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ORIGINAL ARTICLE

Effects of RANKL inhibitor on hormonal parameters in letrozole induced rat model of polycystic ovary syndrome.

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ABSTRACT… Objective: To study the effect of RANKL inhibitor on serum FSH, LH and testosterone level in Letrozole induced rat model of PCOS. **Study Design:** Experimental Animal study. **Setting:** Thirty adult female Wistar Albino rats of 6-8 weeks of age with a weight range of 120–150 gm were recruited from the animal house of Baqai Medical University. **Period:** February 2022 till August 2022. **Material & Methods:** The rats were randomly divided into A, B, and C groups and each contained 10 rats. Group A was considered ass control while Group B served as Letrozole induced PCOS group and Group C served as RANKL inhibitor group. Letrozole dissolved in 0.1% CMC and was given at a dose of 1 mg/kg orally for 21 days to induce PCOS. RANKL inhibitor (Denosumab) was given 0.25 mg/kg body weight subcutaneously for 4 weeks after the induction of PCOS i.e from 21 days to 50th days. At the end of the experiment, blood was taken from intra cardiac puncture and analyzed through ELISA. **Results:** Mean serum level of LH and testosterone level were significantly higher ($P<0.001$) in Letrozole-treated PCOs group which was significantly decreased ($P<0.001$) in Denosumab treated animals but significantly higher ($P<0.05$) than control group. Whereas, mean serum FSH level was significantly ($P<0.01$) lower in the Letrozole-treated group which was significantly increased in Denosumab treated animals but significantly lower ($P<0.05$) than the control group. **Conclusion:** RANKL inhibitor could prevent the hormonal imbalance in letrozole treated PCOs in albino rats.

Key words: Polycystic Ovarian Syndrome, Letrozole, RANKL Inhibitor, FSH, LH Testosterone.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in premenopausal women. One in every 5 to 6 women suffers serious complications in menstrual cycle and fertility. Approximately 5 to 15% women are affected from PCOS globally.[1] The etiology of PCOS is still unknown however it may be a complex mixture of genetic and epigenetic factors.[2-4] The pathophysiology depends upon many factors, including environmental toxins, diet, nutrition, and circadian cycle. Many genes such as CYP11a, CYP17, LH, AMH, FSHR, TNF alpha, and SHBG that are involved in the development of ovaries may contribute the pathogenesis of PCOS.[5]

PCOS is a complex metabolic and hormonal disorder characterized by oligomenorrhea or amenorrhea, hyperandrogenism, and infertility.[6] The main pathophysiological components of PCOS are gonadotropic dysfunction and insulin resistance, studies also reported that about 55-75% of the cases of PCOs have increased LH/FSH ratios. This increased level of LH is due to stimulated GnRH.[7] Moreover, excessive amounts of anti-mullerian hormone (AMH) may also cause symptoms of PCOS due to increased amount of androgen production.[8] Early pubertal years are when the first signs of PCOS appear, together with increased gonadotropin secretion, reactivation of the hypothalamic GnRH pulse generator, and subsequent increased ovarian estrogen production. The loci LHCGR, FSHR, and FSH-b polypeptide (FSHB) genes were found in genome-wide association studies (GWASs), highlighting the neuroendocrine pathophysiology of PCOS.[9]

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Gonads and bone are interlinked as sex steroids act as a potential regulator of skeletal function. It is therefore assumed that endocrine bone factors may also have some local effect on the gonads and their effect on reproductive function. One such example is the receptor activator of the NF-κB ligand (RANKL).[10] The RANKL-RANK ligand scheme is responsible for osteoclastogenesis. It also has effects on a different system further than the skeletal system including the immune system, mammary gland, thymus, and in tooth development. Moreover, spleen cells can differentiate into mature osteoclasts, provided that osteoblasts are absent and soluble RANK-L and macrophage colony-stimulating factors are present.[11] Expression of RANKL indicates a positive relation between estradiol and seminal fluid RANKL and a negative relation with serum testosterone levels.[10]

This study was aimed to observe the effects of RANKL inhibitors on imbalance of FSH, LH and testosterone levels in Letrazole induced PCOs rats. This experiment will provide a better management plan for the hormonal therapy in PCOs subjects.

