

## **Insulin sensitizing agent improves clinical pregnancy rate and insulin resistant parameters in polycystic ovarian syndrome patients with acanthosis nigricans: a randomized controlled study**

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**Keywords:** Acanthosis nigricans, clomiphene citrate, metformin, polycystic ovary syndrome, insulin resistance, pregnancy

### **Abstract**

*Objective:* To investigate the effect of adding metformin to clomiphene citrate (CC) in polycystic ovarian syndrome (PCOS) patients with acanthosis nigricans (AN) who were previously not responding to CC.

*Material and Methods:* A double blinded randomized controlled trial (NCT02562664) included 66 PCOS women with acanthosis nigricans who were CC resistant (at least 3 months). Day 3 follicle stimulating hormone (FSH) level, fasting insulin, fasting glucose and homeostatic model assessment were used to quantify insulin resistance. Participants were randomly assigned to either group I (CC with placebo tablets) or group II (CC with metformin) for three cycles. Insulin resistance parameters as well as clinical pregnancy rate had been evaluated in both groups. The statistical analysis was done using Chi-square and Fischer exact tests.

*Results:* The demographic data was comparable in both groups, however; there was higher cumulative pregnancy rate after three cycles of stimulation in group II (18/33) (54.5%) in comparison with group I (7/33) (21.1%) ( $P=0.03$ ). There was a significant improvement in the insulin resistance parameters after three months of combining clomiphene citrate with metformin as compared with CC alone.

*Conclusion:* Adding metformin to CC in clomiphene citrate resistant PCOS patients who have acanthosis nigricans improves the pregnancy rate and insulin resistant parameters.

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## **Introduction**

Polycystic ovary syndrome (PCOS) is the most common female endocrine disorder, with a prevalence ranging between 6% to 10% based on the National Institutes of Health (NIH) criteria. When the broader Rotterdam criteria are applied; the prevalence reaches as high as 15%.<sup>1</sup> The typical presentation of PCOS includes ovarian dysfunction (anovulation), hyperandrogenism (either clinical or biochemical), and the ultrasonographic picture of polycystic ovaries.<sup>2</sup> The etiology of the syndrome is still unclear and the variability in clinical presentation continues to make the clinical and research implications challenging.<sup>3</sup>

Insulin resistance is a common finding in obese women with PCOS.<sup>4</sup> The cellular and molecular mechanisms of insulin resistance in PCOS differ from other common insulin-resistant states such as obesity and type 2 diabetes mellitus (DM).<sup>5</sup> PCOS and obesity both have a negative effect on insulin action.<sup>6</sup> Pancreatic b-cell dysfunction is also present in PCOS but may be more related to type 2 DM risk factors such as women having first-degree relative with the DM.<sup>7</sup>

Acanthosis nigricans (AN) is a skin disease characterized by velvety, papillomatous, brownish-black and hyperkeratotic plaques. Typically it presents at intertriginous surfaces and the neck. AN is usually associated with obesity, insulin resistance and PCOS.<sup>8</sup> Hyperkeratosis is increased in melanin pigmentation resulting in the dark color of AN.<sup>9</sup> There is a subtle infiltrate composed of lymphocytes, plasma cells, and neutrophils as well as horn

pseudocyst formation. Colloidal iron tissue staining often exhibits infiltration of the papillary dermis with glycosaminoglycans such as hyaluronic acid, especially in patients with PCOS.<sup>10</sup>

Elevated insulin concentrations result in direct and indirect activation of insulin-like growth factor 1 (IGF-1) receptors on keratinocytes and fibroblasts, leading to its proliferation. Other mediator's receptors may also contribute, including Epidermal Growth Factor Receptor (EGFR) and Fibroblast Growth Factor Receptor (FGFR).<sup>11</sup> Hyperinsulinemia may also facilitate the development of AN indirectly by increasing the levels of free IGF-1 in the circulation.<sup>12</sup> Insulin-like growth factor 1 binding protein and insulin-like growth factor 2 binding protein are decreased in obese women with hyperinsulinemia leading to an increase in plasma concentrations of free IGF-1.<sup>13</sup>

