ABSTRACT: The perimenarcheal onset of polycystic ovarian syndrome (PCOS) has long been recognized, through both its pathophysiology and the frequent onset of hirsutism and menstrual irregularities in this age group. However, there is often a delay in diagnosing PCOS in adolescence because menstrual irregularity is frequently thought to be normal in the first 2 or 3 years after menarche, particularly if clinical signs of hyperandrogenism such as hirsutism or acne are mild or absent. This was a Cross-sectional Study consisting of 102 adolescent girls, aged 12 to 19 years, selected from patients attending the Gynecology Outpatient Clinic. In present study, level of insulin resistance along with markers of PCOS was assessed and compared in adolescent women with (cases) or without (controls) menstrual irregularities, a hall mark of PCOS. 18(35.29%) cases versus 01(1.96%) controls the Rotterdam criteria of PCOS. Clinical manifestations of hyper androgenism were not yet present in all cases who met Rotterdam criteria of PCOS, suggesting menstrual irregularity, especially oligomenorrhea (83.3% of all PCOS (cases), as the most precocious marker of PCOS. CONCLUSIONS: Adolescents with persistent menstrual irregularities 2 years after menarche frequently have PCOS and elevated HOMA-IR values than controls, indicating a high probability of developing insulin resistance and metabolic syndrome later in life.

KEYWORDS: polycystic ovary, hirsutism, menstrual irregularities, oligomenorrhea.

INTRODUCTION: Polycystic ovarian syndrome is one of the most common gynecologic and endocrine problems encountered by gynecologists. Adolescent with this condition consult because of menstrual disorders and physical manifestations of excessive androgen production. They are also at high risk for insulin resistance and impaired glucose tolerance. It is important to identify adolescent at risk early because diabetes mellitus is asymptomatic at early stage of insulin resistance and impaired glucose Tolerance. Life style modification can avert development of metabolic syndrome and diabetes mellitus in the adolescents.1,2,3

The menstruations that follow menarche, especially during the first 2 years, are usually an ovulatory, irregular, and occasionally abundant, a condition attributed to the immaturity of the hypothalamus-pituitary-ovary axis in adolescents.4,5,6

After this period, the hypothalamus-pituitary-ovary axis usually acquires normal functioning. The persistence of an ovulatory cycles for more than 24 months after menarche, especially if associated with other characteristics of hormonal disorders, may suggest ovulatory dysfunction of pathologic origin.7,8

The central pathogenesis of this type of anovulation seems to be insulin resistance,3,9,10,11 Which is related to a post-insulin receptor defect that course with compensatory hyper insulinemina,11,12,13 in addition to other factors such as reduction of hepatic insulin clearance and increased pancreatic sensitivity leading to an abnormal biological response, with increased circulating insulin concent-
ration. This hyper insulinemia also seems to be responsible for the development of hyper androgenism that induces anovulation.6,14,15,16

The National Cholesterol Education Program Adult Treatment Panel (ATPIII)17 guidelines define Metabolic Syndrome as having three or more of the following abnormalities: waist circumference in females≥88cm, fasting serum glucose≥110mg/dL, fasting serum triglycerides≥150mg/dL, serum HDL cholesterol<50mg/dL, and systolic and diastolic blood pressure≥130 and 85mm/Hg, respectively. There is consistent evidence that the metabolic syndrome is associated with a high risk for developing type2 diabetes mellitus and cardiovascular disease, as well as with cardiovascular mortality.2,18,19,20 Several studies have demonstrated a high prevalence of the metabolic syndrome in women with PCOS, ranging from 33% to47%.19,21,22,23,24

Recent evaluation of the economic burden of PCOS has concluded that screening, diagnosis, and intervention prevented or ameliorated the serious complication of diabetes mellitus and is therefore justifiable.24,25 Studies conducted in Indian population, for prevalence of insulin resistance and metabolic syndrome in adolescents with PCOS, are few and has met with varying results.26 And hence further study is warranted.

The objective of present study is to evaluate the presence of insulin resistance in adolescents with menstrual disorders at least 2 years after menarche and to determine the presence of PCOS and metabolic syndrome among them.

AIMS AND OBJECTIVES:
1. To evaluate the presence of insulin resistance in adolescents with menstrual disorders persisting 2 years after menarche.
2. To determine presence of polycystic ovarian syndrome in adolescent with menstrual disorder.
3. To determine presence of metabolic syndrome in these adolescents.

MATERIALS AND METHODS:

Study Design: This was a Cross-sectional Study consisting of 102 adolescent girls, aged 12 to 19 years, selected from patients attending the Gynecology Outpatient Clinic. Informed consent was taken.