MATERIAL & METHODS

After the approval from the ethical committee (BMU-EC-01-2022), thirty adult female Wistar Albino rats of 6-8 weeks of age with a weight range of 120–150 gm were recruited from the animal house of Baqai Medical University and total duration of study were 6 months and was started in February 2022 till August 2022. Each animal was weighed and thoroughly evaluated for any gross disease. They acclimatized in their allotted cages for one week before the commencement of this experimental study. The rats were maintained in the animal house under a controlled environment i.e. well ventilated at a temperature of 23+2ºC, humidity 55 ± 5%, and light & dark cycles of 12 hours each. The animals fed on a standard rat diet and water add libitum.[12]

Grouping and Treatment

The rats were randomly divided into three groups (A, B&C) with each containing 10 rats. Each rat's tail was marked for identification.

Group A; were control and was given 5 ml/kg of distilled water, orally for 21 days. While Group B; served as PCOS induced group and Letrozole was dissolved in 0.1% (Carboxymethylcellulose) CMC and given at a dose of 1mg/kg orally for 21 days.[13-15] and Group C: served as RANKL inhibitor group. In this group, Letrozole dissolved in 0.1% CMC and were received as a dose of 1 mg/kg orally for 21 days to induce PCOS. After which RANKL inhibitor Denosumab was given 0.25 mg/kg body weight subcutaneously for 4 weeks (From 21 days to 50th days).[16]

Dissection and Blood Collection

At the end of experimental exposure, the rats of group A and group B were weighed and observations were recorded, and then sacrificed according to the protocol. Group C animals were administered RANKL inhibitors for four weeks after the induction of PCOS and were sacrificed after the last day of the experiment. The blood samples were collected into vacutainers following cardiac puncture, then stored in the refrigerator until it was centrifuged (PLC-05) at 3000 revolutions per minute for 20 minutes and stored them freezer set at -20C. The serum was separated from the blood sample and was used to assess serum LH, FSH, and testosterone levels using rat-specific kits (ELISA).[17]

Statistical Analysis

The data were entered and analyzed using SPSS 21.0. Mean and standard deviation was calculated for numeric variables. One-way ANOVA was done and the post hoc Tukey test was applied to compare the significance between the groups. P-value less than 0.05 was considered to indicate statistical significance.

RESULTS

Body Weight

Final body weight was significantly increased in all groups as compared to their initial body weight. However, the highest weight gain was seen in the Letrozole-treated group as compared to the other groups. When comparing the final body weight of the Denosumab treated group (160.00+4.69) with the final body weight of the Letrozole-treated

group (198.40+5.35), it was significantly lower (P>0.001) but significantly higher (P>0.001) than the final body weight of the control animals (140.1+1.79).

Absolute Ovarian Weight

Letrozole-treated animals showed significantly higher (P<0.01) ovarian weight as compared to the other groups. While the mean ovarian weight of Denosumabtreated group (35.80+2.24) was significantly decreased (P<0.001) as compared to Letrozole- induced group (39.70+2.19) but it was significantly higher (P<0.05) than the control animals (33.70+1.71).

Relative Ovarian Weight

The mean relative weight of the ovary was significantly higher (P<0.01) in Letrozole-treated animalsas compared to other groups. Whereas the mean relative ovarian weight of Denosumab-treated animals (28.05+2.20) was significantly decreased (P<0.01) as compared to Letrozole-treated animals (33.42+1.55) but it was significantly higher (P<0.01) than the control group (26.87+1.75).

Serum Analysis of Hormones

Luteinizing Hormone (LH)

The mean serum LH level was significantly (P<0.001) higher in the Letrozole-treated group as compared to other groups. Whereas the mean serum LH level of Denosumab treated animals (7.59+0.34) was significantly decreased (P<0.001) as compared to Letrozole treated animals (10.43+0.80), but it was significantly higher (P<0.05) than the control group (6.80+0.61) as shown in Table-I and Figure-1.

