ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Study of Some Hormonal and Genetic Variants in Women with Polycystic Ovaries Syndrome in Baghdad City/Iraq

Noor Thamer Hamood Al-Dolaimy[1], Inaam Nori Ali AKhafaji[2]

[1.] *University of Anbar \ College of Education for Pure Science \ Department of Biology / Iraq.*

[2.] *Ministry of Science &Technology/Environment & Water Research &Technology Directorate / Iraq.*

Abstract

The study was carried out to determine the impact of the levels of sexual hormones represented by (LH, FSH, Prolactin and Testosterone) and also to assess the damage in the genetic material of DNA (comet assay) through the standards which are represented by (Tail length, DNA in tail, and Tail moment). Hormonal levels were examined on the second day of the menstrual period for 50 women infected with polycystic ovary syndrome (patients) and 20 women without polycystic ovary syndrome (control). In addition to that, the hereditary caudate test was tested for 34 patients with polycystic ovary syndrome compared to 50 women in the control group. The results showed a significant increase in the level of Testosterone and a significant decrease in the level of Prolactin hormone levels of LH and FSH hormones were within normal values as well as the genetic instability of DNA is more common in women with polycystic ovary syndrome PCOS compared with control group women.

Keywords: *Sexual hormones, PCO and comet assay.*

Introduction

Polycystic ovary syndrome PCOS is also called hyper androgenic, an ovulation, and Stein - Leventhal syndrome. The syndrome is the origin of symptoms that cause hormonal imbalance in women and this includes: irregular or no menstrual periods, heavy periods, excess body and facial hair, acne, pelvic pain, difficulties in pregnancy, the appearance of dark and thick spots on the skin (velvety skin). Polycystic ovary syndrome PCOS is a case in which some hormonal disorders interfere with the Ovulation process where the Adrenal gland and ovaries produce excessive amounts of the

Testosterone, resulting in the production of high and abnormal amounts of LH and abnormal amounts of follicle stimulating hormone (FSH), which causes the ovary to fill up with bags of immature follicles that are unable to produce eggs [2]. In addition to that, the size of the immature follicles means (2-3 times) follicles increases. The increased follicles are arranged around the ovary in a manner similar to a series of pearls [3]. There is an increase in the proportion of LH: FSH by 3:1 as the levels of LH and Testosterone remains high throughout the menstrual period, [4], where as any increase Prolactin hormone leads to the occurrence of excessive prolactin in the blood (Hyperprolactinemia), as Hyperprolactinemia in the blood and PCOS are the most common in the list of causes of infertility in women [5]. The comet assay or the electrical transfer of the gel to a single cell is a very sensitive method in detecting the DNA, which causes mutations, carcinogens, and environmental factors that affect Fertility in couples [6].This study is intended to examine the physiological and genetic factors in this syndrome .

Objective of this Study

- Investigation of the relationship and role of hormonal variables in women infected with polycystic ovaries at specific ages.
- Investigation of the relationship and role of genetic variables in women infected with polycystic ovaries at specific ages.

Material & Methods

During the year 2017, 70 blood samples were collected, 50 of them for women infected with polycystic ovary syndrome and 20 for normal women (Control) ranging from 25-40 years from the women's consultation clinic of Yarmouk Instructional Hospital, samples

were taken from women who were sick and normal on the second day of the menstrual period. 2 ml of venous blood was withdrawn for infected and normal women and was subjected to hormonal and genetic tests carried out at the Center for Environmental Pollutants Research and Genetic Impacts in the Ministry of Science and Technology.

Laboratory Examinations and Tests

Hormonal Analysis

Hormonal tests were performed by placing 3 ml of venous blood in a plain tube and then centrifuged at 3000 cycles / min for 10 minutes and separating the serum from the rest of the sample. Determination of concentration of FSH, LH, Prolactin and Testosterone hormones in the serum :

The hormones in the serum were measured using the TOSOH AIA system analyzer according to the method attached to the measurement kit and equipped from the Japanese company TOSOH.

Genetic Tests and Analysis

2ml of residual venous blood were placed in a test tube containing the

EDTA tube for the purpose of conducting the comet assay.

