Review / Derleme

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Individual effects of GSTM1 and GSTT1 polymorphisms on the risk of polycystic ovarian syndrome: A systematic review and meta-analysis

GSTM1 ve GSTT1 polimorfizmlerinin polikistik over sendromu riski üzerindeki etkileri: Sistematik bir inceleme ve meta-analiz

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Abstract

This study aimed to understand the relationship between two specific genetic variations (GSTT1 and GSTM1 polymorphisms) and the risk of developing polycystic ovarian syndrome (PCOS). PCOS is a common endocrinologic disorder that affects women. Oxidative stress may play a significant role in the development of PCOS. Certain enzymes, such as glutathione S-transferases, help protect cells against oxidative stress. However, previous research on the correlation between these specific genetic variations and PCOS risk has produced inconsistent findings. To address this, a meta-analysis was conducted to examine the potential impact of these genetic variations on PCOS. We conducted a thorough search of the Embase, PubMed, Scopus, Web of Science, and Google Scholar databases to find studies that met our criteria. We used fixed-effects or random-effects models to determine the pooled odds ratios (ORs) and 95% confidence intervals (CIs) of the GSTT1 and GSTM1 polymorphisms related to PCOS. We also performed subgroup analyses based on ethnicity, mean age of participants, and PCOS diagnostic protocols. After screening, we found five studies with 1.607 participants (872 in the PCOS group and 735 in the control group) to be suitable for our meta-analysis. Our analysis showed that GSTM1 and GSTT1 null genotypes were not linked to an increased risk of PCOS (OR: 0.925, 95% CI: 0.755-1.134; OR: 1.175, 95% CI: 0.614-2.247 respectively). Additionally, both Begg's and Egger's tests revealed no publishing bias. This meta-analysis confirmed that there is no association between GSTM1 and GSTT1 polymorphisms and an increased risk of PCOS. However, further studies are required to validate this conclusion.

Keywords: GSTM1, GSTT1, polymorphism, polycystic ovarian syndrome

Öz

Bu çalışmada, iki spesifik genetik varyasyon (GSTT1 ve GSTM1 polimorfizmleri) ile polikistik over sendromu (PKOS) gelişme riski arasındaki ilişkiyi anlamayı amaçladık. PKOS kadınları etkileyen yaygın bir endokrinolojik hastalıktır. Oksidatif stres PKOS gelişiminde önemli bir rol oynayabilir. Glutatyon S-transferazlar gibi belirli enzimler hücrelerin oksidatif strese karşı korunmasına yardımcı olur. Bununla birlikte, bu spesifik genetik varyasyonlar ile PKOS riski arasındaki korelasyona ilişkin önceki araştırmalar tutarsız bulgular ortaya çıkarmıştır. Bu konuyu ele almak için, bu genetik varyasyonların PKOS üzerindeki potansiyel etkisini incelemek üzere bir meta-analiz yapıldı. Kriterlerimize uyan çalışmaları bulmak için Embase, PubMed, Scopus, Web of Science ve Google Scholar veritabanlarında kapsamlı bir araştırma yaptık. PCOS ile ilgili GSTT1 ve GSTM1 polimorfizmlerinin havuzlanmış olasılık oranlarını (OR'ler) ve %95 güven aralıklarını (GA'lar) belirlemek için sabit etkiler veya rastgele etkiler modelleri kullandık. Ayrıca etnik kökene, katılımcıların ortalama yaşına ve PKOS teşhis protokollerine göre alt grup analizleri de yaptık. Tarama sonrasında 1.607 katılımcıyla (PKOS grubunda 872 ve kontrol grubunda 735) beş çalışmanın meta-analizimize uygun olduğunu gördük. Analizimiz, GSTM1 ve GSTT1 null genotiplerinin artan PKOS riskiyle bağlantılı olmadığını gösterdi (sırasıyla OR: 0,925, %95 GA: 0,755-1,134; OR: 1,175, %95 GA: 0,614-2,247). Ek olarak hem Begg's hem de Egger's testleri herhangi bir yayın yanlılığı ortaya çıkarmadı. Bu meta-analiz, GSTM1 ve GSTT1 polimorfizmleri ile artan PKOS riski arasında bir ilişki olmadığını doğruladı. Ancak bu sonucu doğrulamak için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: GSTM1, GSTT1, polimorfizm, polikistik over sendromu

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Introduction

Women of childbearing age with polycystic ovarian syndrome (PCOS) experience various clinical symptoms because of this complex endocrine disorder[1]. The condition is characterized by hyperandrogenism, ovarian dysfunction, and enlarged ovaries with multiple 2-9-mm- sized follicles[2,3]. According to the World Health Organization, 3.4% of women worldwide have PCOS, which amounts to over 116 million women[4].