The patients were divided into two groups, group I (study group) had 51 adolescents presenting with menstrual irregularities such as oligomenorrhea, amenorrhea, or polymenorrhea, and Group II included 51 adolescents with normal menstrual cycles.

Inclusion Criteria (G I):
- Adolescents aged 12 to 19 years at least 2 years post–menarche.
- Menstrual disorders such as amenorrhea, oligomenorrhea (≤6 menses per year), polymenorrhea.
- Patient giving informed consent.

Exclusion Criteria (G I):
- Age>19 years.
- Taking medications [OCP > 3 years, steroids etc] that may interfere with hypothalamus-pituitary-ovary axis.
- Any medical or surgical disease.
Methodology: Detailed history of the patients was taken and thorough clinical examination was done. Height, weight, waist–hip, measurements, Body mass index and presence of Hirsutism and other signs of Hyperandrogenism were noted. Clinical hyperandrogenism was considered to be present when the Ferriman–Gallway index\textsuperscript{27} was ≥8 and when hair loss, acne, or oily skin was present.

On day 3 of menstrual cycle all adolescents underwent following set of biochemical analysis required in our study plan: Serum levels of testosterone, 17-hydroxyprogesterone, and DHEA-S (to establish laboratory hyper androgenism, characterized by testosterone levels ≥120ng/dl, 17-hydroxyprogesterone ≥140ng/dl and DHEA-S (dehydroepiandrostenedionesulfate) levels ≥ 250µg/dl).

Blood glucose and insulin levels obtained after an 8-to12-hour fast was used to diagnose disorders of glucose metabolism. An oral glucose tolerance test with 75g dextrose was applied and glycemia and insulin were determined at 0, and 2 hours. HOMA-IR method was used to evaluate insulin resistance, using the following formula: fasting serum insulin (µU/ml) × fasting plasma glucose (mmol/l)/22.5. An ideal normal-weight individual aged <35 years has a HOMA-IR of 1.0mol×µU/L2 and HOMA value > 3.90mol×µU/L² was considered as insulin resistance\textsuperscript{28}. Fasting lipid profile (TG, HDL-C) was done to determine derangements of lipid metabolism.

Hb, TC, DC, ESR, URINE (Routine and Microscopy), Prolactin (PRL), TSH, T3, T4, FSH, LH, determinations were performed to exclude other causes of anovulation. Luteinizing hormone, follicle stimulating hormone (FSH), PRL, TSH, testosterone, DHEA-S, 17 hydroxyprogesterone (17-OHP), insulin were determined by ELISA and plasma glucose was determined by the exokinase method.

All adolescents underwent transabdominal pelvic ultrasound in follicular phase (day 3 to 5) for the evaluation of them or phological aspect of the ovaries. Adolescents were considered to have PCOS when two of the following three criteria were met-(1) chronic anovulation characterized by persistent menstrual irregularity for over six months; (2) clinical and/or laboratory hyperandrogenism; and (3) ultrasonographic appearance of the polycystic ovary\textsuperscript{29,30}

Metabolic syndrome was considered to be present when adolescents had three or more of the following abnormalities: waist circumference > 88cm, fasting serum glucose ≥110mg/dL, fasting serum triglycerides ≥150mg/dl, serum HDL cholesterol < 50mg/dL, and systolic and diastolic blood pressure ≥ 130 and 85 mm/Hg, respectively\textsuperscript{17}.

The same parameters were determined in group II (control group) and prevalence of PCOS, obesity; insulin resistance and metabolic syndrome were compared between two groups.

Statistical Analysis: This was across-section at study and results were analyzed by applying following statistical methods;

1. Data was analysed by applying student test/Mann-Whitney test wherever applicable.
2. P value <0.05 was considered as significant.

OBSERVATIONS:

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-15</td>
<td>2(3.9%)</td>
<td>7(13.7%)</td>
<td>0.28</td>
</tr>
<tr>
<td>16-19</td>
<td>49(96.07%)</td>
<td>44(88.27%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>51(100%)</td>
<td>51(100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: AGE DISTRIBUTION
Mean age of cases were 17.84 years and that of controls were 17.24 years. P value determined by t-test was not significant.

<table>
<thead>
<tr>
<th>PATTERN OF MENSTRUAL IRREGULARITIES</th>
<th>NO. OF CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligomenorrhoea</td>
<td>40(78.43%)</td>
</tr>
<tr>
<td>Hypomenorrhoea</td>
<td>6(11.76%)</td>
</tr>
<tr>
<td>Amenorrhoea</td>
<td>1(1.96%)</td>
</tr>
<tr>
<td>Polymenorrhoe</td>
<td>4(7.84%)</td>
</tr>
<tr>
<td>Total</td>
<td>51(100%)</td>
</tr>
</tbody>
</table>

Table 2: PATTERN OF MENSTRUAL IRREGULARITIES

Most of cases had Oligomenorrhoea as presentation of menstrual irregularities.

