Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.12.03.Art063

Exploring the Genomic Effects of Pioglitazone on Skeletal Muscle in Polycystic Ovary Syndrome

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Abstract

This study examines the molecular effects of pioglitazone on the skeletal muscle tissue of women who have polycystic ovary syndrome (PCOS), with an emphasis on the medication's ability to improve insulin sensitivity and lower inflammation. Differentially expressed genes (DEGs) that highlight important biological processes and pathways altered by pioglitazone, such as the cytokine-cytokine receptor interactions and PI3K-Akt signalling pathway, were found using gene expression profiling. To predict treatment response and serve as targets for future pharmaceutical development, the study identified hub genes like ESR1 and KRAS as key participants in these pathways. These results highlight the complex function that pioglitazone plays in controlling inflammatory and metabolic processes, which are essential for the management of PCOS. Although the study has several merits, such as the thorough molecular analysis, one drawback is the rather small sample size, which may limit how broadly the results may be applied. Prospective investigations have to concentrate on verifying our findings in more extensive cohorts, examining the clinical significance of the detected biomarkers, and carrying out mechanistic analyses to gain a deeper comprehension of Pioglitazone's impacts. This work advances our knowledge of the molecular mechanisms underlying pioglitazone's effects in PCOS, paving the way for the creation of more individualized and potent treatment plans that will eventually improve patient outcomes.

Keywords: *Gene Expression, Inflammation, Personalized Medicine, Pioglitazone, PI3K-Akt Pathway, Polycystic Ovary Syndrome.*

Introduction

About 10% of women who are of reproductive age have Polycystic Ovary Syndrome (PCOS), an endocrine disorder marked by symptoms like insulin resistance, hyperandrogenism, and irregular menstrual periods [1]. Complex genetic, metabolic, and environmental variables all have a role in the pathophysiology of PCOS and contribute to its expression [2]. Insulin resistance is a major side effect of PCOS that is made worse by changes in muscle metabolism. Insulin resistance raises the risk of type 2 diabetes and cardiovascular illnesses in addition to exacerbating metabolic abnormalities in PCOS patients [3]. Treatment options for PCOS patients with insulin resistance have historically included medication, lifestyle changes, and, more recently, insulin-sensitizing medications such as pioglitazone [4].

Received: 21.08.2024 ***Accepted: 11.09.2024*** ***Published on: 30.09.2024***
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Pioglitazone, a drug in the thiazolidinedione class, has been studied for its potential to improve insulin sensitivity by acting on muscle tissue in addition to other targets [5]. Furthermore, studies have shown that pioglitazone may be useful in lowering fasting insulin levels and enhancing insulin-stimulated glucose excretion in PCOS-affected women [6]. The capacity of pioglitazone to regulate several metabolic pathways, especially those connected to the metabolism of glucose in muscle tissue, sets it apart from other therapies now on the market [7]. There are still unanswered questions regarding the long-term safety and effectiveness of pioglitazone as well as the variations in patient response caused by genetic and environmental factors [8].

To fill in these gaps, this study investigates the molecular effects of pioglitazone on the muscle tissue of PCOS-affected women. This study attempts to pinpoint certain gene expression alterations that underpin the medication's therapeutic benefits by using gene expression profiling from the Gene Expression Omnibus (GEO) dataset GSE8157, which contains information from muscle biopsies obtained before and after pioglitazone treatment [9].

This work is unique because it focuses on the molecular pathways that pioglitazone uses to affect skeletal muscle, which is an essential site for insulin-mediated glucose uptake. In the skeletal muscle of insulin-resistant PCOS women, prior research has shown decreased expression of genes related to mitochondrial oxidative phosphorylation (OXPHOS) [10]. This study explores the changes in gene expression that take place in muscle tissue, which may lead to the discovery of new therapeutic targets. Previous research focused mostly on the clinical results of pioglitazone treatment [11]. This work intends to improve patient outcomes by addressing the metabolic abnormalities associated with PCOS through more effective and tailored treatment regimens by clarifying these biological pathways [12].

Materials and Methods

Data Preparation and Organization

The National Center for Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO) database provided the gene expression data for this investigation. To be more precise, the dataset GSE8157 was obtained [13]. It contains the gene expression patterns from muscle biopsy samples taken from women with PCOS both before and after pioglitazone treatment. Muscle samples from PCOS patients both before and after pioglitazone treatment are included in the collection, along with comparisons to healthy controls [14].