The cause of PCOS is not fully understood, but researchers believe it results from a combination of genetic and environmental factors, including obesity, lifestyle, ovarian dysfunction, hypothalamic-pituitary abnormalities, and oxidative stress[5]. PCOS is widely recognized as the primary contributor to menstrual irregularity, resulting in subfertility[6]. Numerous studies have provided evidence regarding the influence of genetic factors on women's embryonic development and subfertility[7,8]. Some genetic conditions may prevent fertility or improve the effectiveness of treatments for subfertility; therefore, research in this field remains highly intriguing[7,9].

Oxidative stress, acknowledged to have a crucial impact on the pathophysiology of PCOS, is an inequality between oxidants and antioxidants within cells[10]. This condition causes the accumulation of free radicals such as reactive oxygen species (ROS) and peroxides in the cells and DNA damage[11]. Intricate enzymatic and non-enzymatic antioxidant systems regulate ROS production within cells. Among these systems, the enzyme GST plays an essential role in the detoxification of ROS and in defending against oxidative stress and tissue damage[12].

In the human species, GST enzymes comprise eight distinct classes, classified according to their amino acid sequences. These classes include GSTA, GSTM, GSTP, GSTT, GSTK, GSTZ, GSTO, and GSTσ[13]. Epidemiological research has demonstrated that deletion polymorphisms of the genes *GSTM1* and *GSTT1* are prevalent within human populations and have been broadly studied[14].

At present, a limited quantity of research exists investigating the correlation between genetic polymorphisms of GSTT1 and GSTM1 and PCOS. Despite the importance of *GSTM1* and *GSTT1* gene variations and some discrepancies found in previous research, this study seeks to perform a meta-analysis of the data available to determine the influence of these genetic polymorphisms on PCOS.

Materials and Methods

Design and Search Strategy

The investigation followed the PRISMA guidelines for systematic reviews and meta-analyses[15]. A thorough search was conducted on various databases, including Embase, PubMed, Scopus, and Web of Science, to identify eligible studies that explored the possible association between GSTM1 present/null and GSTT1 present/null with PCOS risk. The studies were published up to September 2023. In addition, relevant studies were manually searched on 30 pages of Google Scholar, and the references of the selected articles were carefully checked to identify any further relevant publications. Language restrictions were not applied during the search, and the following keywords were used: ("Glutathione S-transferase" or "GST", "GSTM1" or "GSTT1") and ("Polymorphism*") and ("Polycystic ovarian syndrome" or "PCOS").

Inclusion and Exclusion Criteria

This study focused on research that met specific criteria, including the use of case-control or cohort study designs, the provision of genotype data or odds ratio (OR) with a 95% confidence interval (CI), and the examination of the correlation between GSTM1 and/or GSTT1 polymorphisms and the risk of PCOS. Studies that contained overlapping data, case reports, editorials, reviews, letters, and meta-analyses or did not provide adequate data were not included.

Data Extraction and Quality Assessment

Two authors independently extracted and reviewed the data from each study included in the meta-analysis. The extracted data are presented in Tables 1 and 2. The quality of each case-control study was assessed using the Newcastle-Ottawa Quality Assessment Scale (NOS)[16], as shown in Table 2, with a maximum total score of nine. Articles with a NOS score of seven or higher were considered high quality, whereas those with scores ranging from 5 to 7 were moderate quality.

