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RESEARCH ARTICLE

RELIEVING EFFECT OF METFORMIN ON HYPOTHYROIDISM ATTENDANT POLYCYSTIC OVARY IN RAT MODEL

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Present investigation was undertaken to study the relieving effect of metformin on hypothyroidism attendant polycystic ovary in rat model. Fifty female Sprague-Dawley strain rats were divided into five groups (10 rats each): water control group, oil control group, dehydroepiandrosterone (DHEA) group, metformin group and DHEA + metformin group. Serum levels of total triiodothyronine (tT3), total thyroxine (tT4), thyrotropin (TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) were analyzed after 30 days of injection. DHEA group exhibited PCOS and hypothyroidism but significant amelioration was demonstrated in Met + DHEA group. It was concluded that metformin can relieve PCO and improve the thyroid gland functions.

Key words: Hypothyroidism, Polycystic ovary, Metformin, Dehydroepiandrosterone.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a well-known endocrine disorder concerning the reproductive women. It is commonly related to anovulatory infertility (Zhang *et al* 2015). It is a multifaceted, heterogeneous disorder of indefinite causes and there is a strong proof to be categorized as a genetic disease (Kanagavalli *et al* 2013). Thyroid gland dysfunction which is manifested as hypo-thyroidism, is an universal disorder and has been described in PCOS patients (Nagarathna *et al* 2014). The clinical features of hypothyroidism also include weight gain, menstrual irregularities and infertility and an association has been reported between PCOS and hypothyroidism (Walters *et al* 2012). Most of the times, hypothyroidism is subclinical and diagnosed first time during the evaluation of PCOS (Dahiya *et al* 2012). DHEA which is an androgen of mainly adrenal origin is often raised in women with PCOS. Therefore, DHEA has been used to induce PCOS in various rodent models (van Houten *et al* 2012). Currently, the investigators have been found the optimistic action of metformin on both reproductive and metabolic aspects of PCOS. But, the mechanisms underlying the positive actions of metformin in the management of PCOS stay partly unstated (Paoli *et al* 2013). Recently, several researches have stated a reduction in TSH levels tracking the management by metformin in patients of PCOS (Pappa and Alevizaki, 2013). In this study, the effect of metformin was tested for relieving and protective effect against PCOS and hypo-thyroidism in rats.

Materials and Methods

All drugs and chemicals were obtained from sigma chemical company (St Louis, USA).

Animals

Fifty female Sprague-Dawley strain rats (weighing 130-150 g) were obtained from animal house of National Organization for Drug Control and Research (NODCAR), Cairo, Egypt. Vaginal smears were taken for at least 2 estrous cycles to eliminate non-cyclic animals. Animals

were accommodated for one week in the lab before testing under standard housing conditions (RT 24-27°C) with altering 12 h light and dark cycles (12 h each) and free access to food (standard pellets diet) and water was allowed *ad libitum* unless otherwise specified. All animals' procedure was performed in accordance to the institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals. PCOS was induced by subcutaneous (sc) administration of DHEA. Adult female rats weighing 130-150 g were randomly allocated into five groups (10 rats each): water control group (Gr. I), oil control group (solvent for DHEA) (Gr. II), DHEA group (30mg/kg/day, sc) (van Houten *et al* 2012), (Gr. III), Metformin (Met) (500 mg/kg/day, *p.o.*) (Elia *et al* 2006) group (Gr. IV) and DHEA (30 mg/kg/day, *s.c.*) + Met (500 mg/kg/day, *p.o.*) group (Gr. V).

Handling of blood and tissues sampling

At the end of experiments (30 days), animals were anesthetized using ether. Then, blood samples were collected in non heparinised tubes. Tubes were centrifuged at 3000 r.p.m. for 15 min at 4°C and the produced sera were analyzed to measure the levels of total Triiodothyronine (tT3), total Thyroxine (tT4), Thyrotropin (TSH), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Prolactin (PRL) using AccuBind ELISA Microwells commercial kit. The thyroid glands were isolated and stored in 10% formalin for further histological examinations (Hajioun *et al* 2014).

Statistical analysis

The resultant data were expressed as the mean ± standard error of the mean (SEM) for ten animals in each group. Differences between groups were assessed by one-way analysis of variance (ANOVA). Subsequent multiple comparisons between the different groups were analyzed by Dunnett two-sided multiple comparison tests. Data were statistically analyzed using the statistical package for social science (SPSS 6.0 software). Values at $P < 0.05$ were considered significant (Linares *et al* 2013).

RESULTS

Analysis of blood samples

The results showed high significant differences between rats treated with DHEA (Gr. III) and those treated with oil (negative control group) (Gr. II) with respect to FSH, LH and PRL levels in blood (**Table 1**).

