Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Association of CCR5 gene polymorphism with incidence of polycystic ovarian syndrome

Farah A. Abdulrazzaq [1*] ***and Zina F.H. Al-Obaidi*** [1]

[1] Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrian University, Baghdad, Iraq

*Corresponding author: Farah.a.abdulrazzaq@ced.nahrainuniv.edu.iq

Received: 30/05/2025, Accepted: 24/06/2025, Published: 30/06/2025

This work is licensed under a Creative Commons Attribution 4.0 International License

Abstract

PCOS is a multifactorial and heterogeneous endocrine disorder with a complex pathophysiology. This infertility disorder is increasingly prevalent worldwide, impacting a substantial percentage of women in reproductive age, with an estimated prevalence of approximately 8-13%. This search aimed to investigate the association of (CCR5Δ32) polymorphisms in chemokine receptor gene (*CCR5*) with PCOS.A case control study was organized at Baghdad Teaching Hospital in Baghdad Medical City included 150 women which were divided into three groups, Group 1 Includes (50) normal weight PCOS patients, Group 2 Includes (50) obese PCOS patients and Group 3 Includes (50) healthy women (Control). polymerase chain reaction (PCR)was used to detect and determine the presence of the gene variant (rs333) in the search subjects. The search's findings demonstrated that the majority of the biochemical and endocrine serum biomarkers analyzed including Lipid profile by checking the amounts of Total cholesterol, triglycerides, HDL, LDL and VLDL and hormonal analysis of (LH, FSH, Testosterone, E2 and AMH) exhibited statistically significant variations in PCOS patients. Specifically, PCOS patients' groups showed elevated levels of triglycerides, total cholesterol, LDL, and VLDL, alongside reduced levels of HDL. Furthermore, the hormonal analysis confirmed a significant increase in LH: FSH ratio, LH, total testosterone, and AMH, whereas lower levels of E2, with no significant difference in FSH values between PCOS patient groups and control group. This search found no significant association between the CCR5-Δ32 (rs333) mutation and polycystic ovary syndrome (PCOS). The wild-type *CCR5* genotype was predominant among all participants with a slightly higher frequency in in heterozygous form, in the control group (4%) compared to the obese PCOS group (2%), with no individuals carrying the homozygous Δ32/Δ32 genotype in the studied population.

Keywords: *CCR5*, CCR5Δ32, polycystic ovary syndrome (PCOS), rs333, Luteinizing Hormone, Follicle Stimulating Hormone.

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent endocrine metabolic disease affecting women in reproductive age [1]. PCOS is expected to affect 4–10% of women of reproductive age globally [2]. It is defined by many small subcapsular ovarian follicles developing into cysts under ultrasonic imaging, hyperandrogenism, and chronic anovulation. It may be associated with obesity, dyslipidemia, insulin resistance with compensatory hyperinsulinemia. Also, there is a higher risk of cardiovascular disease (CVD), type 2 diabetes mellitus and several cancers among women with PCOS [3].The Rotterdam criteria are the most widely accepted criteria for diagnosis of PCOS that suggested the diagnosis of PCOS could be done if two out of the following three features are present : (1) Anovulation (oligomenorrhea or amenorrhea), (2) Elevation in circulating androgens levels (hyperandrogenemia) or clinical manifestation of androgen excess like (hirsutism, androgenic alopecia, acne) (3) Presence of polycystic ovary morphology (PCOM) on ultrasonographic [4] . The menstrual cycle and other ovarian functions are regulated by several hormones, it is known as hypothalamus-pituitary-ovary axis[5] . Women's hormone levels are dysregulated will interferes with the ovaries ability to function and causes development of small cysts and this increase in number and impaired maturation of follicles in the ovaries will elevate Anti-Mullerian hormone level since it is produced by granulosa cells and the ovarian follicles [6]. Any disruption in the ovarian or adrenal gland function tends to increase the body's androgen levels. In addition, the anterior pituitary produces luteinizing hormone (LH) and follicle stimulating hormone (FSH) in a 1:1 ratio in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus in women with typical regular monthly menstrual cycles. In women with PCOS this ratio is higher even 2:1 or 3:1 [7]. Obesity and PCOS are sharing a complex bidirectional relationship as a viscous cycle, where each condition can exacerbate the other by multiple metabolic and hormonal pathways [8]. Additionally, dyslipidemia which is Lipid abnormalities are found among PCOS patients. Mild hypercholesterolemia is often encountered in PCOS women with multiple study's findings indicated that various lipid profile patterns may be observed in individuals with PCOS, including decreased levels of high density lipoprotein (HDL) and elevated levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), and lipoprotein concentrations [9]. PCOS is thought to be caused by a combination of lifestyle factors and genetics. Every gene or mutation that has an indirect or direct impact on the ovaries is linked to PCOS [10]. Several genes contribute to PCOS, one of them is C-C chemokine receptor type 5 gene (*CCR5*) which is a receptor of chemokine subtypes expressed by several immune cells involved in immune monitoring and inflammatory response, it is essential for attracting immune cells to inflammatory areas [11]. The *CCR5* gene, which produces the *CCR5* protein, is found on the short (p) arm of chromosome 3 at location 21 on and consists of four exons and two introns [12]. The CCR5Δ32 mutation is characterized by a 32-base pair deletion within the coding region of the *CCR5* gene, specifically affecting the second extracellular loop. This deletion induces a frameshift in the protein-coding sequence, resulting in the production of a prematurely truncated *CCR5* protein that lacks three transmembrane domains. Consequently, the truncated protein is non-functional and fails to localize to the cell surface. This common mutation introduces a premature stop codon, yielding a shortened protein composed of 215 amino acids instead of the full-length 352 amino acids, which markedly reduces the surface expression of the *CCR5* receptor [13].

