

ORIGINAL ARTICLE

The Relationship between Zearalenone Produced by *Fusarium spp* and the Incidence of Polycystic Ovary Syndrome in Women

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ABSTRACT

Key words:

Fusarium spp, Zearalenone, PCOS, CYP19

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Background: Zearalenone (ZEN) is a mycotoxin that the fungus produces *Fusarium spp.*, which reaches humans through food contaminated with mycotoxins. The purpose of this study is to look into the presence of zearalenone in the serum among females suffering from polycystic ovary syndrome, how it influences the levels of FSH, or follicle-stimulating hormone and estrogen, and how it relates to CYP19 gene polymorphisms. **Methodology:** This was accomplished by obtaining blood samples from both healthy women and women with polycystic ovarian syndrome and measuring zearalenone, estrogen, and FSH using ELISA. PCR was also employed to examine the CYP19 gene's SNPs utilizing the HSP92II enzyme. **Results:** The findings show that the amount of zearalenone in polycystic ovarian syndrome women's serum (4.03 ± 1.65 ng/ml) was greater than the healthy women (0.5 ± 0.1 ng/ml). Additionally, It was noted that samples of women with PCOS had higher levels of estrogen (60.86 ± 18.27 pg/ml) than samples of healthy women (48.82 ± 17.90 pg/ml). In contrast, samples of blood from polycystic ovarian syndrome patients had lower levels of FSH (5.22 ± 1.56 ml.U/ml) than samples from healthy women (7.55 ± 1.73 ml.U/ml). The PCR results using the HSP92II enzyme indicate the presence of three the genotype AA (homozygosity) AG (heterozygosity) GG (homozygous) The genotypes in women with PCOS indicate that they are GG, AG, AA (38, 43, 19)% compared to healthy women (20, 35, 45)%, respectively. **Conclusions:** This study indicates the presence of the toxin zearalenone in PCOS-affected women's blood serum. Toxins in the serum contribute to PCOS.

INTRODUCTION

Zearalenone (ZEN), also known as F-2 toxin, ecoestrogen, and mycoestrogen, is an estrogen-like fungal toxin. Produced by several *Fusarium* species such as *F.graminearum*, *F.incarnatum*, *F.verticillioides*, *F. Culmori*, *F.equiseti* and *F. Cerealis* ¹. ZEN is a heat stable toxin. This causes common health issues and is not broken down after normal food processing. ZEN may cause lipid peroxidation, disrupt DNA and protein synthesis, and induce germ cell death, all of which can lead to cytotoxicity ². Through the estrogen signaling route, ZEN (particularly α -ZEN) competitively couples with the estrogen receptor, causing malfunction in the reproductive system. ZEN is a chemical that is stable during processing, milling, and storage ³.

Many cereal grains, including corn, oats, rice, barley, rye, wheat, and sorghum, are frequently contaminated by these fungi. Additionally, it can contaminate dairy products and meat, and it can even penetrate aquatic environments through rain ⁴. PCOS or ovarian syndrome, is a multisystemic metabolic condition. According to research on ZEN, it increases permeability and germ cell death, which causes prematurity and other endocrine diseases because of its

strong affinity for estrogen receptors, ZEN and its metabolites, also referred to as xenoestrogens, have a tendency to accumulate in human reproductive systems, leading to polycystic ovarian syndrome. The primary cause of anovulation and female fertility loss is polycystic ovaries in the female reproductive system and cycle ^{5,6}.

Researchers are becoming more aware of the characteristics and causes of PCOS, and they are particularly interested in how environmental contaminants such as estrogenic mycotoxins may contribute to this condition. One of the characteristics of PCOS is changed expression of the enzymes and genes involved in the genesis of ovulation ^{7,8}.

Zearalenone and polycystic ovarian syndrome have been linked to the single nucleotide polymorphism (SNP) of the CYP19 gene rs2414096 ⁹.