Metformin has a great effect on PCOS patients. This may be due to a reduction of pituitary secretion of LH, a reduction of ovarian secretion of androgens, a reduction of adrenal secretion and finally an increased level of sex hormone binding globulin.<sup>14</sup> Obesity is usually associated with hyperinsulinemia which is responsible for the low responsiveness of PCOS patients to clomiphene citrate.<sup>15</sup> A significant improvement in ovulation and pregnancy rates was reported when adding metformin to clomiphene citrate in clomiphene-resistant PCOS patients.<sup>16</sup>

The current study reviews the effect of adding metformin to clomiphene citrate in PCOS patients with acanthosis nigricans (AN) who were previously did

not respond to CC alone. We hypothesized that adding metformin to this special group of women, AN patients with insulin resistance, may be beneficial for them including an improvement in pregnancy rate and hyperinsulinemia parameters.

## **Materials and Methods**

The current study is a clinically registered double blinded, parallel, RCT (NCT02562664) compassing the effect of adding metformin to CC in PCOS patients who had acanthosis nigricans. The ethical review board of the Faculty of Medicine of the Assiut University approved the study. The participants were recruited from the Outpatient Infertility Clinic of the Woman's Health Hospital. It was carried out in the period between the first of August 2013 and the first of November 2014. This trial was designed and reported according to the revised recommendation of ClinicalTrials.gov for improving the quality of reporting RCTs.

### Eligible participants

All participants who presented to the above clinic with acanthosis nigricans associated PCOS and previously not responding to CC were recruited in the study. The patient was considered eligible if she was under age 40 year, fulfilled at least 2 out of the three criteria of Rotterdam consensus 2003 and had AN. All participants had unsatisfactory ovulation after at least 3 months of CC induction. We excluded women with any contraindications to metformin such as liver disease, heart or respiratory failure, alcohol abuse, and kidney disease. All couples signed an informed consent to participate in the study.

### Randomization

Randomization was done using a computer-generated random table. The participants who consented were randomly assigned to receive either CC with placebo or CC with metformin. Allocation concealment was done using serially numbered closed opaque envelopes. Each envelope was labeled with a serial number and had a card showing the intervention type inside. Allocation was never changed after opening the envelopes (study flow chart, Fig. 1).

### Intervention

All study participants had proper clinical evaluation to ensure the diagnosis of AN (by assistant professor of dermatology D.A.A). Additionally, day 3 follicle stimulating hormone (FSH) level, fasting insulin, fasting glucose and homeostatic model assessment, to quantify insulin resistance (HOMA -IR), were done. Eligible participants were allocated to one of two groups. Group I received 100 mg clomiphene citrate (Clomid, global Napi , Egypt) from day 3 to day 7 of the cycle with placebo tablets which were taken twice daily continuously for three cycles. Group II received the above CC dose plus metformin (Cidophage , Amon , Egypt) 500 mg twice daily continuously for three cycles. Insulin resistance parameters as well as clinical pregnancy rate had been evaluated in both groups. The participants were requested to come to a monthly follow-up in our clinic for 3 months. Each participant had a special follow-up card that included the study serial number, the study group and the required follow-up schedule.