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Materials and Methods

Subjects

This search included 150 women which were divided into three group: Group 1 Includes (50) normal weight PCOS patients, Group 2 Includes (50) obese PCOS patients and Group 3 Includes (50) healthy women (Control). All participants underwent a questionnaire and clinical examination to meet the Rotterdam criteria for PCOS diagnosis. Inclusion criteria required women aged between (17-40) years old, with BMI between (18.5 - 24.9) described as the 'healthy range' and up to 30 described as obesity. Diagnosed with PCOS based on at least two of the following: oligo/anovulation, clinical/biochemical hyperandrogenism, and polycystic ovary morphology on ultrasound. Exclusion criteria encompassed smokers, those undergoing pituitary downregulation, poor ovarian reserve, endometriosis, autoimmune diseases, and endocrine disorders such as diabetes, thyroid dysfunction, hyperprolactinemia, and hypertension.

Biochemical and Hormonal assays

Venous blood samples have collected, serum was obtained for determination of Lipid profile by checking the amounts of Total cholesterol, triglycerides, HDL, LDL and VLDL according to manufacture protocol and automated device by GenoLab-TEK Chemistry Analyzer Smart-150 instrument by Enzymatic Colorimetric method. Also, Hormonal analysis of (LH, FSH, Testosterone, E2 and AMH) hormones done by using the Automated Immune assay by Chemiluminescence Immunoassay Analyzer CL-900i (Mindray Company).

Extraction of Genomic DNA from blood

Each frozen blood sample was thawed; genomic DNA was then extracted directly using the FavorPrep™ genomic blood DNA extraction kit. DNA concentration estimated by the Qubit® 4 instrument.

Genotyping of CCR5Δ32 (rs333) Mutation

We genotyped CCR5Δ32 (rs333) by polymerase chain reaction (PCR) by used:

Forward primer (5′-TGTTTGCGTCTCTCCCAG-3′)

Reverse primer (5′-CAC AGCCCTGTGCCTCTT-3′)

The polymerase chain reaction (PCR) carried out in a reaction vol. of (25 µL) which was composed of (12.5 µl) One*Taq* 2X Master Mix, (0.5 µl) Forward primer, (0.5 µl) Reverse primer, (2µl) Template DNA, the final volume of 25 µL was achieved by using Nuclease-free-water (9.5 µl).

PCR Thermocycling Conditions

The thermocycling conditions were as follows: an initial denaturation step at 94 °C for 4 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 30 seconds. This was followed by a final extension step at 72 °C for 4 minutes, with subsequent storage of the amplified products at 4 °C.

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Electrophoresis on an agarose gel

The amplified PCR products were separated using 2% agarose gel, stained with RedSafe dye, and visualized using a gel documentation system (Cleaver Scientific, UK).

Statistical analysis

The statistics was accomplished *via* the SPSS software version 28 (SPSS 28, USA). the median with interquartile range (IQR). The group variations were analyzed using the non-parametric Kruskall–Wallis test and Dunn pairwise comparisons for continuous parameters and chi-squared ($\chi 2$) for categorical parameters. Also, the Dunn–Bonferroni correction method was performed to keep the overall risk of type I error at 0.05. Furthermore, box and whisker plots were plotted using GraphPad Prism, version 10 (GraphPad, La Jolla, CA, USA).

Results and Discussion

Investigating serum levels of lipid biomarkers, Kruskal–Wallis and post hoc Dunn tests exhibited significant differences in the median levels of TC, TG, LDL, and VLDL among the three groups, with the highest and the lowest median levels in group2 and group3, respectively (Table 1, $P \leq 0.05$). By contrast, the highest and the lowest median HLD levels were in group 3 and group 2, respectively. Notably, it was observed that median HDL levels of PCOS cases in groups 1 and 2 were comparable ($P > 0.05$) and exhibited dramatically lower levels versus healthy women in group 3 (Table 1, $P \leq 0.05$).

Table 1. Median serum levels of lipid biomarkers by polycystic ovarian syndrome sub- groups and control subjects.