The primary enzyme in charge of catalyzing the last stage in the transformation of androgens (C19 steroids, androstenedione, and testosterone) into estrogens (C18 steroids, estrone, and estradiol) in gonadal and extragonadal tissues is cytochrome P450 aromatase, which is encoded by the CYP19 gene. Differences in CYP19 gene may impact human health in two ways: either the amount of estrogen accessible for receptor

binding or the quantity of testosterone accessible for binding to androgen receptors. Zearalenone also affects the *CYP19* gene, and an increase in the androgen to estrogen ratio is a result of *CYP19* polymorphism and may be linked to hyperandrogenic phenotype in PCOS patients¹⁰. A common endocrine condition, PCOS is linked to aberrant estrogen and estrogen receptor (ERS) activity in females. Estrogen Receptor Alpha (ER α) and Estrogen Receptor Beta (ER β) in target tissues are the mechanisms by which estrogens mediate genomic effects. Cellular functions such as Controlling the proliferation and differentiation of the endometrium and ovarian function are affected by modifications in the estrogen receptor signaling pathway. The non-genomic signaling The G-protein Coupled Estrogen receptor (GPER) mediates the action of estrogen, according to recent reports¹¹.

In females, ovarian function is impacted if hormone levels are regularly disrupted. This results in the ovaries developing cysts^{12,13}. Female sexual development and reproduction depend on the main hormone is follicle-stimulating hormone (FSH) that controls ovarian activity. Estrogen regulates intricate feedback processes that affect its secretion. Low estrogen levels in the early phases of the menstrual cycle cause the pituitary gland to release more FSH, but high levels brought on by follicular expansion prevent this release. Any change in estrogen levels can throw off the regular balance of FSH output, which can hinder ovulation and follicular development^{14, 15}. The purpose of this study is to look into if zearalenone is present in the serum. Among female polycystic ovarian syndrome patients, its effects on FSH, or follicle-stimulating hormone and estrogen levels, and its relationship to polymorphisms in the *CYP19* gene.

METHODOLOGY

Blood samples

Venous blood samples were drawn in 8ml from 80 women (60 with PCOS and 20 as controls) and placed 4ml in BD Vacutainer tubes and 4ml in EDTA tubes. Their ages ranged from 20-45 years. All of them were examined for oligoovulation, anovulation, hyperandrogenism and polycystic ovary signs which were clinically diagnostic using by ultrasound imaging by a specialized doctor. After their blood collection stored at -20°C until use.^{16,17}

Elisa technique

Three Microelisa molds Kits were used, one was coated with a specific antibody to zearalenone, another with a specific antibody to estrogen, and the last with a specific antibody to FSH. Then, blood serum was added to the wells in the Microelisa and mixed with the

specific antibody. Then, Horseradish peroxidase (HRP) antibodies specific to zearalenone (Cloud-Clone Corp SEB806Hu), estrogen (Enzo Life Sciences ADI-900-174), and FSH (Thermo Fisher Scientific EH202RB) were added to each well. Then, each Microelisa was coated well and each was incubated. After that, each was washed and TMP was added to each well. The result will be a color change after adding the stop solution, and a spectrophotometer was used to measure the optical density (O.D.) at the designated wavelength. The instructions of the company equipped with the standard preparation were followed.^{17,18}

Polymorphism genotyping analysis

All of the study's blood samples had their DNA extracted using a manually submitted methodology and then the DNA was stored at -80°C for genotyping test¹⁹. By PCR; *CYP19* gene (SNP rs 2414096) was detected using

Forward primer	5.TCTGGAACTTTTGGTTTGAGTG-3
Reverse primer	5.GATTAGCTTAAGAGCCTTTTCTTACA-3

A total volume of 25 ML was used for the three amplifications, which included 12.5 ml of Green Master Mix (PromegaUSA), 50 ng of genomic DNA, and 6.25 pmol of each primer. Five minutes at 94°C, thirty one-minute cycles of 94°C, 60°C, 72°C, and 72°C for ten minutes were among the cycling parameters^{20,21}. The sample was then placed onto a 2% agarose gel after the RFLP PCR technique reaction product had been treated for four hours at 37 degrees Celsius with the *CYP19* gene-specific enzyme HSP92II (MspI). The electrophoresis apparatus was then used to transfer the samples, followed by the addition of Red Safe dye and, lastly, the use of Gel Documentation to image the gel.²²

Statistical analysis

Calculating the mean, the standard deviation, error deviation of the mean, and correlation between the variables were computed using the Statistical Package for the Social Sciences (SPSS) version 25.