Table 1. Basic information about the included studies

First author	Year	Country	Ethnicity	Genotyping method	Cases mean age	Controls mean age	PCOS diagnosis protocol
Babu et al.[17]	2004	India	Asian	PCR	26.5	26.4	Sonography and ultrasound scan
Savić-Radojević et al.[18]	2018	Serbia	European	PCR	16.3	16.7	2003 Rotterdam criteria
Chung et al.[19]	2020	Korea	Asian	PCR	-	-	2003 Rotterdam criteria
Azevedo et al.[20]	2020	Brazil	South-American	PCR	26.0	31.0	2003 Rotterdam criteria
Alves et al.[21]	2020	Portugal	European	PCR	33.0	31.0	Amsterdam ESHRE/ARSM-Sponsored 3rd PCOS Consensus

PCR: Polymerase chain reaction, PCOS: Polycystic ovarian syndrome

Table 2. The scores were related to the quality assessment of the eligible studies and the details regarding the included patient and control groups

First author (year)	NOS score	Case number	Control number	Case GSTM1		Control GSTT1	
				Present	Null	Present	Null
Babu et al.[17]	7	180	72	151	29	60	12
Savić-Radojević et al.[18]	8	35	17	15	19	10	7
Chung et al.[19]	7	478	376	252	226	184	193
Azevedo et al. [20]	8	110	109	129	90	60	50
Alves et al.[21]	8	69	161	34	35	89	72

NOS: Newcastl-Ottawa quality assessment scale

Statistical Analysis

In this study, we used CMA 3.0 software developed by Biostat, USA to analyze our data. Our goal was to investigate the correlation between GSTM1 and GSTT1 polymorphisms and PCOS. To determine statistical significance, we calculated ORs and 95% CIs. A p-value 0.05 indicated a significant result. We also evaluated the presence of interstudy heterogeneity using I2 and p-values. A value of I^2>50% or Q statistic test indicated heterogeneity, whereas a p-value greater than 0.10 for the Q statistic indicated its absence. We used either a random-effects model or a fixed-effects model depending on the presence or absence of heterogeneity In addition, we conducted a meta-regression analysis to explore the effects of participants' age, the method of PCOS diagnosis, and ethnicity. To ensure the reliability of our findings, we conducted a sensitivity analysis. Finally, we assessed potential publication bias using Egger's regression analysis and Begg's funnel plot.

Results

Study Characteristics

Tables 1 and 2 provide a clear summary of the clinical and demographic characteristics of the patients included in this study. The investigation consisted of five case-control studies, with 872 cases and 735 controls. The primary focus of this study was to explore the relationship between GSTM1 and GSTT1 null deletion polymorphisms and the risk of PCOS[17-21]. Table 1 shows that two studies were conducted in Asia, two in Europe, and one in South America. Figure 1 depicts a flow diagram that follows the PRISMA guidance for the literature review[15].

Quantitative Synthesis

Based on statistical analysis, it was found that the absence of the *GSTM1* gene was not significantly linked with PCOS (OR: 0.925, 95% CI: 0.755-1.134) as shown in Figure 2. Similarly, the absence of GSTT1 did not significantly increase the risk of PCOS (OR: 1.175, 95% CI: 0.614-2.247) as shown in Figure 2.

Heterogeneity Test

Table 3 shows that there was variation in the GSTT1 variate between studies, indicating heterogeneity. To investigate this further, subgroup analyses were conducted to examine the