Parameter	Group 1 (N=50)	Group 2 (N=50)	Group 3 (N=50)	*P* value
TC (mg/dL)	171.50 (102-250) a	197.50 (118-245) b	119.50 (90-174) c	0.00
TG (mg/dL)	155.50 (60-257) a	186.50 (74-257) b	103 (75-128) c	0.00
HDL (mg/dL)	51 (42-59) a	50 (44-58) a	57.50 (50-63) b	0.00
LDL (mg/dL)	89 (31-192) a	114 (52-160) b	47.50 (11-96) c	0.00
VLDL (mg/dL)	30.80 (12-51) a	37 (14-51) b	20.30 (15-25) c	0.00

Statistically significant differences are indicated by different letters (a ،b ،c). However, values sharing the same letter are not significantly difference ($p>0.05$).

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Dyslipidemia is a common metabolic abnormality in PCOS patient, Moreover, individuals with PCOS are more likely to exhibit dyslipidemia, characterized by reduced levels of high-density lipoprotein (HDL) and elevated levels of triglycerides, total cholesterol, and low-density lipoprotein (LDL), in comparison to women without PCOS. Dyslipidemia is a substantial risk factor for development of cardiovascular disease (CVD) in PCOS women [14]. The dyslipidemia observed in individuals with polycystic ovary syndrome (PCOS) is likely attributable to underlying metabolic disturbances, particularly hyperinsulinemia and hyperandrogenemia. These hormonal imbalances promote enhanced catecholamine-induced lipolysis in adipose tissue, resulting in increased release of free fatty acids into the circulation. Elevated circulating free fatty acids are subsequently taken up by the liver, stimulating the production and secretion of very low-density lipoproteins (VLDL), which contributes to the development of hypertriglyceridemia. This condition, through disruption of the reverse cholesterol transport pathway, leads to a reduction in high density lipoprotein (HDL) levels and an increase in low density lipoprotein (LDL) concentrations.

Additionally, hyperandrogenism may directly influence lipid metabolism by enhancing hepatic lipase activity, which plays a role in the catabolism of HDL particles.[15] The resulting dyslipidemia in PCOS patients significantly elevates the risk of cardiovascular disease and type 2 diabetes mellitus. It also contributes to a higher incidence of pregnancy-related complications, including gestational diabetes mellitus and gestational hypertension, thereby increasing the likelihood of adverse reproductive outcomes. Moreover, elevated lipid levels in PCOS are associated with an increased risk of nonalcoholic fatty liver disease (NAFLD), and may contribute to the pathogenesis of advanced liver conditions such as decompensated cirrhosis, hepatocellular carcinoma, and other chronic liver disorders[16]

These findings align with existing literatures that indicating obese PCOS women had higher serum triglyceride and cholesterol levels and lower HDL levels than non-obese [17] and a study conducted by Marbaniang *et al*. (2023) reported a significant increase in serum triglycerides, total cholesterol, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels, along with a notable decrease in high-density lipoprotein (HDL) levels among individuals with polycystic ovary syndrome (PCOS). compared to control group [9].Also Several Iraqi studies showed that HDL levels in obese women with PCOS had lower HDL levels than PCOS with normal weigh [18,19]. While the research by Mir *et al.* (2022) do not align with our result that showed HDL higher levels in PCOS patients with significant differences from the control group [20] .

The median levels with IQR of serum hormones of the study's three groups were illustrate in Table 2 and Figure 2. With the exception of FSH levels, which exhibited nearly comparable values ($P > 0.05$) in the three groups, the median values of LH: FSH ratio, LH, T, E2, and AMH significantly differed among the three groups (Kruskal–Walli's test, $P \leq 0.05$). In particular, significantly higher median values of LH: FSH ratio, LH, T, and AMH, whereas lower median levels of E2, were observed in PCOS groups 1 and 2 versus group 3 ($P \leq 0.05$). Additionally, median levels of those hormones in group 1 were nearly comparable with those in group 2 (Dunn's pairwise comparison test, $P > 0.05$).

Table 2. Median serum levels of hormones by polycystic ovarian syndrome sub-groups and control subjects.

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

Parameter	Group 1 (N=50)	Group 2 (N=50)	Group 3 (N=50)	*P* value
LH (mIU/mL)	10.99 (6.07-31.00) a	11.69 (4.08-26.60) a	6.36 (1.83-11.30) b	0.00
FSH (mIU/mL)	6.77 (2.5-0-13.50) a	6.27 (2.25-11.60) a	6.68 (2.66-11.90) a	0.40
LH:FSH ratio	1.68 (1.02-4.92) a	1.80 (1.14-3.80) a	0.90 (0.49-1.30) b	0.00
T (ng/mL)	1.00 (0.36-1.98) a	1.11 (0.36-1.98) a	0.36 (0.11-0.78) b	0.00
E2 (pg/ml)	38.00 (12.15-55.00) a	37.50 (25.00-50.00) a	45.00 (27.00-77.00) b	0.00
AMH (ng/ml)	7.58 (2.16-15.48) a	7.30 (4.00-12.00) a	3.62 (2.00-4.70) b	0.00

Statistically significant differences are indicated by different letters (a ،b) however, values sharing the same letter are not significantly difference (p>0.05).

