an OGTT, whereas HbA1c levels of less than 5.7% indicate that no further tests are required (ADA, 2023).We also estimated whether the use of HbA1c resulted in the diagnosis of more cases of glucose intolerance in our PCOS inhabitants than the OGTT lonely. The objective of this study was to determine whether HbA1c precisely predicts the presence of pre-DM in this patient people when considering 2h OGTT as the gold standard.

2. Aim of Study

To compare the diagnosis of diabetes mellitus in patients with polycystic ovarian syndrome (PCOS) using glycated hemoglobin (HbA1c) and OGTT.

Patients and methods:

This is a cross sectional study enrolled 40 female patients with PCOS, aged 16–45 years who were routinely referred to our outpatient clinic for PCOS evaluation from April (2022) to April (2023). Was carried out at the endocrinological outpatient department of Al imamein kadhimein medical city Teaching Hospital, and National diabetes center.

Inclusion criteria:

1. The subjects were premenopausal women (15-45)
2. PCOS

Exclusion criteria:

1. Diabetes
2. Patients outside of the designated age range (15 -45 years)
3. Disease: hyperprolactinemia, congenital adrenal hyperplasia, thyroid disorders, Cushing's disease, hypertension, anemia and hemaglobinopathy to avoid any misinterpretation of HbA1c [22,23,24].
4. Using medication (e.g. insulin-sensitizing drugs, oral contraceptives, antiandrogens, corticosteroids and GnRH agonists and antagonists during the 90 days prior to enrollment were excluded.

All patients were informed about the study and consent was obtained.

The women with PCOS consulted our outpatient clinic for PCOS-related symptoms such as hirsutism, acne, obesity, infertility or menstrual abnormalities. PCOS was diagnosed using the Rotterdam criteria an established in 2003 [20,21], which requires two of the following: oligo- or anovulation, clinical and/or laboratory evidence of hyperandrogenism, and polycystic ovaries on ultrasound (≥ 12 follicles measuring 2-9 mm in diameter and/or an ovarian volume > 10 mL in at least one ovary). an investigator determined the presence or absence of hirsutism for every woman with a modification of the

Ferriman–Gallwey (mF–G score) method. Hirsutism was defined as an mF–G score > 7 [25]. Conventional assessment of participants included medical history, clinical evaluation, and fasting blood samples. Subjects were measure the weighed on an electronic scale. Body mass index (BMI)was calculated as the weight in kilograms divided by the square of the height in meters (kg/m2) of every one patient was calculated Normal weight was defined as BMI < 25 kg/m2 and overweight/obesity as BMI equal or >25 kg/m2, and waist circumference (WC) was measured by midway point between the lower rib and iliac crest while the patient was upright position, that was more than 80cm abnormal. An OGTT, venous blood specimen was collected from each female at early morning 8:00-10:00 am after an overnight fasting for 8-10 hours in the early follicular-stage of the spontaneous menstrual cycle (day 3–5) oron a random day of the cycle for amenorrhoeic women. Fasting plasma glucose (FPG) was measured to all women by Cobas 311 (Roche Diagnostics, Mannheim, Germany).A 75 g anhydrous glucose was dissolved in 250 mL water and was having by each female under supervision then plasma glucose was measured after 120 minutes. On the same day of measuring 2-h OGTT, HbA1c was measured to all subjects, by a procedure known as high performance liquid chromatography (HPLC). According to ADA criterion, glycemic state was defined as follows: Prediabetes: FPG=100-125 mg/dl. And/or 2-h OGTT=140-199 mg/dl and/or HbA1c=5.7-6.4%; T2DM: FPG ≥126 mg/dl and/or 2-h OGTT ≥200 mg/dl and/or HbA1c ≥6.5% (48 mmol/mol) [10].

Statistical Analysis

Statistical analyses were performed by using SPSS software version 25.0 (SPSS, Chicago). Continuous data were presented as mean and standard deviation, and analyzed with Student t-test. Categorical variables were expressed as number and percentage and analyzed with Chi-square test. Receiver operating characteristic curve (ROC) was used to evaluate predictive value of HbA1c in predicting glycemic disorder considering the 2-h OGTT as a gold standard. A p- value less than 0.05 were considered to indicate a statistically significant difference.

3. Results

3.1. Demographic Characteristics

Table 3-1. Demographic characteristics of the patients (n=40)

Variables	Value
Age, years Mean ± SD Range	30.95±9.63 16-44
Weight, kg Mean ± SD Range	97.25±11.85 55-105
Height, cm Mean ± SD Range	163.25±7.62 137-171
Body mass index, kg/m2 Mean ± SD Range	29.25±5.40 20.1-48.3
Waist circumference, cm Mean ± SD Range	86.88±11.09 70-120

SD: standard deviation

The present study included 40 diagnosed patients with PCOS. The mean age of the patients at presentation was 30.95±9.63years (range 16-44 years). The mean weight, height and BMI was 97.25±11.85 kg, 163.25±7.62 cm and 29.25±5.40 kg/m^2, respectively. Waist circumference ranged from 70-120 cm with a mean of 86.88±11.09 cm (Table 3-1).