Testosterone (T)

The mean serum testosterone level was significantly (P<0.01) higher in the Letrozole-treated group as compared to other groups. Whereas the mean serum testosterone level of Denosumab treated animals (57.00+5.20) was significantly decreased (P<0.001) as compared to Letrozole treated animals (79.70+5.90), but it was significantly higher (P<0.05) than the control group (50.70+5.80) as shown in Table-I and Figure-2.

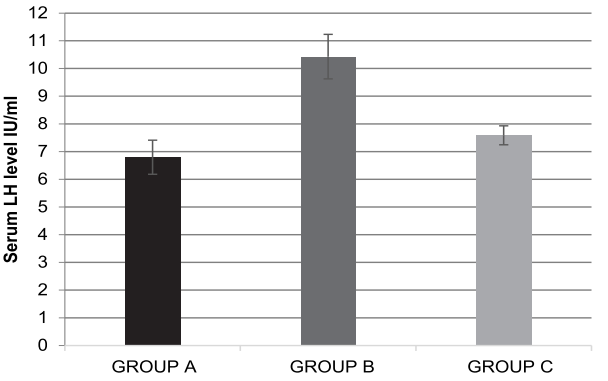

Figure-1. Serum LH level

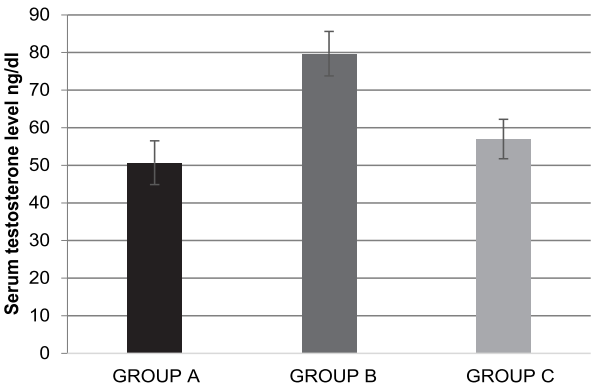

Figure-2. Serum testosterone level

Follicle-Stimulating Hormone (FSH)

The mean serum FSH level was significantly (P<0.01) lower in the Letrozole-treated group as compared to other groups. Whereas the mean serum FSH level of Denosumab treated animals (7.14+0.51) was significantly higher (P<0.001) as compared to Letrozole treated animals (5.23+0.57), but it was significantly lower (P<0.05) than the control group (7.78+0.56) as shown in Table-Iand Figure-3.

	Group A	Group B	Group C	P-Value
LH	6.80+0.61	10.43+0.80	7.59+0.34	<0.001
FSH	7.78+0.56	5.23+0.57	7.14+0.51	<0.001
Testosterone	50.70+5.80	79.70+5.90	57.00+5.20	<0.001

Table-I. Serum hormonal levels
Data were expressed as Mean+ SD

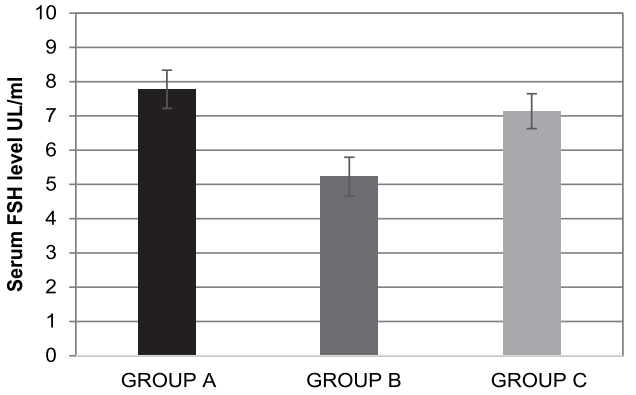

Figure-3. Serum FSH level

DISCUSSION

Excessive ovarian and/or adrenal androgen production is a hallmark of PCOS which leads to disturbance in menstrual cycle and infertility. Despite having multiple therapeutic options for PCOS, most of them are less effective and have serious complications.[18-21] Therefore, this study was designed to explore the effects of Denosumab (RANKL inhibitor) on hormonal alterations in Letrozole-induced polycystic ovary syndrome in female albino rats.