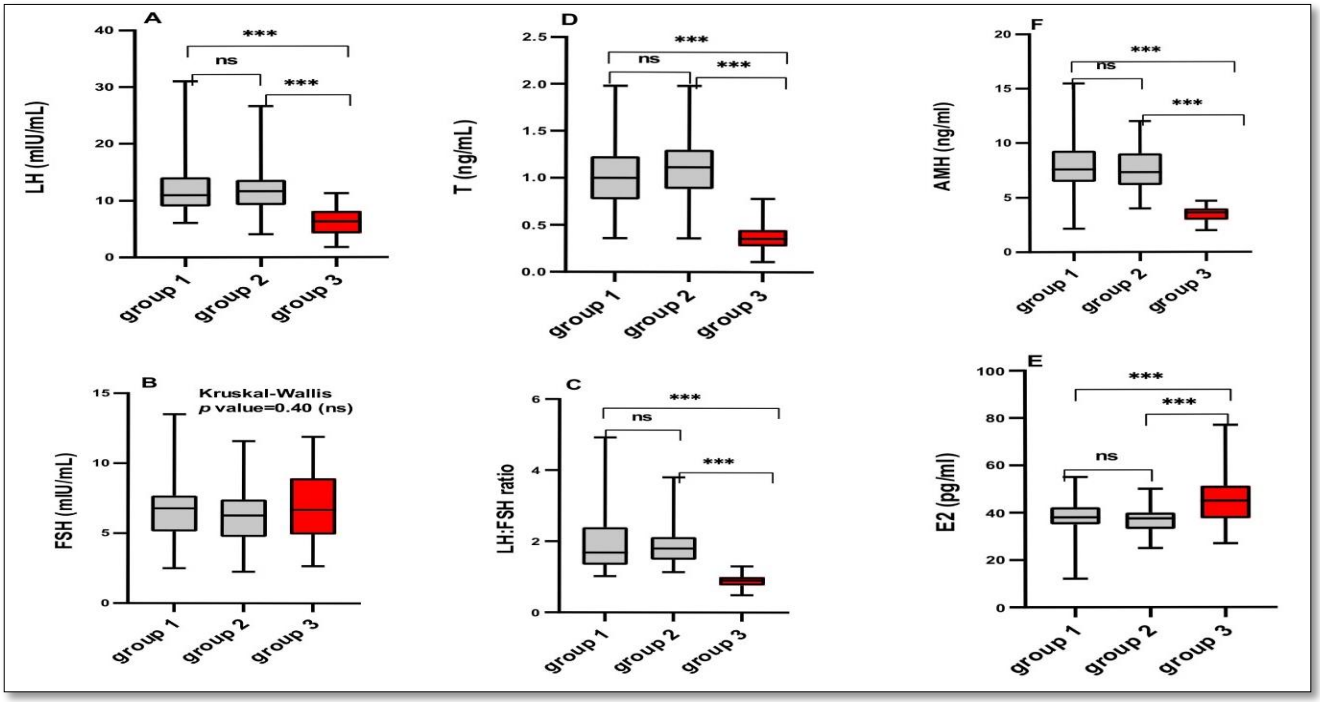

Figure 1. Box-whisker plots to depict median levels with IQR of (A) LH, (B) FSH, (C) LH: FSH ratio, (D) T, (E) E2, and (F) AMH across the study three groups

The elevated levels of luteinizing hormone (LH) and the increased LH/FSH ratio observed in patients with PCOS in this search are consistent with the underlying pathophysiology of the disorder. These

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

hormonal alterations are largely attributed to dysregulation of the hypothalamic–pituitary–ovarian (HPO) axis and disrupt ovarian function, leading to follicular cyst formation [21]. The hypothalamic-pituitary-ovarian (HPO) axis plays a central role in regulating reproductive function through the cyclical secretion of gonadotropic and steroid hormones. This hormonal cascade begins with the hypothalamic release of gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary gland to secrete two key gonadotropins: follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These hormones act on the ovaries to promote follicular development and the production of estradiol, followed by progesterone secretion post-ovulation [22]. In polycystic ovary syndrome (PCOS), a characteristic feature is the persistently increased pulsatility of GnRH, which disproportionately favors LH synthesis over FSH. This results in elevated LH concentrations and an increased LH:FSH ratio (typically exceeding the normal value of <2). The elevated LH levels stimulate androgen production by the ovarian theca cells, primarily through upregulation of cytochrome P450c17, the rate-limiting enzyme in sex steroid biosynthesis.