3.2. Clinical Characteristics of the Patients

The mean systolic and diastolic blood pressure of the patients was 120.15±12.35 mmHg and 70.0±9.53 mmHg, respectively. On the other hand, the mean FPG, HbA1c 2-h OGTT was 95.43±12.29 mg/dl, 5.32±0.46% and 147.6±40.77 mg/dl, respectively (table 3-2).

Table 3-2. Clinical characteristics of the patients

Variables	Value
Systolic blood pressure, mmHg Mean ± SD Range	 120.15±12.35 95.0-150
Diastolic blood pressure, mmHg Mean ± SD Range	 70.0±9.53 60.0-90.0
FBS, mg/dl Mean ± SD Range	 95.43±12.29 66-112
HbA1c Mean ± SD Range	 5.32±0.46 4.6-6.6
OGTT0 Mean ± SD Range	 95.43±12.29 66-112
OGTT2 Mean ± SD Range	 147.6±40.77 110-275

SD: standard deviation, FPG: fasting plasma glucose, OGTT: oral glucose tolerance test.

3.3. Comparison between HbA1c, FPG and 2-h OGTT in Diagnosis of Glycemic Disorders

According to 2h-OGTT, 28 (70%) women had normal glucose level. Among those, only 24 were also normal according to HbA1c, and 16 normal according to FPG. Impaired glucose tolerance was reported in 9 patients (22.5%). Of those, 7 patients were considered normal according to both HbA1c and FPG. Finally, 3 women (7.5%) were considered to have diabetes according to 2h-OGTT, all of whom were detected as normal in both HbA1c and FPG. Despite this variation, statistically, there were no significant variation between the 2h-OGTT with each of HbA1 and FPG (Table 3-3).

Table 3-3. Comparison between HbA1c, FPG and 2-h OGTT in diagnosis of glycemic disorders

Variables	2h-OGTT			Total (n=40)	p-value
	Normal (n=28)	IGT (n=9)	DM (n=3)		
HbA1c Normal Prediabetes DM	24(85.71%) 2(7.14%) 2(7.14%)	7(77.78%) 2(22.22%) 0(0%)	3(100%) 0(0%) 0(0%)	34(85%) 4(10%) 2(5%)	0.580
FPG Normal IFG DM	16(57.14%) 12(42.86%) 0(0%)	7(77.78%) 2(22.22%) 0(0%)	3(100%) 0(0%) 0(0%)	26(57.5%) 17(42.5%) 0(0%)	0.068

3.4. Association of Demographic and Clinical Factors with Glycemic Disorder by 2h-OGTT in Women with PCOS

Due to the small number of diabetic patients and those with IGT, there were merged in one group to facilitate statistical analysis. Two factors were found to be significantly associated with glycemic disorder according to 2-h OGTT. The mean BMI of women with normal IGT/DM was 31.56±56 kg/m 2 which was higher than that of normal women (27.54±4.45 kg/m 2) with a significant difference. Likewise, the mean DBP in normal glycemic patients and those with IGT/DM was 76.29±9.8 mmHg and 83.33±4.43 mmHg, respectively with a significant difference (Table 3-4).

Table 3-4. Association of demographic and clinical factors with glycemic disorder by 2h-OGTT in women with PCOS

Variables	2h-OGTT		p-value
	Normal (n=28)	IGT/DM (n=12)	
Age, years Mean ± SD Range	29.82±9.71 16-44	33.58±9.27 19-43	0.263
Weight, kg Mean ± SD Range	76.58±12.12 65-93	80.39±11.76 55-105	0.153
Height, cm Mean ± SD Range	166.83±4.12 159-171	161.71±8.27 137-170	0.057
BMI, kg/m^2 Mean ± SD Range	27.54±4.45 23.3-33.60	31.56±56 20.9-48.0	0.039
WC, cm Mean ± SD Range	84.67±7.54 78-95	87.82±12.3 70-120	0.112
SBP, mmHg Mean ± SD Range	118.43±13.16 95-150	124.17±9.5 110-135	0.081
DBP, mmHg Mean ± SD Range	76.29±9.8 60-90	83.33±4.43 80-90	0.026

3.5. Association of Demographic and Clinical Factors with Glycemic Disorder by HbA1c in Women with PCOS

As in case of OGTT, diabetic and prediabetic patients were merged in one group. Diabetic and prediabetic patients had higher age and weight (35.33±9.56 years and 80.29±10.44 kg, respectively) than those with normal HbA1c level (30.18±9.57 years and 73.33±13.07 kg, respectively) with significant differences. Other factors were comparable between the two groups with no significant differences (table 3-5).