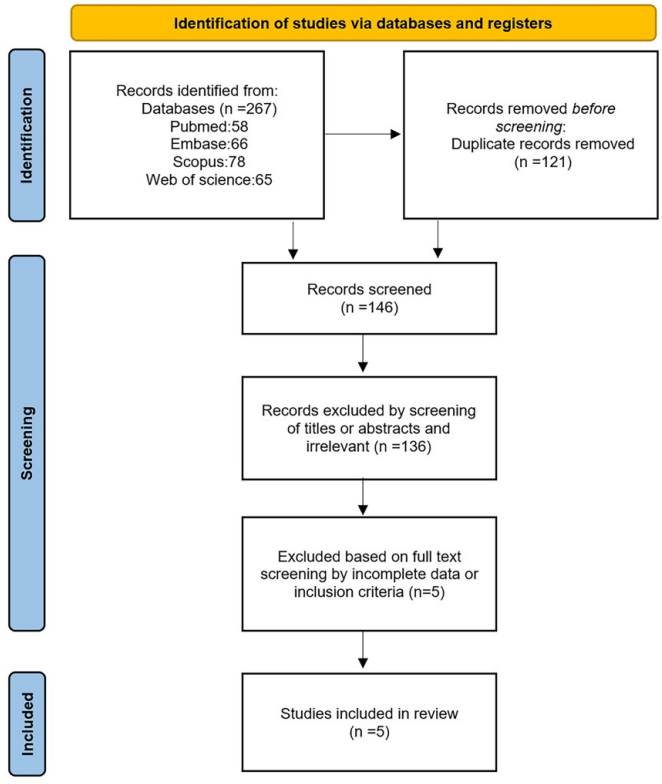

Figure 1. Flowchart of the search strategy

impact of different ethnic groups and PCOS diagnostic protocols on the results of the meta-analysis. However, no significant differences were found among the investigated ethnic groups (p=0.625) and PCOS diagnostic protocols (p=0.458), indicating that they were not the cause of the heterogeneity. Additionally, inter-study heterogeneity in relation to GSTM1 was found to be statistically insignificant (Table 3). Furthermore, the effect of the mean age of study participants on the meta-analysis results for GST1 was investigated using a meta-regression method, and the results showed that no changes were not statistically significant (p=0.1928).

Sensitivity Analysis

We conducted a sensitivity analysis to evaluate how each study affected the results of our meta-analysis. We excluded

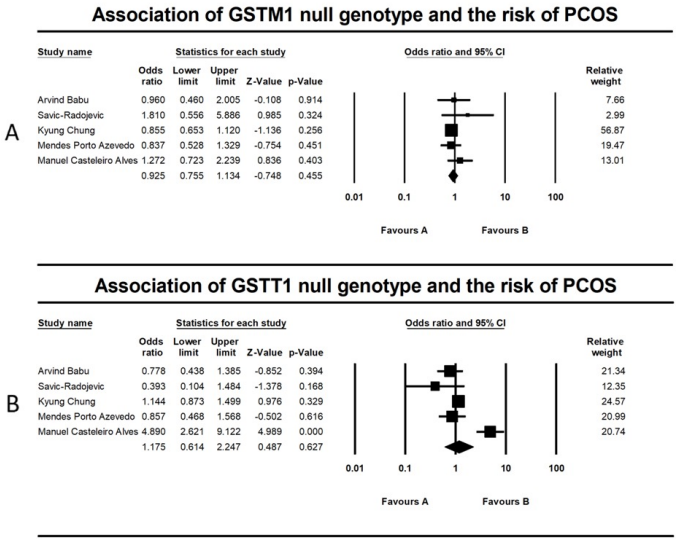

Figure 2. Forest plots of the pooled odds ratios indicating the risk of PCOS related to the null genotypes of GSTM1 (A) and GSTT1 (B)

PCOS: Polycystic ovarian syndrome, CI: Confidence interval

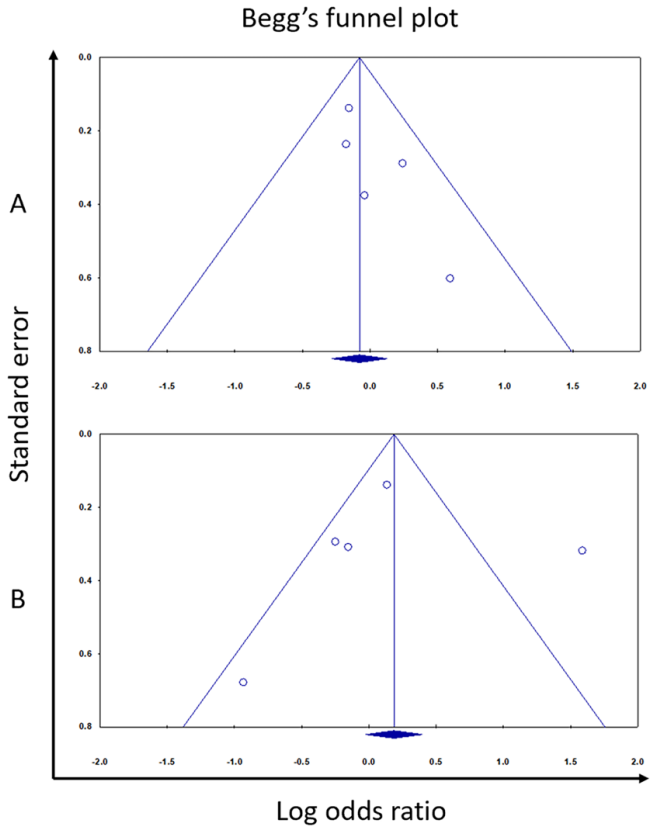

Figure 3. Begg's funnel plots for publication bias test of the studies that investigated the polymorphisms of GSTM1 (A) and GSTT1 (B)