Alkaline Comet Assay

This test was performed according to the protocol described by Tice [7].Various measurements were made for each sample. Several measurements were used in this study:

- Length, height and comet area.
- Density and average density of the comet .
- Diameter, area, density and average head density.
- The percentage of DNA in the head.
- Length, area, density and mean intestine density.
- The percentage of DNA in the sin.
- Force and moment of tail starting from the following equation:

The percentage of DNA in the distance between the center of the head and the center of the tail or the so-called tail moment.

Preparing the Fundamental Slides

- The normal agarose was prepared with a melting rate of 10% by dissolving 500 mg of agarose per 50 ml of distilled water and heated until boiling.
- The prepared slices were dipped up to the top third in the boiling agarose and gently raised, and the bottom of the bowl was surveyed slide to remove the agarose, the slide cover is then placed in a dish on a flat surface to cool. The slide can be dried by air or heated to 50 ° C for the fastest drying. The slides were brought before use for one day and marked before use.
- Low solubility agarose 1% was prepared by dissolving 50 mg of agarose per 50 ml of PBS and heated quietly until the solubility was dissolved and the quantity was placed in transparent containers and kept frozen until needed.
- 100 micro liters of low solubility agarose were taken and added to 100 micro liters of cell suspension and blended, and well mixed and pull by one drop to be placed on the slices containing the normal and standard agarose which has been prepared in advance.

Electrophoresis of Micro Gel Slides

- After at least 2 hours of sample retention in freezer at about 4 ° C, slides are extracted then place it gently from the decomposition solution and stack as close as possible to the horizontal transfer box, close one end.
- Fill the electric transfer tank with the transfer chamber immediately until the liquid level is covers the slides fully (avoiding bubbles above the gel).
- Let the slides settle in the transfer chamber for 20 minutes to allow for DNA degradation and the expression of the damage that marks the rules.
- The power supply was powered by 24 volts (approximately 0.74 volts / cm) and the slides were moved for 30 minutes by raising or lowering the level and slides are migrated for 30 minutes.
- The power supply was closed and the slides were gently lifted and left on a drying bowl. The glass slides were washed three times in a row with a neutral solution of Tris-HCL and PH: 7.
- 80µL of (EtBr) x1 were added and left for 5 minutes and immersed in cooled distilled water to remove the excess dye. The lid of

the slide was then returned and the slide was recorded directly.

- After the registration, the lid of the slide was removed, immersed in absolute alcohol to withdraw the water, left to be dried by air or heated to 50 ° C by drying the fastest for 30 minutes and then stored for working purposes.

Statistical Analysis

After collecting and scheduling the data, the research was statistically analyzed using the Anova statistical contrast program for Hormonal Testing and Alkaline comet assay (SPSS) Statistical Package for Social Sciences in order to find the mean and standard deviation of the morbidity profile of PCOS cases and compare them with women control group.

Result &Discussion

Comparing the Level of Hormones Taken on the Second day of the Menstrual Period in Patients Infected with Polycystic Ovary Syndrome PCOS and Control Group

The results shown in Table 3.1 show that the level of hormones for women with PCOS compared with control group women, were normal values for FSH, LH, and there were no significant differences between the study group and the control group while significant differences were found ($p < 0.05$) at the level of Testosterone for the group of women with PCOS (0.48 ± 0.72) compared with women of control group, (0.12 ± 0.24), whereas the level of prolactin was significantly reduced in women with polycystic ovary syndrome compared with control group women (15.7 ± 7.50) and (15.38 ± 28.25) respectively as shown in the following Table:

Table 3.1: Hormonal conditions, in patients with PCOS and control group number 70

Groups	Parameters	Mean ± S.D	P. value
Control 20	LH	7.54 ±6.34	0.335
	FSH	10.23± 12.42	0.493
	Testosterone	0.24 ± 0.12	0.000
	Prolactin	28.25 ± 15.38	0.000
PCO 50	LH	8.73 ± 3.78	0.335
	FSH	8.94 ± 3.06	0.493
	Testosterone	0.72 ± 0.48	0.000
	Prolactin	15.79 ± 7.50	0.000