On the other hand, significant amelioration was demonstrated in Met + DHEA treated group (Gr. V) when compared with DHEA group (positive control group).

Table 1. Levels of FSH, LH (mlU/ml) and PRL (ng/ml) of the treated groups

Data / Studied groups	Mean ± S.E.				
	Water	Oil	DHEA	Met	Met+DHEA
FSH	18.5±2.8	40.5±2.1	49.23±1.5***	18.2±2.1	42.5±0.41**
LH	6±0.06	5.5±0.3	2.05±0.2***	5.4±0.3	2.5±0.2
PRL	5.4±0.4	5.2±0.3	10.6±1.1***	5.71±0.4	3.13±0.1***

FSH=Follicle Stimulating Hormone, **LH**=Luteinizing Hormone, **PRL**=Prolactin; ** Significance differences at P-value<0.01, *** Significance difference at P-value<0.001

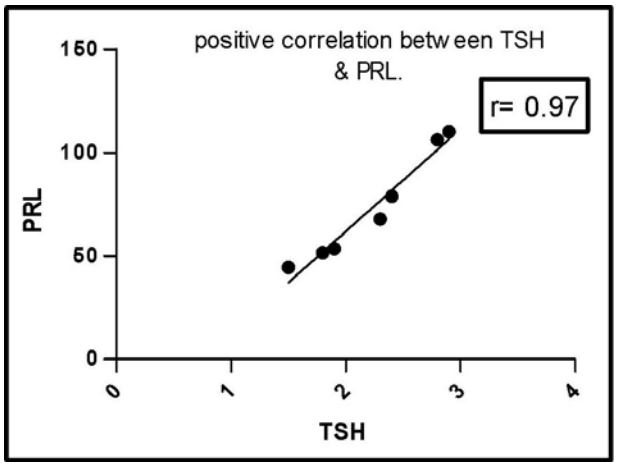
Significant changes in thyroid hormone levels (tT3, tT4 and TSH) were detected when comparing DHEA group (Gr. III) to Group II while amelioration was observed in Met + DHEA treated group (Gr. V) when compared to positive control group (**Table 2**).

Table 2. Biochemical characteristics of thyroid gland in the treated group

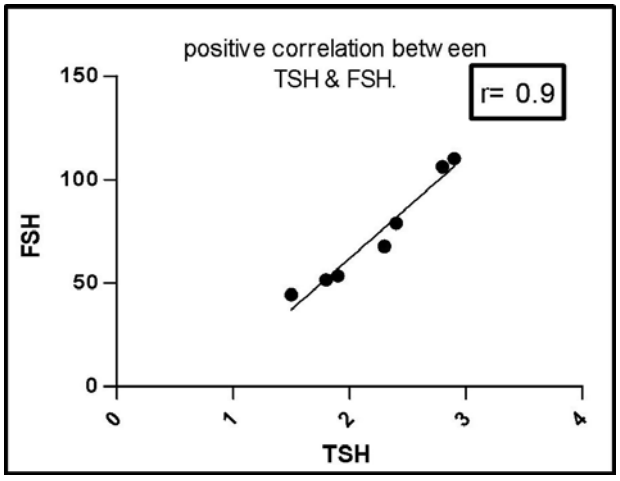
Data/ Studied groups	Mean ± S.E.				
	Water	Oil	DHEA	Met	Met+DHEA
tT3 ng/ml	2.6±0.07	1.6±0.1	0.27±0.02***	2.15±0.1	1.18±0.05***
tT4 μg/dl	4.4±0.1	4.2±0.07	2.1±0.1***	4.1±0.2	4.8±0.1***
TSH μlU/ml	1.8±0.2	1.8±0.1	2.9±0.12***	2.4±0.2	1.5±0.1***

tT3=total triiodothyronine hormone, tT4=total thyroxine hormone, TSH=thyroid stimulating hormone

In addition, a positive significant correlation between TSH and PRL levels (**Figure 1A**) and between TSH and FSH levels (**Figure 1B**) was detected while a negative correlation between TSH and LH was observed (**Figure 1C**).