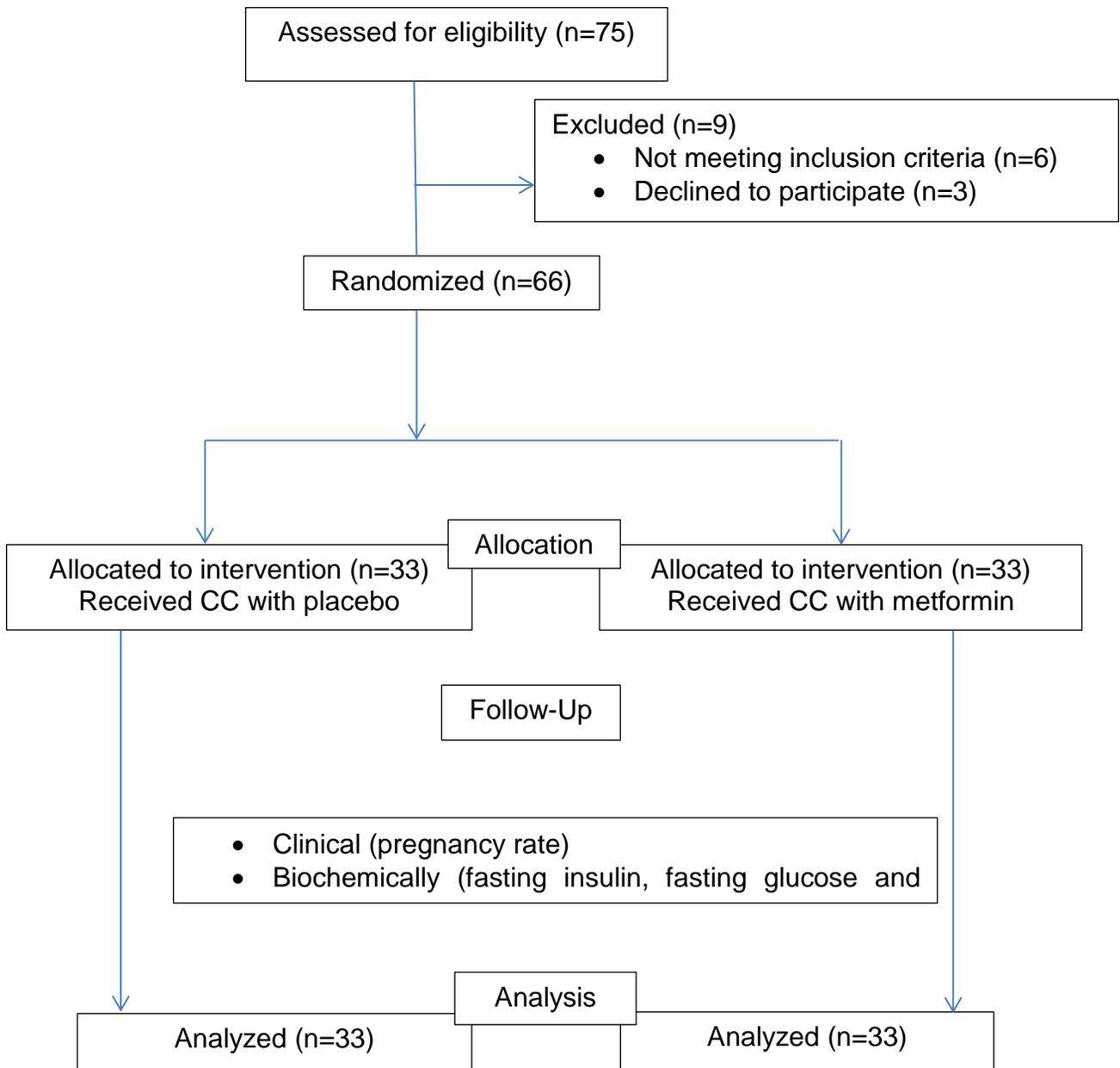


Figure 1: The study flow chart

### Study outcomes

The primary outcome of this study was the cumulative pregnancy rate after 3 months of treatment. Secondary outcomes included improvement of insulin resistance parameters such as fasting insulin, fasting glucose and homeostatic model assessment (HOMA-IR).

### Follow-up schedule

All study participants were followed at the end of the first, second and third months from the start of treatment. During each visit, we evaluated the patients clinically; we asked them to report their last menstrual period to ensure the pregnancy occurrence. When pregnancy occurred, 2D ultrasound was done to evaluate number of pregnancies. Finally, the insulin resistance parameters were assessed at the end of third month.

### Sample size

The sample size calculation was based on the primary outcome (cumulative pregnancy rate). Previous randomized studies reported that the CC with metformin improved pregnancy rate from 8% to 24% after using CC with metformin.<sup>17</sup> Using a two-sided chi-square ( $\chi^2$ ) test with an  $\alpha$  of 0.05, a total sample size was calculated to be at least 66 patients in the 2 groups (33 in each arm) with 80% power assuming a rate of loss to follow-up of 10% (Epi-info™, Centers for Disease Control and Prevention, USA).

### Statistical analysis

The data were collected and entered

into a Microsoft Access database and were analyzed using the Statistical Package for Social Science (SPSS Inc., Chicago, version 16). The demographic characteristics and baseline data were compared between both groups. The outcome variables were calculated using a paired t test to compare continuous variables before and after treatment and using an unpaired t test between groups. For dichotomous variables, chi-square was used to estimate the significance value. For analysis,  $p > .05$  was considered to be significant.