In this present study, the mean final body weight of all groups was significantly increased. Comparatively, the mean final body weight of the Letrozole-induced PCOS group was higher than the others. This finding is in complete agreement with the previous study in which letrozole-induced animals showed weight gain, similar to those associated with PCOS in women.[22] Comparable results were also reported in other studies where Letrozole-induced PCOS rats showed more weight gain than control animals.[23,24] The increased weight gain in PCOS animals or human beings could be due to elevated levels of circulatory androgen which results in hunger and increased food intake.[25] An earlier study also revealed that women with PCOS had elevated levels of lipids and non-high-density lipoprotein cholesterol that contribute to increased body weight.[26] PCOS-induced insulin resistance and hyperinsulinemia also cause adipocyte lipogenesis or proliferation that leads to adiposity and weight gain.[27-29] Denosumab-treated rats showed less weight gain as compared to the PCOS-induced rats. To the best of our knowledge, this is the first study that reports Denosumab decreased PCOS-induced weight gain. This reduction of weight gain could be due to decreased adiposity or decreased hunger and food intake because of the decreased level of androgens.

The present study showed that mean ovarian weight was significantly increased in PCOS induced group as compared to other groups. This result corresponds to the previous study that showed a significant increase in ovarian weight in PCOS induced group due to the Letrozole anabolic effect, ovarian fat, and might be due to the formation of many follicular cysts.[30-33] However this finding is in contrast to the previous studies that showed no significant change in ovarian weight of PCOS induced group and suggested that other factors such as genetic and environmental might play a role in increasing the ovarian weight.[34,35] While mean ovarian weight was lower in the group treated with denosumab as compared to the PCOS-induced group. This decline in mean ovarian weight shows the positive effects of Denosumab, which might be due to decrease expression of NF-κB, improvement in the hormonal profile, the restoration of the shape of normal follicles, or a decline in adipose tissue.

Among all, the Letrozole induced (PCOS) group showed significantly increased levels of LH and testosterone level as compared to other groups. These findings are in line with the previous studies that showed high levels of LH and testosterone in Letrozole induced group.[36] As explained in previous studies hormonal imbalance in PCOS may be due to dysfunction in the hypothalamus-pituitary axis, insulin resistance, irregular estrus cycle, decrease estradiol synthesis, and inhibition of aromatase enzyme activity.[32,37-39] In Denosumb treated group the LH and testosterone levels were significantly decreased as compared to Letrozole induced group. This decrease in LH and testosterone levels in Denosumab treated group might be due to the effect of Denosumab on increased aromatase activity that helps in the reduction of androgen by the aromatization process or by the conversion of testosterone into estrogen.

By further hormonal investigation, the FSH level of PCOS induced group was decreased in comparison with the Denosumab treated and control group. This finding corresponds with the previous research that indicates the decreased level of FSH in Letrozole induced group.[40] According to previous research there is a correlation found between insulin resistance, elevated level of LH, and decreased level of FSH in PCOS subjects.[41] In the Denosumab-treated group FSH levels were higher than those in the Letrozole-induced group but less than the control. Denosumab appears to be having a positive effect on the PCOS-induced group, as indicated by the increase in FSHlevel thatmight be due to the decreased expression of NF-κB, enhance insulin sensitivity, and lessen adiposity.

CONCLUSION

The present study concluded that RANKL inhibitors could ameliorate the body and ovarian weight gain and biochemical alterations in rat models of polycystic ovary syndrome.

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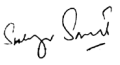
3	Surriyya Sarwat		
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