Differentially Expressed Genes (DEGs) Identification

GEO2R online analysis tool provided by GEO, the DEGs between the sample groups (PCOS control vs. PCOS following pioglitazone treatment) were found [15]. Genes that exhibit differential expressions under various experimental circumstances can be found by comparing many sample groups using GEO2R. For p-value correction, the Benjamini-Hochberg (False Discovery Rate) approach [16] and the Benjamin and Yakutieli method [17] were chosen to manage the false discovery rate and consider possible gene correlations. To graphically illustrate the DEGs and highlight those with notable expression changes, a volcano plot was created using GEO2R [13].

Network Construction for Protein-Protein Interaction (PPI)

The software Cytoscape was utilized to investigate the discovered DEGs for possible protein-protein interactions (PPI) [18]. Molecular interaction networks can be seen with the popular open-source program Cytoscape. To build the PPI network, the known and expected interactions between the identified proteins were integrated using the Cytoscape STRING database plugin [19].

Identification of Hub Genes

The CytoHubba plugin was used in Cytoscape to find hub genes in the PPI network, which are important nodes with a high level of interaction [20]. The CytoHubba plugin was used to choose hub genes. The three-centrality metrics including maximum clique centrality (MCC), degree centrality, and closeness centrality were used to pinpoint the most important genes in the interaction network that may be crucial to the pathophysiology of PCOS and the reaction to pioglitazone therapy can be identified with the use of these metrics [21].

The KEGG Pathway Enrichment Analysis with Gene Ontology (GO)

SR Plot was used to conduct pathway enrichment studies for GO and Kyoto *Encyclopaedia* of Genes and Genomes (KEGG) to obtain insights into the biological processes, molecular functions, and pathways related to the identified DEGs [22, 23]. Extensive enrichment analysis is possible with this bioinformatics tool. The DEGs' functional roles were revealed by the GO analysis results, and the major metabolic and signalling pathways in which these genes are engaged were found by the KEGG pathway analysis [23]. The enrichment analysis results were visualized to highlight the most significant GO terms and pathways [21].

Results

Identification of DEGs

The difference in gene expression between control and post-Pioglitazone (Pio) treatment conditions is shown visually in the volcano plot (Figure 1). The log2 fold change in gene expression is represented by the x-axis, and the -log10 adjusted p-value (Padj) is displayed on the y-axis. Red dots indicate genes with significantly higher expression levels following Pio treatment when compared to the control. With a Padj < 0.05 and a log2 fold change larger than 0, these genes are situated on the right side of the volcano plot. Table 1 lists the top 10 highly elevated genes from the investigation, which revealed several important upregulated genes.

Blue dots indicate genes that significantly decreased expression after receiving Pio therapy. These genes have a Padj < 0.05 and a log2 fold change less than 0, placing them on the left side of the volcano plot. Table 2 provides an overview of the top 10 downregulated genes. Black indicates genes with no discernible changes in expression (Padj ≥ 0.05). These genes do not match the criteria for differential expression and are centred around the volcano plot's origin.

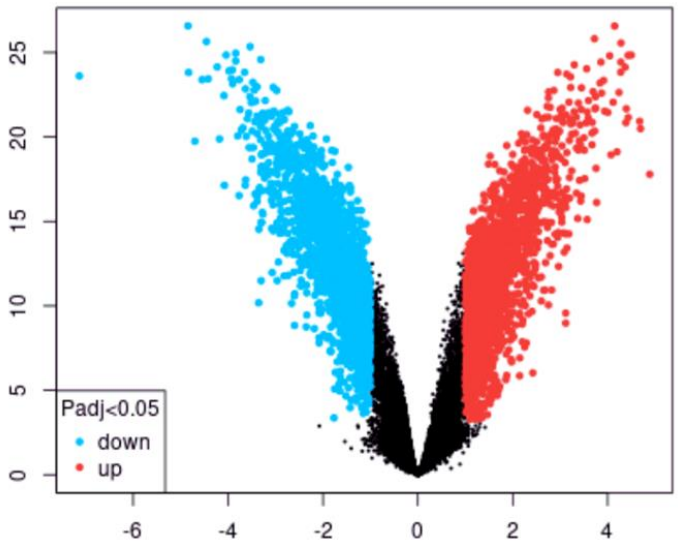

Figure 1. Volcano plot – GSE8157: Control vs After Pioglitazone.