### **Results**

Out of 75 recruited patients, 66 consented to participate. Of those nine women; three did not meet the inclusion criteria and six women were not willing to participate in an RCT. Basal characteristics of the study participants are given in Table 1 and showed that the two study groups were similar in mean age, duration of infertility, weight and height. Moreover; the studied women were also similar in the ovarian volume, antral follicle count (AFC), FSH, LH and prolactin (Table 1).

There were significant improvements in the insulin resistance parameters after three months of combining CC and metformin when compared with CC alone. After 3 months of treatment patients of group II had statistically significant improvement of fasting glucose (P value=0.001), fasting insulin (p value= 0.005) and HOMA- IR (P value= 0.001) (Table 3 and 4).

**Table 1: Patient demographic data**

	Group I (CC+ placebo) n=33		Group II (CC+ metformin) n=33		P. value
	Mean	SD	Mean	SD	
Age (years)	26.12	5.24	24.91	3.34	0.05
Duration of infertility(years)	5.00	3.10	3.94	1.70	0.05
Wt (Kg)	78.03	8.11	78.58	8.18	0.787
Ovarian Volume(mL)	11.97	2.37	11.32	1.87	0.220
Galactorrhea	0.33	0.48	0.27	0.45	0.599
AFC	13.15	1.72	13.39	2.03	0.602
FSH(MIU)	5.50	1.34	5.44	1.73	0.866
LH(MIU)	10.12	2.20	10.42	1.88	0.558
Prolactin(ng/mL)	16.11	5.23	15.34	5.72	0.571
Endometrial thick(mm)	8.69	2.04	9.04	2.27	0.515
Number of follicles at cycle 1	2.00	0.82	1.89	0.92	0.657
Number of follicles at cycle 2	1.77	0.76	2.04	0.94	0.261
Number of follicles at cycle 3	1.70	0.63	1.96	1.00	0.270
Progesterone day 21(ng/ml)	14.79	6.33	14.95	4.67	0.932

Wt weight, AFC antral follicle count, FSH follicular stimulating hormone, LH luteinized hormone

**Table 2: Cumulative pregnancy rate after 3 months of treatment**

	Group I (CC+ placebo) n=33		Group II (CC+ metformin) n=33		P. value
	No.	%	No.	%	
PR from cycle 1	2	6.1	3	9.1	NS
Singleton	2	6.1	0	0.0	NS
Twin	0	0.0	3	9.1	NS
Abortion	0	0%	1	3%	NS
PR from cycle 2	5	15.2	12	36.4	NS
Singleton	4	12.2	10	30.3	NS
Twin	1	3.0	2	6.1	NS
Abortion	0	0%	1	3%	NS
PR from cycle 3					
Abortion	0	0%	1	3%	NS
Three cycles PR	7	21.2	18	54.5	0.033*

\*Statistically significant difference ( $p < .05$ ). PR pregnancy rate

**Table 3: Insulin parameters before treatment**

	Group I (CC+ placebo) n=33		Group II (CC+ metformin) n=33		P. value
	Mean	SD	Mean	SD	
Fasting glucose( mg/dL)	102.12	8.85	99.70	9.23	0.280
Fasting insulin (mg/dL)	11.57	2.73	12.60	3.91	0.364
HOMA_IR (mg/dL)	2.88	0.63	3.07	0.87	0.467
Progesterone D 21(ng/ml)	14.79	6.33	14.95	4.67	0.932

HOMA\_IR homeostatic model assessment of insulin resistance

**Table 4: Insulin parameters after 3 months treatment**

	Group I (CC+ placebo) n=33		Group II (CC+ metformin) n=33		P. value
	Mean	SD	Mean	SD	
Fasting glucose( mg/dL)	96.85	6.84	89.76	6.22	0.001*
Fasting insulin( mg/dL)	12.06	3.12	7.83	2.95	0.005*
HOMA_IR( mg/dL)	2.90	0.71	1.72	0.67	0.001*

\*Statistically significant difference ( $p < .05$ ). HOMA\_IR homeostatic model assessment of insulin resistance

## Discussion

Our data showed that adding metformin to clomiphene citrate for three cycles of ovulation induction in PCOS patients resistant to clomiphene citrate with acanthosis nigricans not only improved the cumulative pregnancy rate but also the insulin resistance parameters e.g. fasting insulin, fasting glucose and HOMA-IR.