Moreover, this endocrine imbalance is exacerbated by a feedback mechanism wherein elevated androgen levels further increase GnRH pulse frequency. This is thought to occur via a reduction in the negative feedback sensitivity of the hypothalamic-pituitary axis to sex steroids, thereby creating a self-reinforcing loop that maintains elevated LH and androgen levels—contributing to the pathophysiology of hyperandrogenism in PCOS [23] Although hyperandrogenism is a central feature in the pathophysiology of polycystic ovary syndrome (PCOS), it is closely interlinked with insulin resistance, compensatory hyperinsulinemia, and abdominal adiposity. These metabolic disturbances collectively contribute to increased androgen production. Insulin, in particular, plays a pivotal role by exerting an inhibitory effect on the expression and enzymatic activity of aromatase—an essential enzyme responsible for the conversion of androgens to estrogens. Suppression of aromatase activity limits this conversion, thereby enhancing the androgenic milieu and further exacerbating the hormonal imbalance characteristic of PCOS [24] . The study's outcomes support the findings of previous investigations, that found the LH-FSH ratio and serum total testosterone were significant increase in women with PCOS [25] and another study showed that the mean of LH and LH/FSH ratio, is increased in PCOS patient groups when compared with control [26].
AMH levels have been proposed as a substitute marker for polycystic ovary morphology as well as for diagnosing PCOS. Women with PCOS are noted to have higher levels of Anti-Mullerian hormone than normally women this correlation was attributed to the multiple antral follicles in ovary [27]. Elevated levels of anti-Müllerian hormone (AMH) may contribute to ovulatory dysfunction by promoting the accumulation of antral follicles, as well as to hyperandrogenism through the inhibition of aromatase activity, both of which are commonly observed in women with polycystic ovary syndrome (PCOS). Furthermore, a strong positive correlation has been consistently reported between the number of ovarian follicles detected via ultrasound and circulating AMH concentrations in individuals with PCOS. [28]. A similar pattern has been observed in prior studies, supporting the current findings that found AMH levels were significantly higher

in PCOS patients than in controls across various BMI categories [29]. Gorzko et al., showed there was a significant negative correlation between AMH levels and BMI for Polish women with PCOS [30].

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

The study conducted by Al-Fartosy et al. showed there was increased levels of LH, LH/FSH ratio, AMH in patients with PCOS as compared to control group [31]. Kumar & Agarwal, reported that obese women with PCOS are at an elevated risk of having long-term complications and hence need early management, Obese PCOS women also have higher LH levels, LH:FSH ratio and total serum testosterone [32].

The 32 bp deletion in the *CCR5* gene (CCR5-Δ32) reported in groups 1 and 2, comparable to group 3, was not statistically notable (P > 0.05, Table 3).

Our finding showed the frequency of (rs333) genotypes in PCOS patients was (100%) *CCR5* wild type in group 1, (98%) in group 2 and Control group 3 was (96%).

Higher heterozygosity frequency was documented in control group compared to patient group that Mutant *CCR5* (Δ32+Δ32*) in control was (4%) compared with PCOS in group 2 was (2%). However Mutant CCR5Δ32* is (0%) in all study group as shown in Table 3. The wild-type amplicon corresponded to a 233bp band while the CCR5Δ32 allele corresponded to a 201bp band as shown in Figure 2.

Table 3. Association of CCR5 Δ32 (rs333) genotypes with polycystic ovarian syndrome sub-groups susceptibility

	N	CCR5 Wild	Mutant CCR5 (Δ32+Δ32*)	Mutant CCR5Δ32*	DF	χ2	*P* value
Group 1	50	50 (100%)	0 (0%)	0 (0%)	1	2.04	0.15
Group 2	50	49 (98%)	1 (2%)	0 (0%)	1	0.34	0.55
Group 3	50	48 (96%)	2 (4%)	0 (0%)	---	---	---