Table 1. Top 10 Upregulated Genes

ID	Gene Symbol	Log2(fold change)	Log p-value
231586_at	SPATA42	4.676	20.926
1555437_at	EARS2	4.434	21.153
222259_s_at	SPO11	4.386	20.845
220237_at	ATG3	4.369	24.131
215019_x_at	ZNF528	4.275	23.829
1569973_at	SEPT7P2	4.24	22.632
232188_at	AKAP13	4.198	19.115
207952_at	IL5	4.071	18.939
232992_at	SAYSD1	3.914	23.344
233423_at	TLDC1	3.872	22.594

Table 2. Top 10 Downregulated Genes

ID	Gene Symbol	Log2(fold change)	Log p-value
1561411_at	LINC01222	-4.832	23.817
242077_x_at	MB21D1	-4.7	19.743
235604_x_at	ZNF493	-4.55	23.385
215686_x_at	TFAP2B	-4.086	22.443
241631_at	ARHGEF40	-4.078	17.134
203755_at	BUB1B	-4.041	24.843
240065_at	FAM81B	-3.928	23.445
1557900_at	SIM2	-3.911	23.97
237408_at	DCUN1D1	-3.904	23.153
224341_x_at	TLR4	-3.84	24.497

Network of Protein-Protein Interaction (PPI) and Identification of Hub Genes

After the data were pre-processed, 2,291 genes were identified and prepared for Cytoscape analysis to look at protein-protein interactions. The Cytoscape tool was used to create a PPI network based on the genes that showed differential expression. The network provides insights into the interactions between the proteins encoded by these genes and identifies hub genes that are crucial to the organization and functionality of the network.

Genes and proteins are represented as nodes in the PPI network, which is shown in Figures 2A, 2B, and 2C, and their interactions are represented as edges. MCC, Degree, and Closeness are the three centrality criteria that were used to determine the top ten hub genes. Hub genes are frequently engaged in important biological processes and are essential for preserving the integrity of the network. Table 3 lists the hub genes determined by MCC, Degree, and Closeness centrality scores.

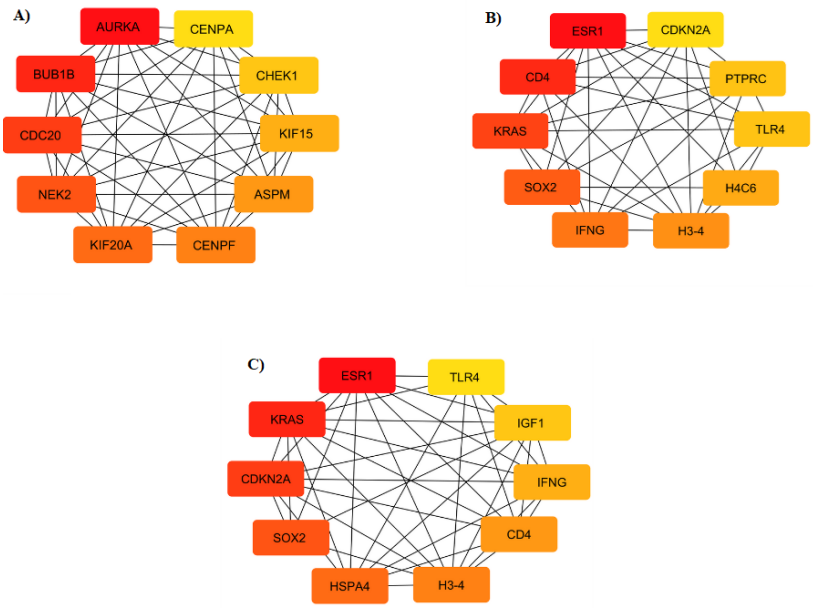

Figure 2. Centrality Criteria A) MCC, B) Degree, and C) Closeness, were Used to Determine the Top Ten Hub Genes.

Pathway Analysis using KEGG and GO.

In the context of PCOS, GO and KEGG pathway analyses were carried out to obtain a deeper knowledge of the biological activities and pathways associated with the DEGs. GO analysis provided insights into the biological processes, molecular functions, and cellular components associated with the DEGs. Significant enrichment in processes like inflammatory response, cell cycle regulation, and apoptotic process was found by the GO Biological Process analysis (Figure 3A). These processes have previously been linked to the pathophysiology of PCOS, where inflammation and dysregulation of cellular processes are important contributing factors. The overrepresentation of functions including enzyme activity, DNA binding, and transcription factor activity was revealed by the GO Molecular Function (MF) analysis (Figure 3B), which reflects underlying molecular mechanisms that contribute to the onset and progression of PCOS. Furthermore, these DEGs were found in key cellular regions such as the cytoplasm, membrane, and nucleus by the GO Cellular Component (CC) analysis (Figure 3C). These findings indicate that these proteins play important roles inside the cell and are relevant in the context of PCOS pathophysiology.