RESULTS

The findings show that the amount of zearalenone in the serum of women with polycystic ovarian syndrome (4.03 ± 1.65 ng/ml) was greater than that of women in good health (0.5 ± 0.1 ng/ml) as shown in the (Figure 1).

It was also noted that estrogen levels were greater in female samples with polycystic ovary syndrome (60.86 ± 18.27 pg/ml) in contrast to healthy females (48.82 ± 17.90 pg/ml) according to (Figure 2).

As for FSH, it was noted that it was lower in females with polycystic ovarian syndrome's blood samples (5.22 ± 1.56 ml.U/ml) compared to healthy females (7.55 ± 1.73 ml.U/ml), as shown in the (Figure 3).

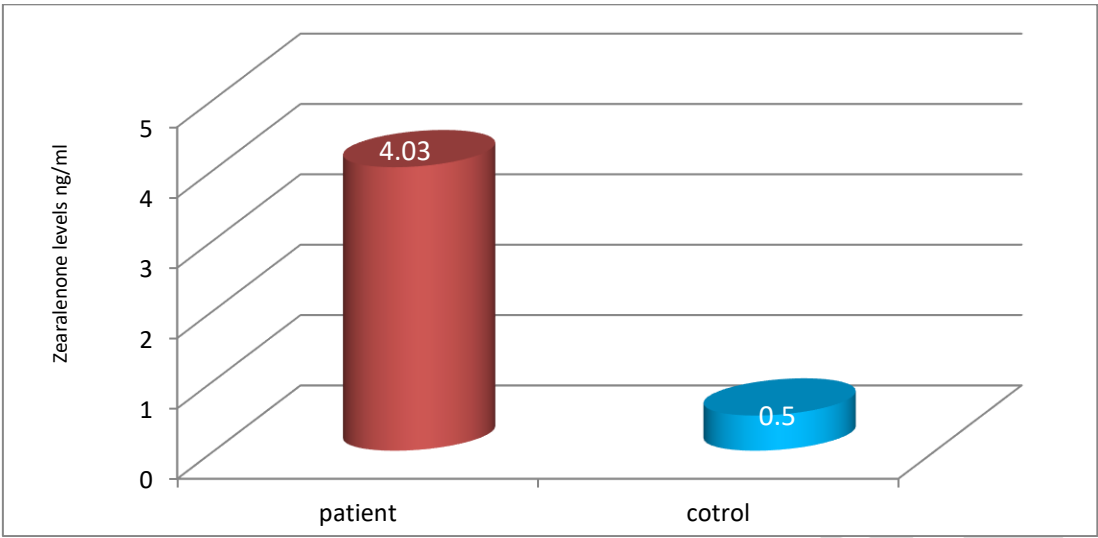


Fig. 1: Zearalenone levels among females in the study group (PCOS and healthy)