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

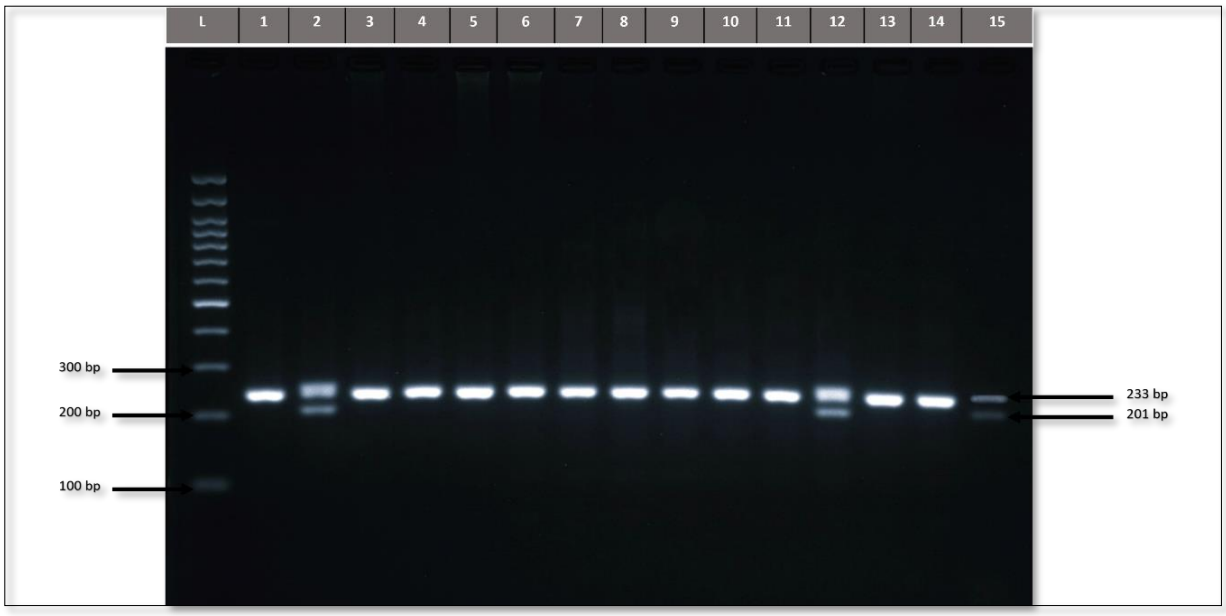

Figure 2. Agarose 2% Gel electrophoresis image for CCR5 gene showing SNP Δ32 bp
The lower band mutant sample with 201 bp while higher band 233 bp represent for wild sample

Our research is one of the first studies that studied the relationship between this SNP and PCOS, and we were preceded and rely by research done in University of Tabuk, Saudi Arabia, find out CCR5Δ32 (rs333) are possible loci for developing PCOS in the Tabuk residents. Their results reported the mutation between PCOS patients and matched controls was significant (p<0.0.0001) and they found the frequency of CCR5Δ32bp (rs333) genotypes in PCOS patients was CCR5Δ32 wild type (59.095%), *CCR5* heterozygous (Δ32+Δ32*) (31.81%), and *CCR5* homozygous (Δ32*) (0%) and for controls CCR5Δ32 (99%), *CCR5*(Δ32+Δ32*) (1%), and *CCR5*(Δ32*) (0%). Therefore, functional alterations in *CCR5* due to the Δ32 mutation could influence the inflammatory and metabolic profile in PCOS [33]

CCR5, a critical co-receptor for R5-tropic HIV entrance into host cells, the expression level of *CCR5* on $CD4^+$ T cells is a crucial factor in regulating and HIV infection predicting and progression, that HIV utilizes *CCR5*, expressed on T cells and macrophages, to facilitate viral entrance. The genetic variant CCR5Δ32 impairs the *CCR5* expression on the cell surface [34] heterozygosis CCR5Δ32 promotes a reduction in the functional *CCR5* expression on the cell surface compared to *CCR5* wild-type cells. Consequently, if heterozygous individuals infected with HIV, will have a small protection against HIV progression and homozygous individuals (Δ32/Δ32) expressed virtually total protection against HIV infection, since there is no *CCR5* expression is confirmed on cell surface [35] There are several meta-analyses of the association between rs333 and the risk of numerous autoimmune diseases, like susceptibility to systemic lupus erythematosus (SLE) [36] .

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

In the Iranian Turkmen population 400 randomly selected Turkmen populations that achieved the CCR5*Δ*32 polymorphism may be absent [37]. In Iraqi study stated that the *CCR5* gene mutant type was (0%) not detected in all study groups, on the other hand, the (CCR5-wild type) was(100%), in their study groups which indicates that CCR5Δ32 deletion has a significant negative correlation in the prediction of COVID-19 infection [38].

Conclusions

The findings of this search indicate that patients with polycystic ovary syndrome (PCOS) exhibit significant alterations in lipid profiles and hormonal levels compared to healthy controls. The CCR5-Δ32 polymorphism showed no significant association with PCOS in this study population with a higher frequency of the heterozygous genotype in the control group compared to PCOS patients. The remaining patients were predominantly of the wild-type genotype, with no homozygous (Δ32/Δ32) was detected.

Acknowledgments

The authors are grateful to their respective College of Biotechnology for their support.

Author's Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University/College of Biotechnology]. This approval underscores our commitment to ethical research practices and the well-being of our participants.
- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Biotechnology], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Author's Contribution Statement

Farah A. Abdulrazzaq: Contributed to the conception and design of the study, conducted some experiments, data rearrangement and drafted the initial manuscript.

Zina F.H. Al-Obaidi: conducted some experiments, collection a part of literature review and conducted some characteristics of the products.