Table 3. Top 10 Hub Genes by Centrality Measures

Gene Symbol	MCC Rank	Degree Rank	Closeness Rank
ESR1	1	1	1
CDKN2A	2	3	2
KRAS	3	2	3
TLR4	4	4	4
CD4	5	5	6
SOX2	6	7	7
IFNG	7	6	5
HSPA4	8	9	8
H3-4	9	8	10
IGF1	10	10	9

Numerous relevant pathways with considerable enrichment among the DEGs were found by KEGG pathway analysis (Figure 4). Specifically, the well-known PI3K-Akt signalling system is essential to the endocrine and metabolic abnormalities linked to PCOS since it regulates cell proliferation, survival, and metabolism. Additionally, there was a high enrichment in the Cytokine-Cytokine Receptor Interaction pathway, which may have a role in regulating immunological responses and inflammation. These two factors are frequently linked to PCOS. Furthermore, the identified Cell Cycle pathway suggests that the regulation of cell proliferation and apoptosis may be involved in the pathogenesis of PCOS.

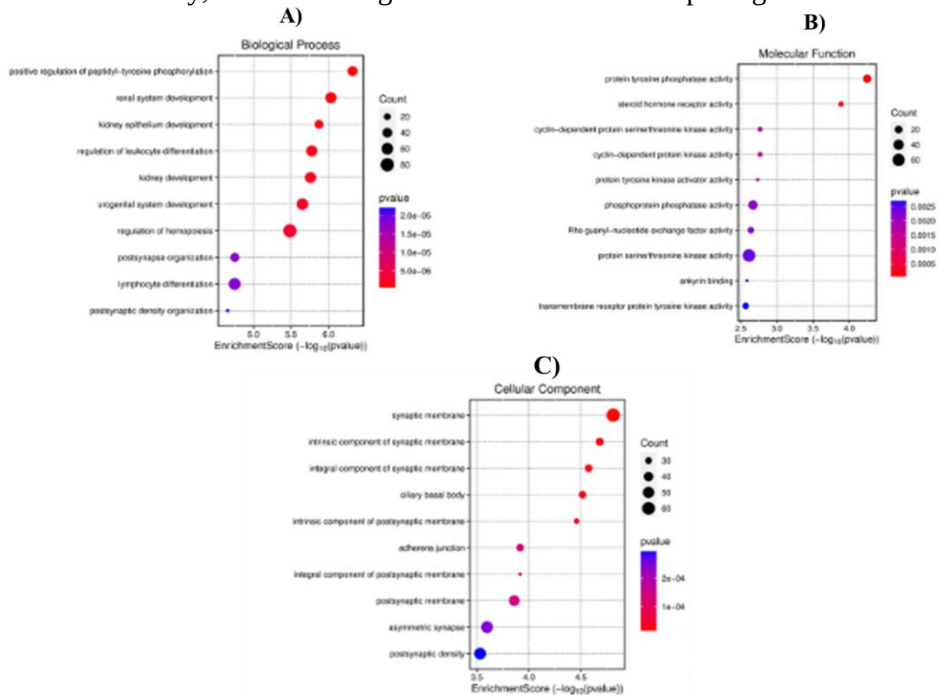

Figure 3. A) Biological Process Cnet Plot, B) Molecular Function Cnet Plot, C) Cellular Component Cnet Plot.

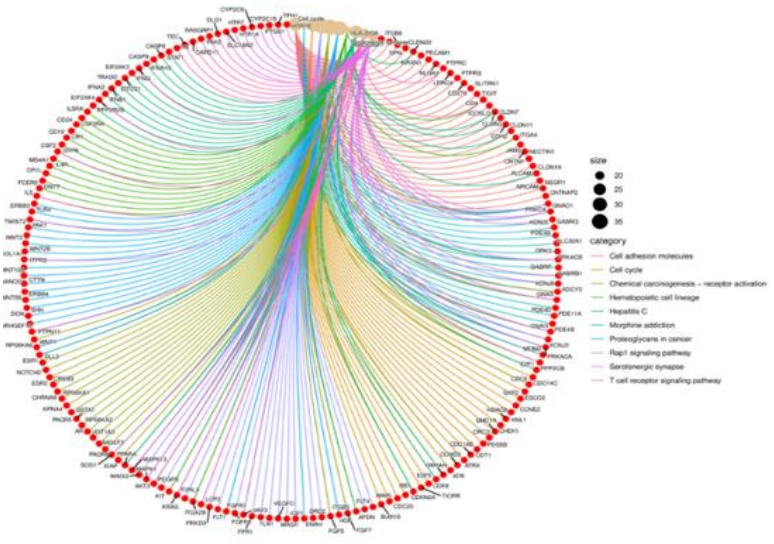

Figure 4. KEGG pathway Cnet plot.