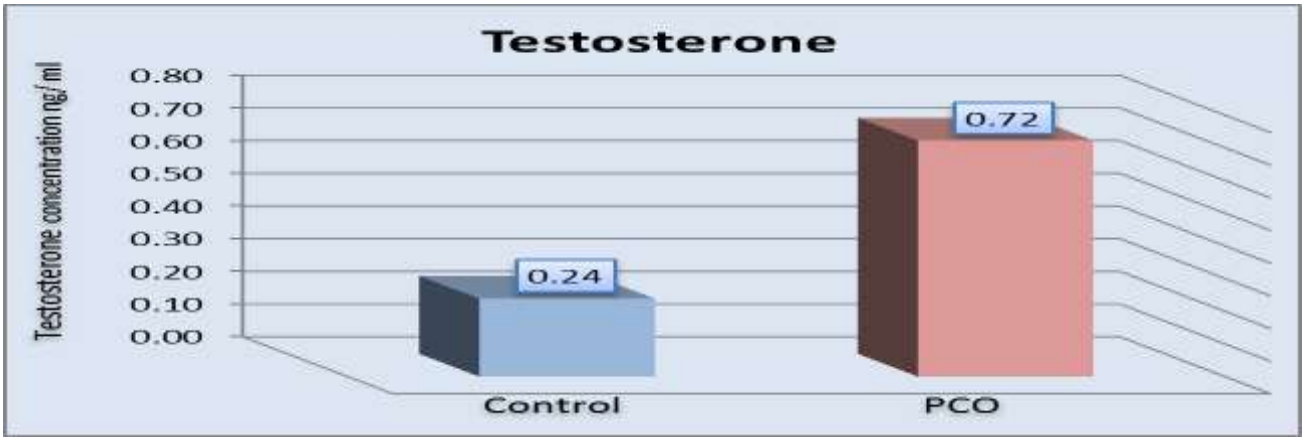

Figure 3.1: Comparison of Testosterone level among the group of women with PCOS and control group women

It was found in the study of Aghadavod and his group [8] (2015) which corresponded to the current study significant increase in hormone Testosterone in women infected with PCOS with an increased BMI, it can be said that Testosterone affects the early stages of follicular growth and follows ovulation where it has an important role in early induction of follicle growth, the increase in this proportion may be attributed to increased production of previously isolated androgens Adrenal gland [9]. The hormonal picture of the current study showed that in patients infected with PCOS, the ovaries have a significantly higher concentration of LH hormone (3.78 ± 8.73) compared to the FSH hormone concentration (8.94 ± 3.06) and where the FSH / LH ratio was very high 0.97 compared with control group 0.73 women. This is consistent with several studies which showed a rise in LH hormone and FSH / LH compared with FSH in women infected with PCOS compared to control group women [10, 11].

This hormonal trait can be explained by increasing the pulse frequency of GnRH hormone from hypothalamus which may favor the production of subunits ß of LH on subunits ß of FSH [12]. In a study conducted by Abd alwahab and his group [13] (2016) showed a significant decrease in serum Prolactin level (10.33 ± 18.67) in women infected with PCOS compared to women of control group (5.35±16.95) as shown in Figure 3.2, moreover there is no relative relationship between PCOS and hyperprolactinemia and this is due to hypothyroidism where Muderris and his group [14] (2011) reported that long-term hypothyroidism contributes to increased ovarian size or cyst formation. In addition to that, the levels of serum hormones are restored due to the effectiveness of the thyroid gland, which caused a decrease in the size of the ovaries and the separation of ovarian tissue together with the recurrence of PCOS-like characteristics .