3.6. Association of Demographic and Clinical Factors with Glycemic Disorder by FPG in Women with PCOS

Weight and BMI were much higher in patients with IFG (83.09±11.77 kg and 31.6±5.67 kg/m 2) than those with normal FPG (75.06±10.1 kg and 27.0±3.76 kg/m 2) with significant differences. Other factors were comparable between the two groups with no significant differences (table 3-6).

Table 3-5. Association of demographic and clinical factors with glycemic disorder byHbA1c in women with PCOS

Variables	HbA1c		p-value
	Normal (n=34)	Prediabetic/DM (n=6)	
Age, years Mean ± SD Range	30.18±9.57 16-44	35.33±9.56 17-43	0.044
Weight, kg Mean ± SD Range	73.33±13.07 60-93	80.29±10.44 55-105	0.040
Height, cm Mean ± SD Range	164.94±4.3 157-171	157.67±14.18 137-167	0.069
BMI, kg/m^2 Mean ± SD Range	28.57±4.31 20.9-37	30.1±10.3 23.3-48.0	0.118
WC, cm Mean ± SD Range	85.68±8.65 70.-112	88.0±21.53 70-120	0.123
SBP, mmHg Mean ± SD Range	117.5±11.31 95-130	121.32±12.33 100-150	0.132
DBP, mmHg Mean ± SD Range	76.47±9.33 60-90	80.0±10.95 70-90	0.079

SD: standard deviation, IGT: impaired glucose tolerance, DM: diabetes mellitus, BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table 3-6. Association of demographic and clinical factors with glycemic disorder by FPG in women with PCOS

Variables	FPG		p-value
	Normal (n=23)	IFG (n=17)	
Age, years Mean ± SD Range	30.29±10.05 16-44	31.29±9.87 19-43	0.716
Weight, kg Mean ± SD Range	75.06±10.1 60-90	83.09±11.77 55-105	0.015
Height, cm Mean ± SD Range	163.94±5.41 151-171	161.72±10.05 137-169	0.238
BMI, kg/m^2 Mean ± SD Range	27.0±3.76 23.3-34.7	31.6±5.67 20.9-48.0	0.019
WC, cm Mean ± SD Range	84.71±11.0 70.-112	88.48±11.09 70-120	0.293
SBP, mmHg Mean ± SD Range	118.9±13.7 95-135	121.1±11.48 100-150	0.583
DBP, mmHg Mean ± SD Range	76.49±9.81 60-90	79.39±9.52 70-90	0.318

SD: standard deviation, IGT: impaired glucose tolerance, DM: diabetes mellitus, BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure.

Predictive Value of HbA1c in Predicting IGT

Receiver operating characteristic (ROC) was used to evaluate the predictive value of HbA1c in predicating IGT. The area under the curve (AUC) was 0.763, 95% CI= 0.618-0.909, p=0.005. The test's sensitivity and specificity were 77% and 65%, respectively, at a cut-off value of HbA1c= 5.22% (Figure 3-1).

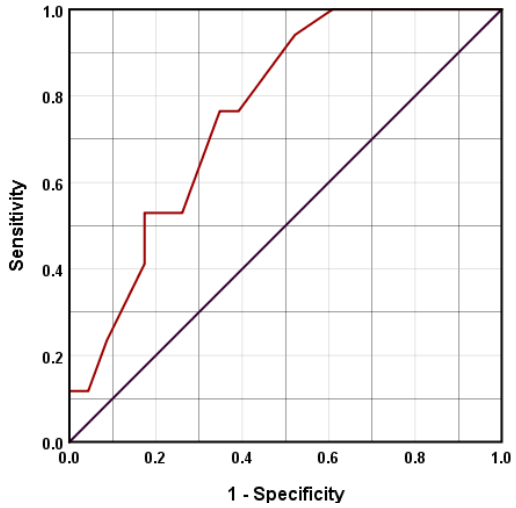

Figure 3-1. Receiver operating characteristic curve of HbA1c in the context of predicting IGT in women with PCOS