Acanthosis nigricans is one of the clinical parameters indicating insulin resistance especially in patients with PCOS.<sup>9</sup> Hence, the basis for the hypothesis that AN patients would benefit from the addition of metformin to

clomiphene citrate by increasing glucose hepatic utilization accompanied by an improved metabolic profile, a significant reduction in hyperandrogenemia, and an improvement in ovulation and menstruation pattern.<sup>18</sup>

In developing countries where it is financially prohibitive to perform the insulin parameters for all clomiphene citrate resistant patients, AN can be used as clinical sign indicating insulin resistance and thus recommending the addition of metformin to clomiphene citrate in their treatment protocol.

The same data were published by

Neveu et al. who proved that metformin is better for ovulation induction than CC alone and they suggested using of metformin as a first line for ovulation induction in patients with PCOS regardless of their weight and insulin levels. However; they found no significant difference in the pregnancy achievement which differs from our study as we found more cumulative pregnancy rate with using CC with metformin.<sup>19</sup> Our results were in agreement with data published by Khorram et al. who concluded that adding metformin with CC reduced serum insulin, insulin resistance and increased SHBG levels, resulting in an improved response to CC.<sup>20</sup>

Ben Ayed and colleagues support our results because they found a significant improvement of the ovulatory response to clomiphene citrate in PCOS women when adding metformin.<sup>21</sup> The same results were also observed by Dasari et al. who reported that the ovulatory rate and the pregnancy rate with the metformin-CC combination was higher when compared with CC alone.<sup>17</sup>

The conclusion of the systematic review conducted by Siebert et al. is that CC alone is superior to metformin alone regarding live birth rate and ovulation. The combination (CC+ metformin) is superior to CC alone as a primary method for ovulation induction and to achieve pregnancy in PCOS.<sup>22</sup> Kar et al. reported similar results and proved that metformin is as good as CC in terms of live birth rate and the combination of CC and metformin gave the highest ovulation and live birth rate.<sup>23</sup> The last published study was by Shigiyama et al. in 2016 who successfully treated a lean PCOS patient with type 1 diabetes by

metformin. However; they concluded that the hyperinsulinemia associated with type 1 diabetes potentially exacerbates PCOS through hyperandrogenism. Therefore, metformin is recommended for lean PCOS with type 1 diabetes as well as for common obese PCOS with type 2 diabetes.<sup>24</sup>

Rosiglitazone is another insulin sensitizer that acts through increased production of insulin-sensitive adipocytes and increased glucose uptake. Many published studies suggest using it in PCOS due to its beneficial effect on hyperandrogenism, insulin resistant and anovulation in both lean and obese women with PCOS.<sup>25</sup> Yilmaz et al. found that both metformin and rosiglitazone increased insulin sensitivity in obese and lean patients with PCOS and they also found that rosiglitazone seemed to be more effective in decreasing the androgen levels and in achieving a slightly higher improvement in menstrual disturbance than metformin.<sup>26</sup> Dereli et al. demonstrated in their study that rosiglitazone improved the ovulatory dysfunction, hirsutism, hyperandrogenemia, and insulin resistance of PCOS with minimal adverse effects. In addition; this drug may be a good choice for patients with PCOS, especially for the ones who failed to show satisfactory results in metformin therapy.<sup>27</sup>

It was noticed that the gastrointestinal side effects of metformin were dramatically improved when the patients used them during meals as recommended by previous studies.<sup>28</sup> This improvement enabled us to continue using metformin with CC for three successive months.

In our study we selected patients with acanthosis nigricans assuming that those patients would have more response to the addition of metformin to CC for three cycles of stimulation. Limitations of our study include small sample size and not evaluating the effect of metformin on improving AN from the dermatological perspective. Still more data is needed to support the effect of insulin sensitizers on improving the pregnancy rate in the CC resistance PCOS patients who have acanthosis nigricans.

### **Conclusion**

The addition of metformin to CC in clomiphene citrate resistant PCOS patients who have acanthosis nigricans improves both pregnancy rate and hyperinsulinemia parameters.

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