<table>
<thead>
<tr>
<th>BMI</th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;18-&lt;25</td>
<td>21(41.17%)</td>
<td>47(92.15%)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥25</td>
<td>29(56.86%)</td>
<td>4(7.84%)</td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>1(1.96%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>51(100%)</td>
<td>51(100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: BODY MASS INDEX (KG/M²)

30(58.82%) cases versus 4(7.84%) controls had BMI≥25. Mean value of BMI in cases was 25.29 and that of controls were 22.18. p value obtained with t-test was 0.001, which was highly significant.
Mean value of waist in cases was 82.43 cms and that of controls were 72.62. p value obtained with t-test was 0.001, which was highly significant. ≥88Cm waist size was taken as criteria for central obesity in NCEP-ATPIII criteria.

Mean value of BP (systolic) in cases was 120.98mmHg and that of controls were 113.80mmHg. p value obtained with t-test was 0.001, which was highly significant.

Mean value of BP (diastolic) in cases was 79.03mmHg and that of controls were 71.27mmHg. p value obtained with t-test was 0.001, which was highly significant.

Altogether there were 19(37.25%) cases who were hypertensive; with 7(13.72%) cases having both systolic and diastolic, 4(7.84%) only systolic and 8(15.68%) having only diastolic hypertension.

14(27.45%) of cases versus none among controls had hirsutism, Acne was present in 9(17.64%) cases and 4(7.84%) controls, whereas 3(5.88%) cases versus none of controls had temporal pattern of hair fall.

P value obtained by Mann-Whitney test was 0.001, which was highly significant.

7(13.72%) of cases versus 3(5.88%) of controls had elevated testosterone levels. P value obtained by Mann-Whitney test was 0.001, which was highly significant.
Table 9: SERUM DHEAS

<table>
<thead>
<tr>
<th>Serum DHEAS</th>
<th>Cases</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥250µg/dl</td>
<td>4(7.84%)</td>
<td>1(1.96%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

4(7.84%) among cases versus 1(1.96%) among controls had elevated DHEAS values. P value obtained by Mann-Whitney test was 0.001, which was highly significant.

None of the cases or controls had increased Prolactin, TSH, or progesterone levels.

Table 10: HOMA –IR

<table>
<thead>
<tr>
<th>HOMA-IR</th>
<th>Cases</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>22(43.13%)</td>
<td>47(92.15%)</td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>25(49.01%)</td>
<td>4(7.84%)</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt;3.9</td>
<td>4(7.84%)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

HOMA-IR was elevated in 29(56.82%) cases and 4(7.84%) controls. Insulin resistance was present in 4(7.84%) cases. 4(7.84%) cases had insulin resistance (HOMA-IR>3.9), 25(49.01%) cases had elevated HOMA-IR values (HOMA-IR>1), whereas only 4(7.84%) controls had elevated HOMA-IR value. P value calculated by Mann-Whitney test was 0.001, which was highly significant.

Table 11: METABOLIC SYNDROME

<table>
<thead>
<tr>
<th>Metabolic syndrome</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11(21.56%)</td>
<td>03(5.88%)</td>
</tr>
</tbody>
</table>

Of the 11(21.56%) cases with metabolic syndrome, 9 had elevated HOMA-IR value, whereas of 3(5.88%) controls who had metabolic syndrome, 1 had elevated HOMA-IR value.

Table 12: PCOS (ROTTERDAM CRITERIA)

<table>
<thead>
<tr>
<th>PCOS</th>
<th>cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18(35.29%)</td>
<td>01(1.96%)</td>
</tr>
</tbody>
</table>

18(35.29%) cases versus 01(1.96%) controls met the Rotterdam criteria of PCOS. Of the 18 cases with PCOS, 14 had elevated HOMA-IR (10 with HOMA-IR>1; 4 with insulin resistance i.e. HOMA-IR>3.9). In 1 control with PCOS, HOMA-IR was >1 but <3.9.

Table 13: PATTERN OF MENSTRUAL IRREGULARITIES IN CASES WITH PCOS

<table>
<thead>
<tr>
<th>PATTERN OF MENSTRUAL IRREGULARITIES</th>
<th>No. of Cases with PCOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligomenorrhoea</td>
<td>15</td>
</tr>
<tr>
<td>Hypomenorrhoea</td>
<td>2</td>
</tr>
<tr>
<td>Amenorrhoe</td>
<td>0</td>
</tr>
<tr>
<td>Polymenorrhoe</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
</tr>
</tbody>
</table>


So oligomenorrhea, hypo menorrhea and polymenorrhea are the alarming pattern of menstrual irregularities for early detection of polycystic ovarian syndrome in adolescent age group.