4. Discussion

Screening for a disease is fundamental when that situation constitutes an important health problem, a reasonable treatment for patients with known disease exists, and facilities for diagnosis and treatment are obtainable. Furthermore, there should be a recognizable latent or early symptomatic stage. There should be an effectual screening test or examination and that test should be acceptable to the patients [35]. However, whether glycemic status must be evaluated in every female suffering from PCOS or in specific subgroups, as well as which is the preferable method for this evaluation, are to date unanswered questions. With consideration to which patients should be evaluated, there are at present time two points of view. One, supported by the Endocrine Society, the Androgen Excess and Polycystic Ovary Syndrome Society, as well as by the guidelines on PCOS diagnosis and management developed in Australia, suggests universal screening in all female with PCOS [11,36,37]. on the other side, a number of experts recommend screening in female with at least one or more risk factors, such as age >40 years, family history of either T2DM or gestational diabetes mellitus, and/or obesity [12,29,38]. The majority of the studies advocacy of these recommendations did not assess in detail the effect of age, obesity, on the development of glycemic disorder and, thus, seem to increase debate over this matter.There is controversy among experts as to whether fasting plasma glucose (FPG), OGTT, or glycated hemoglobin (HbA1c) is the best laboratory method to evaluate glycemic state in a women with PCOS, despite solid data strongly pointing to OGTT as being the most precise [17,39]. Female identified with PCOS are vulnerable to have diabetes (about 10 times higher than the general population) [40]. In our study, FPG misinterpreted the diagnosis of glycemic disorder in more than half of female with IFG, and no patients with T2DM considered them as normal. Weight and BMI were much higher in patients with IFG (83.09±11.77 kg and 31.6±5.67 kg/m 2) than those with normal FPG, with significant differences. These findings suggest that FPG could be an insufficient tool to screen for glycemic disorder in female with PCOS which was also reported by an Austrian cohort study of 671 young and non-obese women with PCOS [42]. One possible explanation for the failure of FPG as a screening method might be the fact that insulin secretory defects have been suggested to be present in PCOS (Holte et al., 2014) and this considered that the OGTT test was the best screening method for impaired glucose and diabetes in female with PCOS.In our study, the ability of HbA1c to distinguish female with PCOS who have normal metabolism from those with abnormal glucose metabolism was investigated. When using cut-off value of HbA1c= 5.22% with the highest specificity and sensitivity, substantially lower than the 5.7% used by the ADA, about less than two third of the women were below the cutoff value even though they were having an abnormal glucose state by the 2-h OGTT. Diabetic and prediabetic patients had higher age and weight than those with normal HbA1c with significant differences. The test's sensitivity and specificity were 77% and 65%, respectively. Therefore, normal HbA1c value should be undergo a formal OGTT may identify dysglycemia in PCOS.in our present study revealed, dysglycemia was consistent with that showed in Europe (Ehrmann DA BR et al 2019), also seen in American female with PCOS. These patients can be significantly benefited from early lifestyle modulation and/or medical therapy. According to Androgen Excess Society (AES) guidelines, 2-h OGTT is recommended as a gold standard test to detect dysglycemia in female diagnosed with PCOS especially those with one or more risk factors for diabetes mellitus [11]. Overweight or obese was dominant in female diagnosed with PCOS in our study; more than half of enrolled patients were either overweight or obese.Which was the same to that disclosed in a cohort study from Alabama [41] .this patients were markedly having mean 2-h OGTT and more dysglycemia determined by 2-h OGTT than normal BMI patient with PCOS. Amazing, prediabetes was diagnosed in lean body with PCOS patients by 2-h OGTT. This finding in our study against the ADA and AES recommendations to evaluate for T2DM in asymptomatic women with PCOS if their BMI equal or more than 25kg/m2 [11]. many female with IGT/DM ,false negative (23%) and false positive (35%) be mistaken as normal or abnormal blood glucose by HbA1c.rendering HbA1c as insufficient screening test for glycemic disorder in female with PCOS, particularly during the detection of prediabetes and type2 DM, this result correspond with study Celik C et al (2013) on a Turkish female with PCOS ,that HbA1c show some potential weakness. But HbA1c as yet has advantage over 2-h OGTT in evaluating chronic glycemic control over 2-3months earlier, less day to day variability and convenient to patient [19]. also by the way in our study, we find FPG misdiagnosed of women with hyperglycemia or IGT or even normal glucose in comparison with OGTT. therefor suggest that FPG consider weak test for evaluation of glycemic disorder in patients with PCOS, this also agreement with Lerchbaum E et al 617(2013) in Austrian female with PCOS [42] and Altemimi MT, et al. (2021) in southern Iraq [44]. Thus, the OGTT consider being most reliable test to diagnosed dysglycemia among patients diagnosed with PCOS. But limitation of our study is sample size.

5. Conclusion

T2DM and prediabetes common in PCOS patient, and when compared with the 2-hr OGTT, HbA1c is insufficient screening tool for prediabetes and T2DM screening in female with PCOS, our study suggest that OGTT should be performed in all patient with PCOS

Recommendation

Larger lady size sample.

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