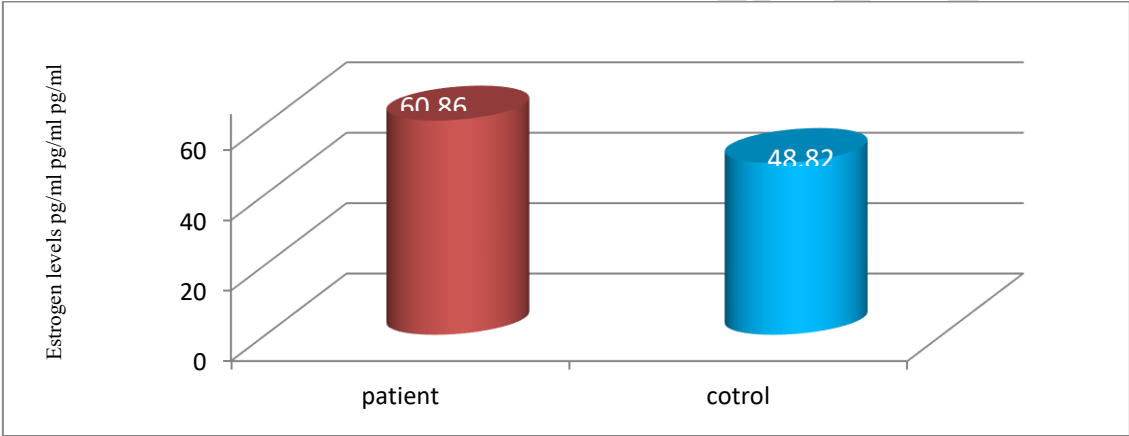


Figure 2. Estrogen levels in healthy females and those with polycystic ovarian syndrome

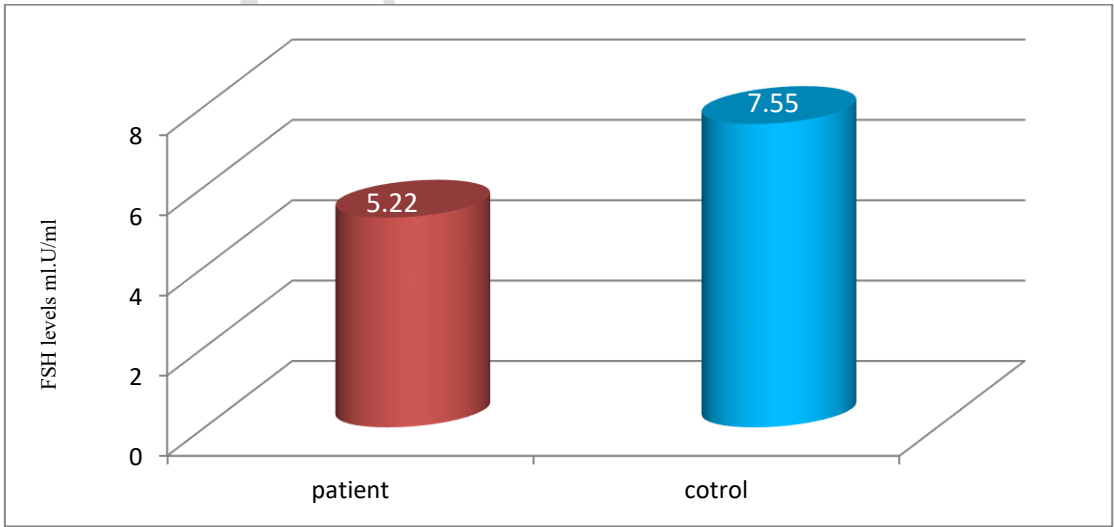


Fig. 3: FSH Polycystic Ovarian Syndrome levels in females and those in good health

The PCR results using the *HSP92II* enzyme indicate the presence of three genotypes, as it produces two fragments, one with a size of 161 bp and the other with a size of 28 bp for the genotype AA (homozygosity). While three fragments 189-161-28bp give the AG genotype (heterozygosity). And one fragment is 189 bp

long and is the GG (homozygous) genotype. as shown in the (Figure 4)

The genotypes in females with PCOS indicate that they are GG, AG, AA (38, 43, 19)% compared to healthy females (20, 35, 45)%, respectively, as shown in (Figure 5).