Discussion

The results of this study greatly contribute to the broader understanding of gene expression patterns in PCOS, especially concerning the effects of pioglitazone therapy. The complicated molecular mechanisms behind PCOS are highlighted by the discovery of DEGs and the subsequent enrichment of biological processes and pathways. Pioglitazone alters gene expression to improve insulin sensitivity and lower inflammation, two crucial aspects of PCOS treatment. This work offers insights into this process by identifying essential pathways, such as the cytokine-cytokine receptor interactions and PI3K-Akt signalling pathway. These results provide a molecular basis for the therapeutic effects of Pioglitazone, thereby enhancing our understanding of its role in PCOS treatment [1, 24].

The study's main goal was to investigate the molecular effects of pioglitazone on PCOS-affected women's muscle tissue. The study specifically sought to identify the precise changes in gene expression that account for pioglitazone's therapeutic advantages. Along with the identification of significant DEGs, other enriched pathways found include the cytokine-cytokine receptor interactions and PI3K-Akt signalling pathway. To improve insulin sensitivity and lessen the metabolic abnormalities connected to PCOS, pioglitazone is believed to modify certain cellular pathways [25, 26].

The results of this study are consistent with previous reports emphasizing the roles of dysregulated cellular processes, inflammation, and insulin resistance in PCOS. The outcomes of this study also corroborate earlier discoveries regarding the enrichment of the inflammatory response and pathways involving interactions between cytokines and their receptors [27, 28]. Additionally, research indicates that in PCOS patients, the PI3K-Akt signalling pathway is critical for regulating metabolic processes [14]. However, the identification of a few hub genes, such as KRAS and ESR1, offers fresh perspectives on the pathophysiology of PCOS and suggests that these genes may have more functions than previously believed, particularly in response to pioglitazone [18, 29].

Personalized therapy approaches can be directed by identifying the genes and pathways that pioglitazone medication modulates. Pioglitazone, for instance, maybe more beneficial to patients with gene expression profiles, which could result in improved clinical outcomes. Moreover, knowledge of Pioglitazone's effects on the cytokine-cytokine interactions and PI3K-Akt signalling pathway can help with the selection of combinatorial therapy, especially for patients with chronic inflammation or severe insulin resistance. These results also demonstrate the possibility of employing gene expression profiles as biomarkers to anticipate a patient's reaction to pioglitazone, hence enabling more individualized treatment strategies [21, 30].

Pioglitazone appears to work across several important biological pathways, based on the observed alterations in gene expression. Pioglitazone may improve insulin sensitivity by enhancing cell growth, survival, and glucose metabolism, as shown by the overexpression of genes implicated in the PI3K-Akt signalling pathway [15]. On the other hand, Pioglitazone may have an anti-inflammatory impact, as suggested by the downregulation of genes linked to the inflammatory response [16]. This could help lessen the chronic low-grade inflammation that PCOS patients experience. Pioglitazone medication has a considerable impact on the regulation of the cell cycle and apoptosis, according to the analysis of pathways and networks. By modifying the expression of important regulatory genes like CDC20 and BUB1B, pioglitazone may be able to correct the dysregulated cell proliferation seen in PCOS, according to the enrichment of the cell cycle pathway [13]. Pioglitazone may affect cell survival pathways, potentially lowering the probability of hyperplasia or other

proliferative disorders linked to PCOS, as evidenced by the apoptotic pathway's participation [19].

DEGs are functionally annotated with links to biological processes by KEGG pathway enrichment analysis and GO. According to the GO Molecular Function category's enrichment of DNA binding and transcription factor activity, pioglitazone may affect transcriptional regulation of gene expression, potentially by modifying the activity of important transcription factors linked to inflammation and glucose metabolism [20]. These functional insights are essential for comprehending the molecular mechanisms by which pioglitazone carries out its therapeutic effects.