Iraqi journal of Bioscience and Biomedical
Published Online First: 30/ June /2025.
p-ISSN: 3007-5491 / e-ISSN:3007-5505

References

1. Ravat, F. K., Goswami, J. R., Nair, S. M. & Thummar, K. N. A review of metabolic and microbial influences on women with polycystic ovarian syndrome. *Steroids*, 109512 (2024).
2. Singh, S. *et al.* Polycystic ovary syndrome: etiology, current management, and future therapeutics. *Journal of Clinical Medicine* **12**, 1454 (2023).
3. Zhu, T., Cui, J. & Goodarzi, M. O. Polycystic Ovary Syndrome and Risk of Type 2 Diabetes, Coronary Heart Disease, and Stroke. *Diabetes* **70**, 627-637, doi:10.2337/db20-0800 (2020).
4. Dason, E. S., Koshkina, O., Chan, C. & Sobel, M. Diagnosis and management of polycystic ovarian syndrome. *CMAJ* **196**, E85-E94 (2024).
5. Peng, J. *et al.* Integrated transcriptomic hypothalamic-pituitary-ovarian axis network analysis reveals the role of energy availability on egg production in layers. *Animal Nutrition*, doi:https://doi.org/10.1016/j.aninu.2024.08.009 (2024).
6. Crosbie, E. & Kenny, L. C. *Gynaecology by Ten Teachers*. (CRC Press, 2024).
7. Saadia, Z. Follicle stimulating hormone (LH: FSH) ratio in polycystic ovary syndrome (PCOS)-obese vs. non-obese women. *Medical Archives* **74**, 289 (2020).
8. Calcaterra, V. *et al.* Probiotics and polycystic ovary syndrome: a perspective for management in adolescents with obesity. *Nutrients* **15**, 3144 (2023).
9. Marbaniang, R. S., Singh, Y. A., Kshetrimayum, V., Naorem, S. & Rosyka, L. Study of lipid profile in polycystic ovarian syndrome: a case control study in tertiary care hospital. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology* **12**, 3561-3566 (2023).
10. Hiam, D. *et al.* The genetics of polycystic ovary syndrome: an overview of candidate gene systematic reviews and genome-wide association studies. *Journal of clinical medicine* **8**, 1606 (2019).
11. Jasinska, A. J., Pandrea, I. & Apetrei, C. CCR5 as a Coreceptor for Human Immunodeficiency Virus and Simian Immunodeficiency Viruses: A Prototypic Love-Hate Affair. *Front Immunol* **13**, 835994, doi:10.3389/fimmu.2022.835994 (2022).
12. Santana, D. S. *et al.* The Influence Between C-C Chemokine Receptor 5 Genetic Polymorphisms and the Type-1 Human Immunodeficiency Virus: A 20-Year Review. *AIDS Res Hum Retroviruses* **39**, 13-32, doi:10.1089/aid.2022.0111 (2023).
13. Panda, A. K., Padhi, A. & Prusty, B. A. K. CCR5 Δ32 minorallele is associated with susceptibility to SARS-CoV-2 infection and death: An epidemiological investigation. *Clinica chimica acta; international journal of clinical chemistry* **510**, 60 (2020).
14. FATHI, Z. H., YOUNUS, Z. M., MAHMOOD, S. M. & MOHAMMAD, J. A. Levels of adiponectin, malondialdehyde and lipid profile in women with polycystic ovary syndrome. *ACTA Pharmaceutica Sciencia* **62** (2024).
15. Al-Taie, F. K. & Al-Jawadi, Z. The impact of obesity on infertile women with polycystic ovaries in Iraq. *Raf J Sci* **28**, 1-9 (2019).
16. Zhou, X., Tian, Y. & Zhang, X. Correlation and predictive value of systemic immune-inflammation index for dyslipidemia in patients with polycystic ovary syndrome. *BMC Women's Health* **24**, 564 (2024).

17. Shallal, M. M., Mahmood, N. M. & Hussein, Z. A. Total L-carnitine and insulin resistance in non-obese and obese Iraqi women with polycystic ovary syndrome. *Journal of the Faculty of Medicine Baghdad* **65**, 20-26 (2023).