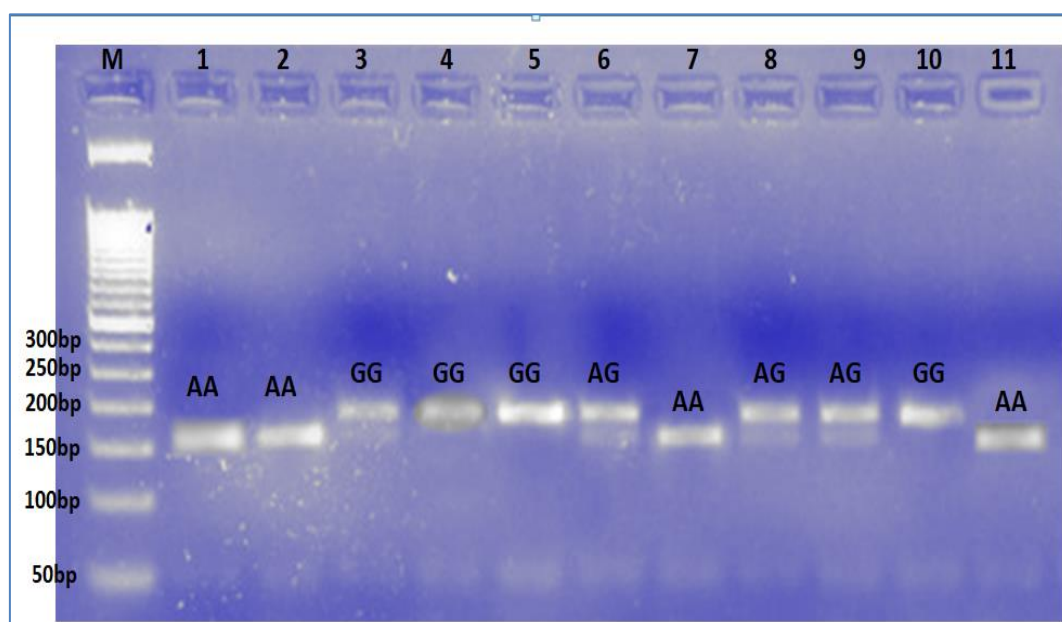


Fig. 4: DNA band under UV light

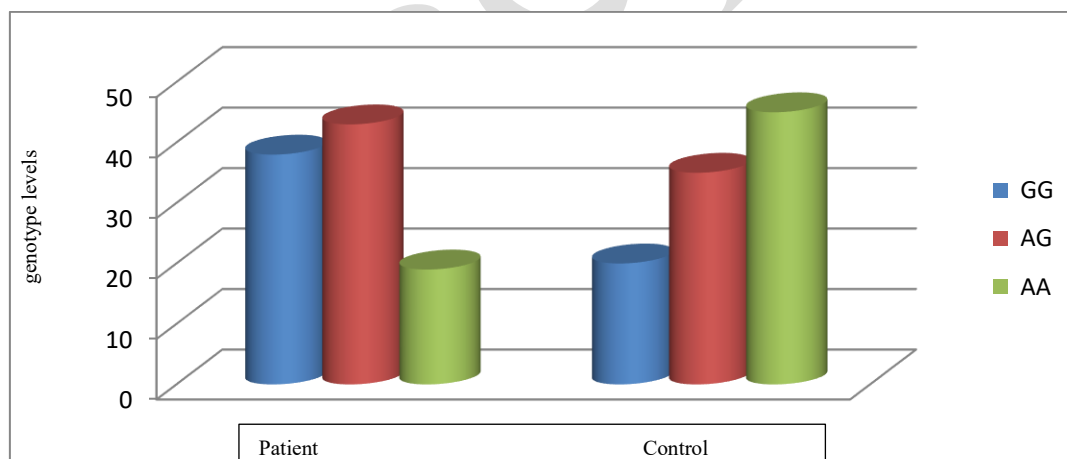


Fig. 5. Genotype levels in PCOS and healthy females

DISCUSSION

Since zearalenone levels were discovered to be more prevalent in females with polycystic ovarian syndrome (4.03 ± 1.65 ng/ml) compared to healthy females (0.5 ± 0.1 ng/ml), it affects 20% of women worldwide^{23,24}. *Fusarium* toxins found in grains, various baked goods, maize oil, processed meals, eggs, red and