Table 3. Heterogeneity analysis of the investigated studies

Variables	I2	Q-test's p-value	p-value of Begg's test	p-value of Egger's test
GSTM1	0.000	0.561	0.220	0.123
GSTT1	84.42%	<0.001	0.806	0.944

one study at a time and found that the combined odd ratios remained consistent. This highlights the statistical reliability of our findings.

Publication Bias Analysis

We created funnel plots (see Figure 3) to check for publication bias. Additional investigation using Egger's regression and Begg's rank correlation tests revealed no significant publication bias for GSTM1 (p=0.220; p=0.123) and GSTT1 (p=0.806; p=0.944). However, because the funnel plot for GSTT1 appears uneven, we used the non-parametric "trim and fill" test to correct for potential publication bias, but the results did not change (data not shown).

Discussion

The enzyme GST is a member of the supergene family of phase II metabolic enzymes, holds a pivotal position in detoxification processes, and plays a fundamental role in decreasing oxidative stress[22]. The most important isozymes are GSTM1 and GSTT1, which exhibit a high degree of polymorphism[23]. Several studies have presented evidence indicating that null genotypes of GSTM1 and GSTT1 may be associated with an increased risk of female reproductive system diseases as well as PCOS[24-27]. Nevertheless, the outcomes of the studies related to PCOS have not been systematically summarized and analyzed, and the overall effect remains uncertain.

This study did not reveal any statistically significant correlation between the null genotypes of GSTM1 and GSTT1 and the occurrence of PCOS. The findings of our investigation agree with those of most prior studies. Babu et al.[17], Savić-Radojević et al.[18], Chung et al.[19], and Azevedo et al.[20] are similar to the results of our study. We could not find an association between the polymorphisms of GSTM1 and GSTT1 and increased risk of PCOS. Contrary to the present study's findings, Alves et al.[21] reported a significant correlation between the null genotype of GSTT1 and susceptibility to PCOS. The most probable and reasonable explanation for the incongruous outcomes of Alves's investigation was its limited sample size, which could have led to diminished statistical potency. In another study conducted by Başkıran et al.[27], the serum level of GST was investigated, and their findings, as opposed to our study, indicated a significant reduction in the serum level of GST among patients diagnosed with PCOS compared with healthy controls. The observed dissimilarity may be attributed to the lack of examination of different GST isozymes in Başkıran's study[27], and the reduction in GST expression may be ascribed to the decline

in inoenzymes other than GSTM1 and GSTT1. The significant correlation observed between *GSP01* gene polymorphism and susceptibility to PCOS in the study by Miraghaee et al.[28] proves this claim.

After considering the overall heterogeneity, we proceeded to conduct a subgroup analysis. The results of the subgroup analysis on GSTT1 single null genotype polymorphism indicated that none of the investigated populations and PCOS diagnostic protocols did not significantly change the overall results. Accordingly, the potential source of heterogeneity is associated with sampling errors or variables other than ethnicity, diagnostic protocols, and age. However, to comprehensively scrutinize heterogeneity, further investigations should be conducted across diverse ethnic groups in the future.

This study presents several advantages. The present meta-analysis investigates the impact of null genotypes of GSTM1 and GSTT1 on the risk of PCOS for the first time. Notably, the studies incorporated in our meta-analysis were meticulously chosen based on stringent inclusion and exclusion criteria. As a result, by using high-quality articles, the credibility of our research findings was enhanced. There are some limitations that must be acknowledged, such as the limited number of primary original studies that meet the criteria for review. This poses a significant limitation in assessing interactions between genes and the environment in the progression of PCOS.

Conclusion

This meta-analysis showed that null genotypes of GSTM1 and GSTT1 may not increase PCOS risk. However, further investigations are recommended to confirm these findings.

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Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: M.H.M., S.F., Design: M.H.M., S.F., Data Collection or Processing: R.H.M., N.A., Analysis or Interpretation: M.H.M., S.F., Literature Search: M.H.M., Writing: M.H.M., S.F.

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