18. Shenta, A., Saud, K. & Al-Shawi, A. Assessment the correlations of hormones, lipid profiles, oxidative stress, and Zinc Concentration in Iraqi Women with Polycystic Ovary Syndrome. *Reports of Biochemistry & Molecular Biology* **9**, 270 (2020).
19. Hussein, S. R. M., Sadiq, A. M., Johar, S. A. & Nasrawi, A. J. M. Insulin level, lipid profile, and HOMA index in lean and obese patients with polycystic ovary syndrome. *Journal of Medicine and Life* **16**, 1258 (2023).
20. Mir, R. *et al.* Biochemical characterization and molecular determination of estrogen Receptor-α (ESR1 PvuII-rs2234693 T> C) and MiRNA-146a (rs2910164 C> G) polymorphic gene variations and their association with the risk of polycystic ovary syndrome. *International Journal of Environmental Research and Public Health* **19**, 3114 (2022).
21. Hajam, Y. A., Rather, H. A., Kumar, R., Basheer, M. & Reshi, M. S. A review on critical appraisal and pathogenesis of polycystic ovarian syndrome. *Endocrine and Metabolic Science* **14**, 100162 (2024).
22. Lonardo, M. S. *et al.* Hypothalamic-Ovarian axis and Adiposity Relationship in Polycystic Ovary Syndrome: Physiopathology and Therapeutic Options for the Management of Metabolic and Inflammatory Aspects. *Curr Obes Rep* **13**, 51-70, doi:10.1007/s13679-023-00531-2 (2024).
23. Sanchez-Garrido, M. A. & Tena-Sempere, M. Metabolic dysfunction in polycystic ovary syndrome: Pathogenic role of androgen excess and potential therapeutic strategies. *Molecular metabolism* **35**, 100937 (2020).
24. Su, P., Chen, C. & Sun, Y. Physiopathology of polycystic ovary syndrome in endocrinology, metabolism and inflammation. *Journal of Ovarian Research* **18**, 34, doi:10.1186/s13048-025-01621-6 (2025).
25. Alhassan, S. M., Elmugadam, A., Abubaker, N. E. & Elfadil, G. A. Testosterone level correlates significantly with luteinizing hormone to follicle-stimulating hormone ratio among women with polycystic ovary syndrome: A cross sectional study. *F1000Research* **11**, 152 (2022).
26. Sharif, Y. H. THE Polycystic ovaries syndrome prevalence in relation to body mass index among patients in AL-Diwaniyah city-Iraq: Polycystic ovaries syndrome prevalence in relation to body mass index among patients in AL-Diwaniyah city-Iraq. *AL-QADISIYAH MEDICAL JOURNAL* **19**, 80-84 (2023).
27. Sivanandy, M. S. & Ha, S. K. The Role of Serum Anti-Mullerian Hormone Measurement in the Diagnosis of Polycystic Ovary Syndrome. *Diagnostics* **13**, 907 (2023).
28. van der Ham, K. *et al.* Anti-Müllerian hormone as a diagnostic biomarker for Polycystic Ovary Syndrome (PCOS) and Polycystic Ovarian Morphology (PCOM): a systematic review and meta-analysis. *Fertility and sterility* (2024).

29. Abdul-Hakeem, A., Al-bayati, A. & abdulkader, m. POLYCYSTIC OVARY SYNDROME-BODY MASS INDEX: HORMONAL AND METABOLIC IMPLICATIONS. (2025).
30. Gorzko, A. *et al.* Evaluation of Anti-Müllerian Hormone (AMH) Serum Levels in Patients with Polycystic Ovary Syndrome (PCOS) Depending on Body Mass Index (BMI). *Journal of Clinical Medicine* **14**, 2677 (2025).
31. Al-Fartosy, A. J. M., Awad, N. A. & Mohammed, A. H. Intelectin-1 and endocrinological parameters in women with polycystic ovary syndrome: Effect of insulin resistance. *The Ewha Medical Journal* **43**, 1-11 (2020).
32. Kumar, N. & Agarwal, H. Early clinical, biochemical and radiological features in obese and non-obese young women with polycystic ovarian syndrome: a comparative study. *Hormone and Metabolic Research* **54**, 620-624 (2022).
33. Almasoudi, K. S. *et al.* Exploring the Association of Biochemical Characterization and Genetic Determinants of TNF-α, CXCR2, and CCR5 Delta 32 Mutation with Predisposition to Polycystic Ovary Syndrome. *Life* **14**, 949 (2024).
34. Yukselten, Y., Wishah, H., Li, J. A. & Sutton, R. E. Targeting CCR5: A central approach to HIV treatment and cure strategies. *Virology* **603**, 110375, doi:https://doi.org/10.1016/j.virol.2024.110375 (2025).
35. Ellwanger, J. H., Kaminski, V. d. L., Rodrigues, A. G., Kulmann-Leal, B. & Chies, J. A. B. CCR5 and CCR5Δ32 in bacterial and parasitic infections: Thinking chemokine receptors outside the HIV box. *International Journal of Immunogenetics* **47**, 261-285 (2020).
36. Mlicka, D. *et al.* Variability of the rs333 in Polish patients with lupus erythematosus. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii* **38**, 131-136 (2021).
37. Norasi, E., Rastegar, M., Hosseini, S. D., Aghcheli, B. & Tahamtan, A. Prevalence of CCR5 Delta 32 Genetic Variant in the Turkmen Population of Golestan Province, Northeast of Iran. *Biomed Res Int* **2023**, 8823863, doi:10.1155/2023/8823863 (2023).
38. Altaie, Z. & Salman, J. *The clinical impact of chemokine receptor CCR5 Δ32 mutation in SARS- CoV-2 infected patients*. (2024).