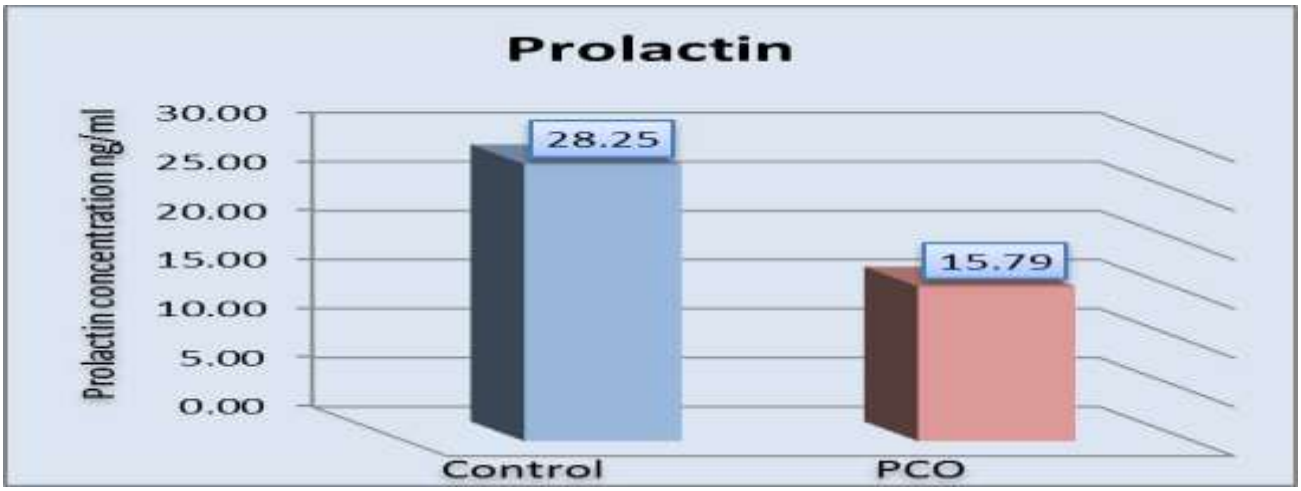

Figure 3.2: Comparison of the Prolactin level among the group of women with PCOS and control group women

Comet Assay (Single Cell Gel Electrophoresis)

The values of damages to DNA were shown using the Score software analysis image comet.The DNA standards adopted were important to determine the extent of damage: Tail length ،the ratio of DNA in the tail (PX), and the tail start moment, as shown in Table 3.2.

Table 3.2: Alkaline comet indicators for PCOS patients and comparing them with control group

Groups	Mean ± St. Deviation		
	Tail length	%DNA in Tail (px)	Tail moment
20 Control	9.59 ±7.50	16.49 ± 14.32	2.63 ± 3.75
34 PCO	15.56 ± 5.35	38.83 ± 10.48	7.34 ± 3.08

The results showed a significant increase ($p < 0.001$) in the Tail length of the comet on the second day of the menstrual period (number=34) at (5.35±15.56) compared with control group women (number = 20) at (7.50 ± 9.59) as shown in Figure 3.3.