<table>
<thead>
<tr>
<th>PATTERN OF MENSTRUAL IRREGULARITIES</th>
<th>No. of Cases with HOMA-IR&gt;1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligomenorrhoea</td>
<td>20</td>
</tr>
<tr>
<td>Hypomenorrhoea</td>
<td>5</td>
</tr>
<tr>
<td>Amenorrhea</td>
<td>0</td>
</tr>
<tr>
<td>Polymenorrhoea</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
</tr>
</tbody>
</table>

Table 14: PATTERN OF MENSTRUAL IRREGULARITIES IN CASES WITH HOMA-IR

Out of 29 patient with HOMA-IR value>1, 20 cases where presented with oligomenorrhic type of menstrual irregularities and 5 with hypomenorrhic pattern followed by 4 with polymenorrhic pattern of menstrual irregularities.

**DISCUSSION:** There are major difficulties in the early diagnosis of PCOS during adolescence because of the clinical characteristics and endocrine changes that occur during the first years after menarche, which lead to a confusion of the clinical signs and symptoms of PCOS with the physiologic alterations during pubertal period.\(^{31}\)

In a recent study by Van Hoof fetal,\(^ {5}\) the presence of oligomenorrhoea at 15 years of age was found to be a better predictor of menstrual irregularity at 18 years, more than elevated levels of testosterone, androstenedione, LH, clinical manifestations of hyperandrogenism or an ultrasound image compatible with micro polycystic ovaries, regardless of patient BMI. According to Ehrmannetal.\(^ {21}\) menstrual irregularity may be considered to be physiological during the first years after menarche only if there are no associated signs of hyperandrogenism.

In the present study, mean age of cases were 17.84 years and that of controls were 17.24 years (p value<0.28). Oligomenorrhoea (78.43%) was the most common pattern of menstrual irregularity,
followed by hypomenorrhoea (11.76%). Acne and temporal pattern of hair fall was present in 9(17.64%) and 3(5.88%) respectively. 14(27.45%) of adolescents with menstrual irregularity presented with hirsutism (Ferriman-Gallway score more than 8) suggesting a diagnosis of PCOS, while none of the adolescents with regular menstrual cycles presented with hirsutism (p value<0.05).

On the other hand, Avvadetal.7 stated that the presence of hirsutism can be simply the expression of increased skin sensitivity to normal levels of circulating androgens and does not necessarily indicate an abnormal ovulatory mechanism in these patients.

The literature shows that the levels of free testosterone, LH, and the LH/FSH ratio in adolescents with menstrual irregularity with no clinical signs of hyperandrogenism are similar to those of patients with PCOS and higher than those of adolescents with regular menstrual cycles,3,7,15,31 suggesting a greater risk to develop the syndrome during adult age.

In our study adolescents with menstrual irregularity had significantly higher LH levels (p value<0.5), but not FSH levels (p value=0.04); however, only 13 of 18 adolescents who met the Rotterdam criteria of PCOS had raised LH levels. The mean testosterone and DHEA-S levels in adolescents with menstrual irregularity were significantly higher than the control group, although only seven adolescents with menstrual irregularities and 3 normal adolescents presented with a serum testosterone level higher than normal limits. Perhaps because the evaluation was precocious, clinical manifestations of hyperandrogenism were not yet present in all cases who met Rotterdam criteria of PCOS. These findings agree with other authors who point to menstrual irregularity as the most precocious marker of PCOS.3,5,6,8

A current speculation is that PCOS may be hereditary, with genetic predisposition and clinical expression related to environmental factors. Battagliaetal16 reported an increased incidence of polycystic ovaries detected by ultrasound in daughters of patients with PCOS, even of pre pubertal age, with no increase in circulating androgens or LH, but with increased ovarian and uterine volume and a higher incidence of early pubarche in initial stages. In our study 3 adolescent with menstrual irregularity had family history suggesting of PCOS.

A relation between PCOS and increased serum insulin levels have been described by many investigators (Dunaifetal.32Holteetal.33). It seems that insulin –like growth factor (IGF-like) has the capacity of stimulating the follicular theca to produce ovarian androgens. The molecular structure similarity between insulin and IGF-like permits a cross match in the theca’s receptors, so high serum levels of insulin, which happen in cases of peripheral insulin resistance (receptor defect), leads to increased secretion of androgens by the ovary, more than its capacity to convert androgen in estrogen.