The results of this study may impact PCOS treatment plans in the future, especially regarding the creation of more individualized medicine techniques. Clinicians may be able to better personalize treatments and improve patient outcomes by identifying particular gene expression profiles that respond to pioglitazone. Furthermore, these findings imply that pioglitazone's therapeutic efficacy might be increased by combining it with other treatments that also target the identified pathways, such as insulin sensitizers or anti-inflammatory drugs [18, 21]. The discovery of biomarkers is facilitated by identifying genes that express differently in response to pioglitazone treatment. Some genes, such as the hub genes ESR1 and KRAS, may be useful as biomarkers for PCOS patients' early diagnosis, prognosis, or therapy response prediction. The goal of future research should be to explore the clinical value of these indicators and validate them in bigger cohorts [14].

The development of targeted therapeutics for PCOS may potentially be guided by the particular gene expression profiles found in this study. Patients who do not respond well to current medications may benefit from exploring novel therapeutic options, such as focusing on the PI3K-Akt signalling pathway or cytokine-cytokine interactions. Future research should consider the promising avenue of using gene expression patterns to inform the selection of targeted medicines [30]. Further investigation is required to answer the questions these results raise. Larger cohort studies are first required to corroborate the results and establish the applicability of the detected alterations in gene expression and pathway enrichments. To have a better understanding of pioglitazone's mode of action, mechanistic studies examining the drug's direct impact on the identified pathways and hub genes should also be carried out. Understanding how these variations in gene expression relate to PCOS patients' long-term clinical outcomes may potentially be aided by longitudinal research [17, 21].

The study's main drawback is its rather small sample size, which could restrict how broadly the results can be applied. To confirm the findings and make sure the detected variations in gene expression are typical of the larger PCOS community, larger cohorts are required [13]. The reliability of the data sources is another factor to take into account, especially the gene expression data from the GEO database. Despite the well-established nature of the dataset included in this investigation, it is crucial to understand that biases may be introduced by differences in sample handling, data processing, and analysis methodologies, which could have an impact on the findings [31]. It may be difficult to get firm conclusions on the effects of pioglitazone in patients with diverse PCOS traits or varied degrees of insulin resistance included in the study [20]. This could result in heterogeneity in the gene expression patterns.

Selection bias and confounding factors are examples of potential biases that could affect the study's outcomes.

Pioglitazone's therapeutic potential has been further enhanced by the identification of important pathways and hub genes involved in insulin sensitivity, inflammation, and cell cycle regulation. The development of targeted medicines, biomarker discovery, and

personalized medicine are all significantly impacted by these findings. The main goals of future research should be to confirm these findings in bigger cohorts, investigate the clinical value of discovered biomarkers, and investigate novel treatment approaches based on the profiles of detected gene expression. This study advances our understanding of the molecular effects of pioglitazone, which helps to further ongoing attempts to enhance the management of metabolic diseases related to PCOS [1, 14].

Conclusion

The purpose of this study is to clarify the molecular effects of pioglitazone on the expression of genes in the skeletal muscle tissue of women who have PCOS, with an emphasis on how it affects inflammation and insulin sensitivity. The research offers a thorough understanding of how Pioglitazone modulates important metabolic and inflammatory pathways, particularly the cytokine-cytokine receptor interactions and PI3K-Akt signalling pathway. This is achieved by identifying differentially expressed genes (DEGs) and analyzing related biological processes and pathways. The results support the study's goals and provide a biological basis for the therapeutic effects of pioglitazone in PCOS. Notably, the study shows that hub genes like *KRAS* and *ESR1* are essential to these pathways, indicating that these genes may be used as targets for future therapeutic development and biomarkers for predicting treatment response. These findings highlight the many ways in which pioglitazone affects mechanisms that are essential for controlling PCOS, especially by improving insulin sensitivity and reducing chronic inflammation. Clinically, the findings imply that customized treatment plans based on unique gene expression profiles may optimize Pioglitazone's effectiveness. Pioglitazone in combination with other medications that target the identified pathways may result in more successful management of PCOS using this tailored strategy. Further mechanistic research is required to comprehend Pioglitazone's direct impact on the identified pathways to create novel therapeutic approaches based on these molecular insights. The study's findings contribute to our knowledge of the molecular processes behind pioglitazone's actions in PCOS, laying the groundwork for further investigations into creating more specialized and potent therapies. The knowledge acquired may greatly advance the clinical management of PCOS, improving patient outcomes and advancing the development of therapies for metabolic disorders.

Acknowledgements

The authors thank the Centre for Global Health Research, Saveetha Medical College, and Hospital for providing the facilities and support for preparing this article.

Conflict of Interest

The authors declare that there is no conflict of interest in this study.

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