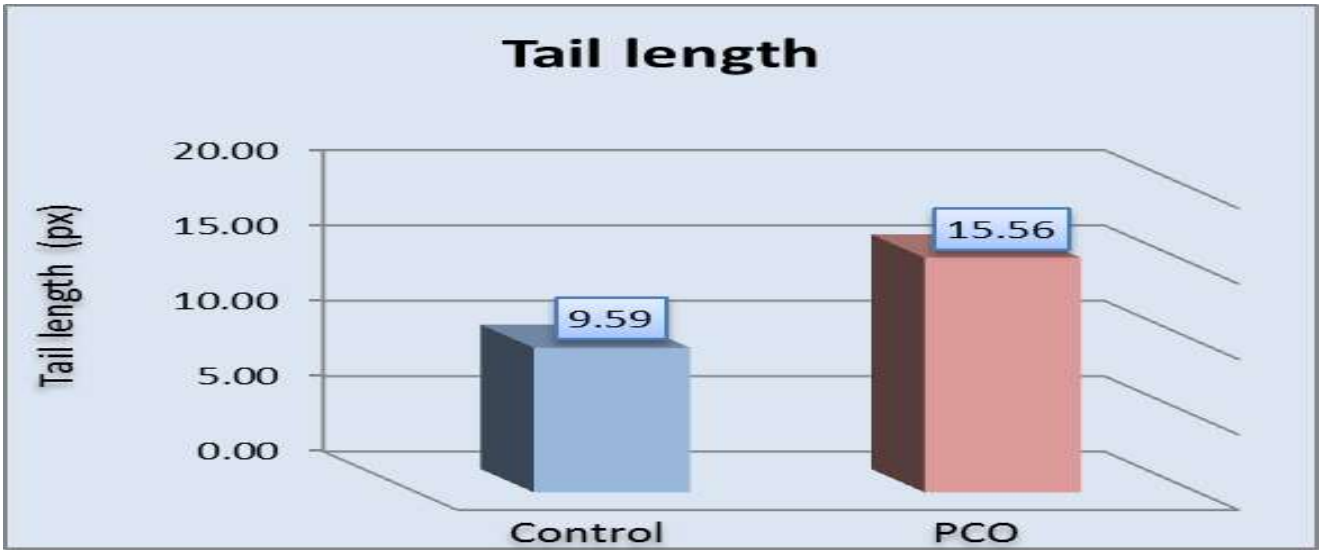

Figure 3.3 Comparison of the group of women with PCOS and control group women

As for %DNA in tail, it was found that women infected with PCOS had a significant increase (p <0.000) in the DNA ratio of the tail compared to the DNA ratio of the tail of the control group as well As shown in Fig. 3.4. A significant increase (p <0.000) was also found in the rate at which the tail starts moment as well as Table 3.2.

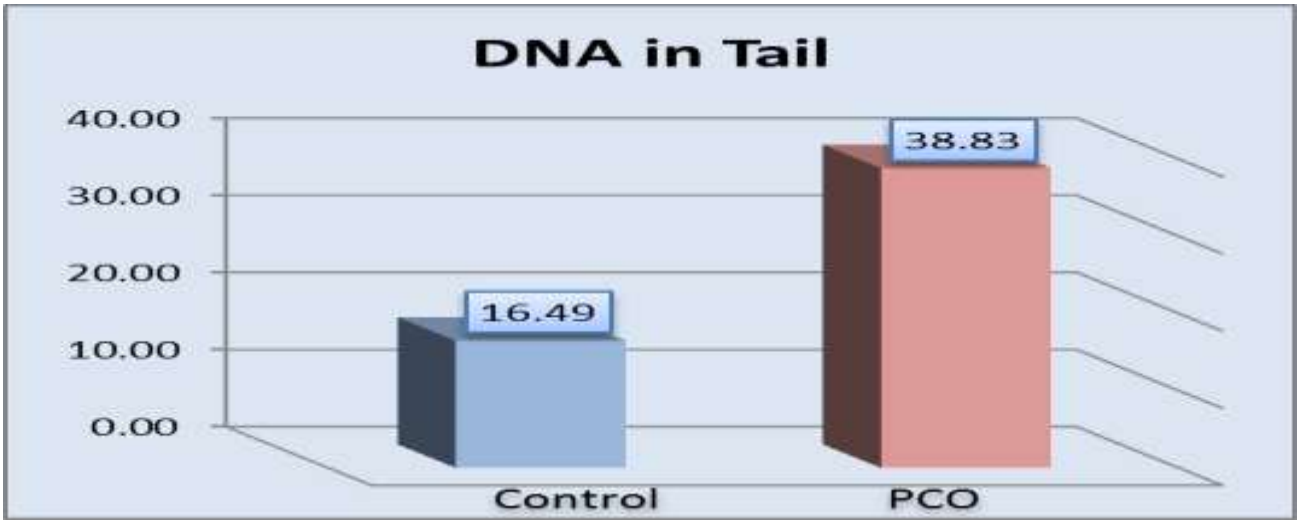

Figure 3.4: Comparison of the group of women with PCOS and control group women

Single cell gel electrophoresis is very sensitive to measuring the damage in the single-acid strip nuclear DNA. [15,16]. The obvious advantage of a comet screening technique that distinguishes it from other techniques that measure damage to the single-stranded DNA is its ability to measure heterogeneity within complex populations. When the culprit is examined, the damaged cell takes the appearance of the comet with the head and tail areas. [7] This is due to the presence of some genetic and environmental factors that caused the syndrome [17]. As a result of genetic instability in PCOS patients, which resulted in tail length, DNA in tail and tail moment compared with control group women. This study is consistent with the study of AL-Ahmed and his group [6] (2016), where the results showed a significant increase in the rate of small nuclei and comet of women with the syndrome compared to control group women.