(1A)



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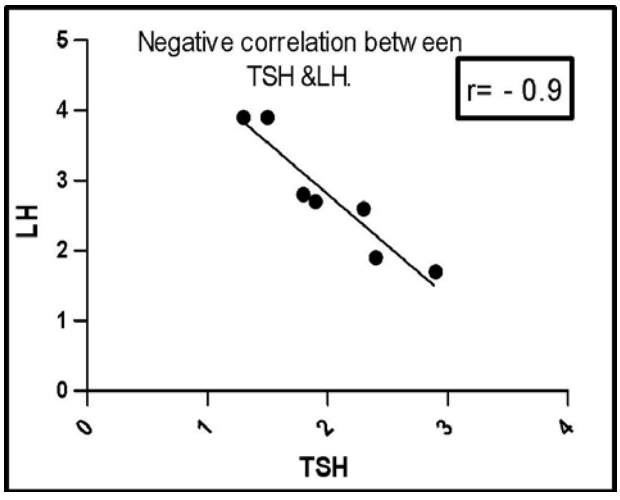

(1C)

Fig. 1. Correlation between TSH and PRL (A), TSH and FSH (B), TSH and LH (C)

Histological examination of thyroid gland of studied groups

Photomicrograph of thyroid tissue of a control group, showing normal architecture, thyroid follicle highlighted as F with intact thyrocytes by an arrow, colloidal secretion by an arrow head is shown in **Figure 2** whereas photomicrograph of thyroid tissues of a group treated with DHEA, demonstrating thyroid follicle (F1) with desqumated cells in their lumen, vacuolated cytoplasm of some thyrocytes (Arrow) with flattened nuclei (Arrowhead) is shown in the **Figure 3**. **Figure 4** displays photomicrograph of thyroid tissues of a group treated with Met, showing normal thyroid histological pattern (F) with colloidal secretion indicated by an arrow. **Figure 5** shows photomicrograph of thyroid tissues of a group treated with DHEA+Met, showing thyroid follicle (F) with desquamated cells in their lumen indicated by an arrow, whereas thyroid follicle with colloidal depletion is shown by F2.

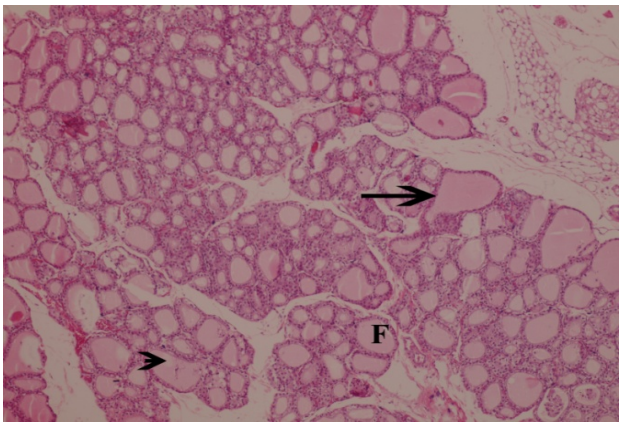

Fig. 2. Photomicrograph of thyroid tissue of control group, showing normal architecture, thyroid follicle (F) with intact thyrocytes (Arrow), colloidal secretion (Arrowhead).H&E, X:40

DISCUSSION

To our knowledge, there are a few studies concerning the thyroid function in PCOS women of reproductive age (Janssen *et al* 2004). Hypothyroidism itself can worsen PCOS signs (Dahiya *et al* 2012) and TSH is mainly the critical marker of hypothyroidism (Mortimer, 2011). Thyroid responsively by the ovaries could be elucidated by the existence of the thyroid hormone receptors on human oocytes. TSH also concerns estrogen metabolism and reduces secretion of sex hormone binding globulin (Soldin *et al* 2013). In this study, DHEA treated

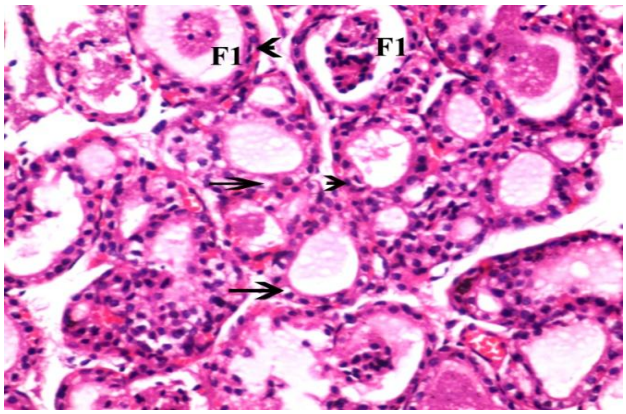

Fig. 3. Photomicrograph of thyroid tissues of a group treated with DHEA, demonstrating thyroid follicle (F1) with desqumated cells in their lumen, vacuolated cytoplasm of some thyrocytes (Arrow) with flattened nuclei (Arrowhead). H&E, X:200