During puberty, insulin sensitivity is usually decreased, causing increased secretion of this hormone30. Some studies24,34,35,36 have assessed insulin resistance in adolescents with PCOS. The method considered to be the gold standard for the diagnosis of insulin resistance is the hyperinsulinemic euglycemic clamp,37 a venous glucose tolerance test frequently used in experimental and scientific investigations but still very difficult to perform in clinical practice. In the present study we calculated the insulin resistance with HOMA-IR method, which showed more elevated values in patients with menstrual irregularities than in controls (P value<0.01), probably demonstrating the lower insulin sensitivity of these patients.

We also calculated the fasting and postprandial glucose levels. None of the adolescents, either cases or controls, had abnormal values, probably because the elevation in the glucose levels begins
only when even with high serum insulin levels the receptor resistance is such that the glucose levels cannot be controlled; in this stage the patient may present glucose in tolerance, a step that leads to type II diabetes.

According to Reisetal and Moralesetal, the effects of insulin resistance are potentiated in obese patients with PCOS. In the present study, HOMA-IR was elevated in 29(57%) cases and 4(8%) controls (p value<0.01). Insulin resistance was present in 4(8%) cases. 4 cases had insulin resistance and remaining 25 cases had elevated HOMA-IR values, whereas only 4 controls had elevated HOMA-IR value. Of 29 cases, who had elevated HOMA-IR value, 20(69%) had BMI≥25kg/M2 None of the cases or controls had abnormal fasting or postprandial glucose levels.

In our study, of the 11 cases who met the criteria of metabolic syndrome laid by NCEP-ATPIII-9(82%) had elevated HOMA-IR value, whereas of 3 controls who had metabolic syndrome only1 (33%) had elevated HOMA-IR value. Our finding further strengthens the association of prevalence of PCOS with insulin resistance and metabolic syndrome.

Dietary orientation, physical exercise, and awareness of the importance of treatment have been given priority in the treatment of this endocrine disorder, leading to a reduction of as much as 58% in the incidence of diabetes in this population.19,23,24

In our study there was a significant association between pattern of menstrual irregularity, elevated HOMA-IR value, and PCOS, with Oligomenorrhea and hypomenorrhea being more frequent association. 15 out of 18 cases with PCOS and 20 out of 29 cases with elevated HOMA-IR presented with oligomenorrhea, the most common pattern of menstrual irregularity in this series.

Based on these findings, we concluded that adolescents with persistent menstrual irregularities, especially oligomenorrhea and hypomenorrhea, 2 years after menarche more frequently have the diagnosis of PCOS and also present with more elevated HOMA-IR values and metabolic syndrome than controls, indicating a great probability of the presence of insulin resistance and providing a unique opportunity to detect patients early in their lives and offer measures that would prevent development of metabolic complications and reproductive abnormalities.

Since our study was conducted on small group of patients, who sought treatment mainly for abnormal menstrual patterns, hence, specific controlled studies on the population of patients with PCOS are necessary in order to improve the diagnosis, treatment and measures of preventing metabolic repercussions of this endocrinopathy.

**SUMMARY AND CONCLUSION:** Polycystic ovarian syndrome (PCOS) is a complex endocrinopathy with wide ranging variation and clinical manifestation. In present study, level of insulin resistance along with markers of PCOS was assessed and compared in adolescent women with (cases) or without (controls) menstrual irregularities, a hallmark of PCOS. 18 (35.29%) cases versus 01(1.96%) controls met the Rotterdam criteria of PCOS.HOMA-IR was elevated in 29(57%) cases against 4(8%) controls and Insulin resistance (HOMA-IR>3.9) was present in 4(8%) cases. Of 29 cases, who had elevated HOMA-IR value, 20(69%) had BMI≥25kg/M2 Of the 11 cases who met the criteria of metabolic syndrome, 9 had elevated HOMA-IR value; whereas, of 3 controls who had metabolic syndrome only 1 had elevated HOMA-IR value. Oligomenorrhea was the most common menstrual irregularity among adolescents with elevated HOMA-IR, BMI≥25kg/M2 or metabolic syndrome.

Based on these findings, we conclude that adolescents with persistent menstrual irregularities 2 years after menarche frequently have PCOS and elevated HOMA-IR values than controls, indicating a high probability of developing insulin resistance and metabolic syndrome later in life.
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FINANCIAL OR OTHER COMPETING INTERESTS: None

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Date of Submission: 24/12/2014.
Date of Peer Review: 25/12/2014.
Date of Acceptance: 29/12/2014.
Date of Publishing: 17/04/2015.