white meat, pasta, and chocolate are the cause of the rise in zearalenone²⁵. This is due to zearalenone, A non-steroidal mycotoxin that functions similarly to estrogen that is thought to be one of the main symptoms of polycystic ovary syndrome. It causes ovarian cysts, uterine swelling, irregular ovulation, hormonal imbalance, chronic inflammatory signs (ovarian tissue inflammation), and poor egg maturation²⁶. By binding

to 17 β -estradiol receptors, the rise in zearalenone also results in hyperestrogenism. This mimics endogenous estrogen and impairs reproductive function by interfering with sex hormone receptors and interfering with their synthesis²⁷. This is in line with findings showing that estrogen levels were greater in females with polycystic ovarian syndrome. (60.86 $\pm$ 18.27 pg/ml) than women in good health (48.82 $\pm$ 17.90 pg/ml). These findings were consistent with a study by Jin et al 2009²⁸, that found that women with polycystic ovarian syndrome had greater estrogen levels (223.6 $\pm$ 136.2 pMol/L) than women in good health (166.1 $\pm$ 142.5 pMol/L). Because *Fusarium spp.* produce toxins and zearalenone is a toxin that is harmful to human health, This could be as a result of mycotoxin contamination in 25% of global agricultural production, and they are present in practically every stage of food production, processing, storage, and distribution³. Zearalenone inhibits the release of FSH by attaching itself to the body's estrogen receptors. FSH release decreases in response to increases in estrogen or zearalenone, and this in turn impacts the ovulation process in women²⁹. This was comparable to our study, which found that blood samples from women with polycystic ovarian syndrome had lower levels of FSH (5.22 $\pm$ 1.56 mIU/ml) than those from healthy women (7.55 $\pm$ 1.73 mIU/ml). These results aligned with the findings of³⁰, who found that FSH was found to be significantly involved in Polycystic ovarian syndrome develops in women with the condition because their levels were lower. Additionally, research shows that zearalenone affects the ovarian cells' expression of the CYP19 gene, which raises the production of endogenous estrogen and causes reproductive system abnormalities in women^{31, 32,33}. According to the findings, Compared to healthy women (20–45%), women with PCOS had a higher prevalence of the GG genotype (38%) and a lower prevalence of the AA genotype (19%). The GG genotype is more prone to PCOS than the AA genotype²⁸. This is because aromatase activity and the natural conversion of androgens to estrogens, which are in charge of maintaining normal androgen levels and shielding the ovaries against PCOS, may be linked to the AA genotype. Similar findings were obtained by Mehdizadeh et al.¹⁰ in their investigation of the relationship between the CYP19 gene and They discovered that Iranian women with polycystic ovarian syndrome possessed the genotypes GG, AG, and AA. (41.4%, 44.3%, and 14.4%, respectively) In contrast to healthy women (22.9%, 52.8%, and 24.3%), Because the CYP19 SNPrs2414096 gene is found in an intron, regulatory sequences may be impacted by variants of the gene. They came to the conclusion that the regular operation of several hormones that shield the ovaries from polycystic ovarian syndrome may be linked to the AA genotype.

CONCLUSIONS

Polycystic ovarian syndrome is caused by an increase in zearalenone in women's blood caused by the fungus *Fusarium spp.* Polycystic ovarian syndrome is caused by an increase in zearalenone, which also causes an increase in estrogen and a decrease in FSH. The high genotype GG was shown to be associated with polycystic ovarian syndrome in contrast to AA-genotyped healthy women, indicating a connection between zearalenone and the CYP19 gene and polycystic ovary syndrome.

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Declarations: On January 17, 2024, the University of Mosul, College of Environmental Sciences, Department of Environmental Sciences, granted ethical approval for the project under No. 57. Every procedure was carried out in compliance with the authorized rules and standards.

Conflict of interest: The authors declare no conflict of interest.

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