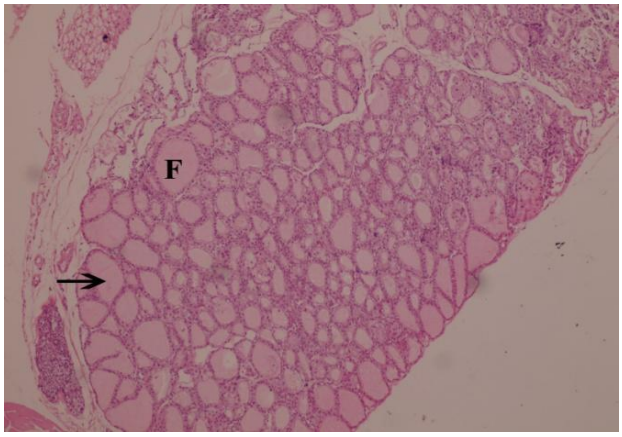

Fig. 4. Photomicrograph of thyroid tissues of a group treated with Metformin (Met), showing normal thyroid histological patteren (F) with colloidal secretion (Arrow). H&E, X:40

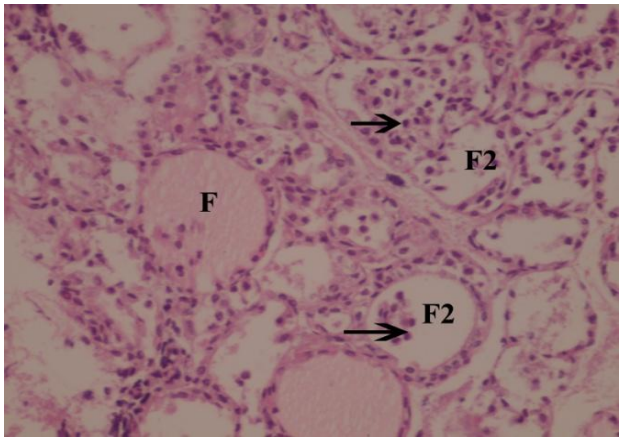

Fig. 5. Photomicrograph of thyroid tissues of a group treated with DHEA+Met, showing thyroid follicle (F) with desqumated cells in their lumen Arrow), thyroid follicle with colloidal depletion (F2). H&E,X:200

group shows PCOS, in addition to hypothyroidism. The relation between PCOS and hypothyroidism is due to adrenal androgen excess in women with PCOS which appears to occur from overexcited secretions of adrenocortical hormones. In response to adrenocorticotrophic hormone motivation, both more willingly form impairments of function of the hypothalamic-pituitary adrenal (HPA) axis (Schmidt *et al* 2011). This results in weakness of the pituitary gland, so no more signal received by the thyroid gland to release enough thyroid hormone (Rastogi and LaFranchi, 2010). Hypothyroidism is also demonstrated in histological inspections where cells of gland are damaged. This is known as primary hypothyroidism (Akeno *et al* 2011) which is in an agreement with El-Hafez (El-Hafez *et al* 2013). TSH is the mainly responsive marker of hypothyroidism (Janssen *et al* 2004). So, we tried to correlate it with PRL, FSH and LH levels. Significant positive correlation between TSH and PRL levels were due to compensative raise in the secretion of thyrotropin-releasing hormone (TRH) followed by an augmented motivation of pituitary prolactin discharge, in response to hypothyroid state. The elevated level of TRH in hypothyroidism and the lack of T4 response from the thyroid gland force cause proliferation and hypertrophy in both pituitary gland thyrotrophs (increasing TSH) and lactotrophs (increasing PRL), and this result is in harmony with Jokic and Wang (Jokic and Wang, 2011). In addition, positive significant correlation between TSH and FSH and negative correlation between TSH and LH are due to hypertrophy pituitary gland thyrotrophs that decreases the pituitary response to gonadotropin releasing hormone (GnRH), resulting in obstruction in LH secretion. This result is in accordance to Cakir (Cakir *et al* 2012). On the other hand, rats treated with DHEA + Met (Gr. V) showed normal cells as shown in the histological examination for thyroid. This indicates that metformin has preventive effect against PCOS and hypothyroidism and also, it has TSH lowering effect. The mechanism of lowering effect of Metformin is unclear up to date but it has been demonstrated that metformin could crosses the blood-brain barrier and has a central mechanism of TSH inhibition that could thus be an attractive elucidation. Although it stimulates the adenosine monophosphate–kinase (AMPK) in the periphery, metformin suppresses AMPK activity in the hypothalamus and may have offsets hypo-

thalamic T3 action on the TSH secretion (Pappa and Alevizaki, 2013). There is also verification that the metformin treatment increases hypothalamic dopaminergic tone (Morteza Taghavi *et al* 2011).

CONCLUSION

The present study concluded that DHEA induced PCOS in rats causing hypothyroidism and metformin has protective effects against both PCOS injury and hypothyroidism.

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