References

1. Sheehan T (2003) Polycystic Ovarian Syndrome: Diagnosis and Management, Chines. Medical & Research, 1:13-27.
2. Bauer J, Cooper-Mahkorn D (2008) Reproductive dysfunction in women with epilepsy: menstrual cycle abnormalities, fertility, and polycystic ovary syndrome. Int. Rev. Neurobiol., 83:135-55.
3. Balen A, Homburg R, Franks S (2009) Defining polycystic ovary syndrome. Bmj, 338. Baillargeon, J., & Nestler, J. (2005). Polycystic Ovary Syndrome: A Syndrome of Ovarian Hypersensitivity to Insulin? JCEM, 91(1): 22-24.
4. Speroff L, Fritz M A (2011) Clinical gynecologic endocrinology and infertility (7th Ed.) (pp. 38-1189). Philadelphia, PA: Lippincott Williams & Wilkins.
5. Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, Wass JA (2011) Endocrine Society. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. J. Clin. Endocrinol. Metab., 96(2):273-88. Review.
6. AL-Ahmed HIA, Mahmood RA, Abdul-Fattah SY(2016) Micro-nuclei Formation and Comet Assay in Women with Polycystic Ovarian Syndrome (PCOS). Iraqi Journal of Science, 57(3B):1952-1958.
7. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobay- ashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single Cell Gel/Comet Assay: Guidelines for in vitro and in vivo Genetic Toxicology Testing. Environ Mol Mutagen, 35:206-221.
8. AghadavodE,ZarghamiN,FarzadiL,ZareM, BarzegariA,MovassaghpourAA,NouriM(2015)Evalution of relationship between serum levels of anti-mullerian hormone

,androgen ,and insulin resistant with retrieval oocyte in over weight patients with polycystic ovary syndrome. Adv Biomed. Res., 4:76.1-7.

9. Seljic TL, Brown AJ (2007) Polycystic ovary syndrome diagnosis and treatment. Am.J.Med.,1 20(2):128-132.
10. Ali IN, Alhiti MAJ, Jawad HG (2016) The Correlation between the Blood and Hormonal Disorders with Syndrome of Polycystic Ovaries in Women of the Baghdad City. European Journal of Biomedical and Pharmaceutical sciences, 3(9): 69-65 .
11. Sharquie KE, Al-Bayatti AA, Al-Ajeel AI, Al-Bahar AJ, Al-Nuaimy AA (2007) Free testosterone, luteinizing hormone/follicle stimulating hormone ratio and pelvic sonography in relation to skin manifestations in patients with polycystic ovary syndrome. Saudi. Med. J. 28: 1039-1043 .
12. Trikudanathan S (2015) Polycystic ovarian syndrome. Medical. The clinics, 99: 221-235.
13. Abd alwahab HS, Khazali EAA, Hasan NS (2016) Evaluation of TSH, T4, T3 and Prolactin in Iraqis PCOS Women. World Journal of Pharmaceutical Sciences, 4(6): 333-337.
14. Muderris, II, Boztosun A, Oner G, Bayram F (2011) Effect of thyroid hormone replacement therapy on ovarian volume and androgen hormones in patients with untreated primary hypothyroidism. Annals of Saudi medicine, 31(2):145-51
15. Endoh D, Okui T, Ozawa S, Yamato O, Kon Y, Arikawa, Hayashi M (2002) Protective effect of a lignan-contain-ing flaxseed extract against CCl4- induced hepatic injury. Journal of Veterinary Medical Science, 64: 761-765.
16. Mohamed T, Takamizawa A, Hosaka Y, Endoh D, Oikawa S (2011) Hepatocyte apoptosis in dairy cattle during the transition period. Canadian Journal of Veterinary Research. In press.
17. Maysoon M, Najeeb M, Saleem (2017) Effect of Polycystic Ovary Syndrome and Hormones Disorder on Enzymes Gammaglutamyl Transferase, Oxaloacetic Transaminase, and Proteins, Journal of Al-Nahrain University, 20(2):31-41.

*Corresponding Author Email: noorma91@yahoo.com, inaambio@yahoo.com

