NEUROPSYCHOPHARMACOLOGIA HUNGARICA

Journal Volume: 22
Journal Issue: 1

ISSN: 1419-8711
Journal Year: 2024

ROLE OF THE NUCLEAR FACTOR KAPPA B PATHWAY (NF-KB)INPOLYCYSTIC OVARY SYNDROME DEVELOPMENT

Husham Ahmed Shaker[1], Dr. Rayah Sulaiman Baban[2], Dr. Enas Adnan Abdulrasol[3]

[1 a] PhD. Student, Department of Biochemistry, Alnahrain Medical College, University of Alnahrain, Iraq.
[1 b]Ministry of Education, Iraq
[2]Professor, Department of Biochemistry, Alnahrain Medical College, University of Alnahrain, Iraq.
[3]Professor, Department of Obstetrics & Gynecology alnahrain Medical College University of alnahrain, Iraq.
Email: hisham_a72@yahoo.com

Abstract

Objective: To explore the involvement ofNuclear Factor Kappa Bpathway(NF-κB) in the development of Polycystic ovary syndrome (PCOS) pathogenesis and the possibility of using the parameters of this pathway in the screening and diagnosis of PCOS.**Methods:** 120women, of which 60 had PCOS and 60 appeared to be healthy. This study was done from January 2022 to December 2023. The average age of participantsranged between18 to 40 years. Age, body mass index (BMI) (kg/m^2), fasting glucose, insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), total testosterone, NF-κB,Inhibitor of nuclear factor kappa-B kinase(IKKβ), interleukin-1 receptor-associated kinase 1(IRAK1),Interleukin 6 (IL-6), Follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured for both groups. **Results:** Serum NF-κB, IKKβ, IRAK1,IL-6, LH, FSH, HOMA-IR, and testosterone of PCOS patients were discovered to be remarkably higher than those of the control group, There is no significant difference in age for both groups, while there is a significantly higher in BMI of PCOS compared to controls. A significantpositive correlation was observed between testosterone and NF-κB pathway parameters (NF-κB, IKKβ, IRAK1). Likewise, a strongpositive correlation was detected between serum HOMA-IR and NF-κB pathway parameters. **Conclusions**: The NF-κB affects numerousfeatures of PCOS, including hyperandrogenemia and insulin resistance. Inhibition of the NF-κB could be an attractive target for PCOS therapeutic advantage. Indeed, NF-κB, IKKβ, and IRAK1 can be used as new markers for screening and diagnosis of PCOS.

Keywords:PCOS, NF-κB, insulin resistance, hyperandrogenemia.

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the common endocrine illnesses, affecting around 8-13% of women of childbearing age.PCOS is considered a complex endocrinological condition connected with dysregulations of the psychological, metabolic, and reproductive systems, it is a serious public health problem. The most precipitating disorder impacting female fertility is PCOS, which is characterized by anovulation. Following the first description of this ailment by Stein and Levanthal in 1935, several research on the pathogenesis of the illness were

conducted (Yasmin et al., 2022, Syabakhash et al., 2020). However, the disorder's genesis is not well known. The ovaries seem larger on ultrasound, with a higher quantity of immature follicles. The fundamental hallmark of this illness is uneven levels of sex hormones and prolonged anovulation caused by hyperandrogenism, in the lack of particular adrenal and/or pituitary problems (Guo et al., 2022). Persistent hormonal imbalance causes the production of many tiny antral follicles and an irregular menstrual cycle, eventually leading to female infertility. Cardiovascular illnesses, insulin resistance, abdominal obesity, psychiatric problems, infertility, and cancer are all linked to PCOS. These pathophysiologies relatedto PCOS are linked to each other **(**Siddiqui et al., 2022).

2. The role of insulin resistance and hyperandrogenism

Insulin resistance can be considered one of the key causes of the symptoms of PCOS, leading to hyperinsulinemia, which plays an important role in the control of androgen production; hence, hyperinsulinemia triggers excessive ovarian androgen production, resulting in PCOS (Zhao et al, 2023). Between 44% and 70% of women with PCOS plus obesity had higher insulin resistance than controls. These findings imply that PCOS-associated obesity affects insulin sensitivity and that their cohabitation may improve insulin resistance (Calcaterra et al, 2021). Understanding the effect of hyperinsulinemia on androgen production is difficult. It is not possible to inject insulin into healthy people for a lengthy period and notice a genuine physiological effect. Several trials used brief infusions of insulin(Fox et al., 1993; Micic et al., 1988). Insulin's short-term influence on androgen production appears to occur in a dose-dependent way in women with PCOS, with a considerable increase in androgen production when insulin levels exceed those found in an insulin resistance state (Micic et al., 1988).

In women with PCOS, the ovaries produce around 60% of androgens, while the adrenals provide approximately 40%. Hyperandrogenism has been shown to produce preferential deposition of intra-abdominal fat and an increase in tiny subcutaneous adipocytes. This can eventually lead to metabolic malfunction through lipotoxicity (Dumesic et al., 2016). PCOS can also be caused by hyperandrogenism via beta-cell dysfunction, with inadequate insulin production to compensate for insulin resistance or an improved earlier insulin response to glucose (Navarro et al., 2015). It is believed that over 80% of women who display indications or symptoms of hyperandrogenism, such as alopecia, hirsutism, or acne, have PCOS (Sirmans and Pate, 2013).

3. Nuclear factor kappa b pathway

In 1986, David Baltimore and Ranjan Sen discovered the nuclear factor-kappa B (NF-κB) in lymphocyte nuclei. NF-κB has a role in inflammation, cell adhesion, proliferation, growth signaling, differentiation, apoptosis defense, and host immunological response. Abnormal NF-κB signaling can cause several diseases, including cancer, AIDS, atherosclerosis, arthritis, diabetes, inflammation, and immunological disorders (Albensi, 2019).

Both glia and neurons express NF-κB, which makes up the NF-κB complex. The NF-κB complex exists in cytoplasm as an inactive molecule where the activation of NF-κB.The canonical and noncanonical (or alternative) pathways are the two main signaling pathways that participate in the activation of NF-κB. Despite having different signaling mechanisms, both pathways are crucial in regulating immunological and inflammatory responses (Figure 1) (Liu et al., 2017, Alharbi et al., 2021). The fundamental mechanism for the activation of canonical NF-κB involves the degradation of IκBα, which is activated via its phosphorylation by the IκB kinase complex.After degradation, the residual NF-κB dimer moves to the nucleus and binds to the target genes(Sen & Smale, 2010).

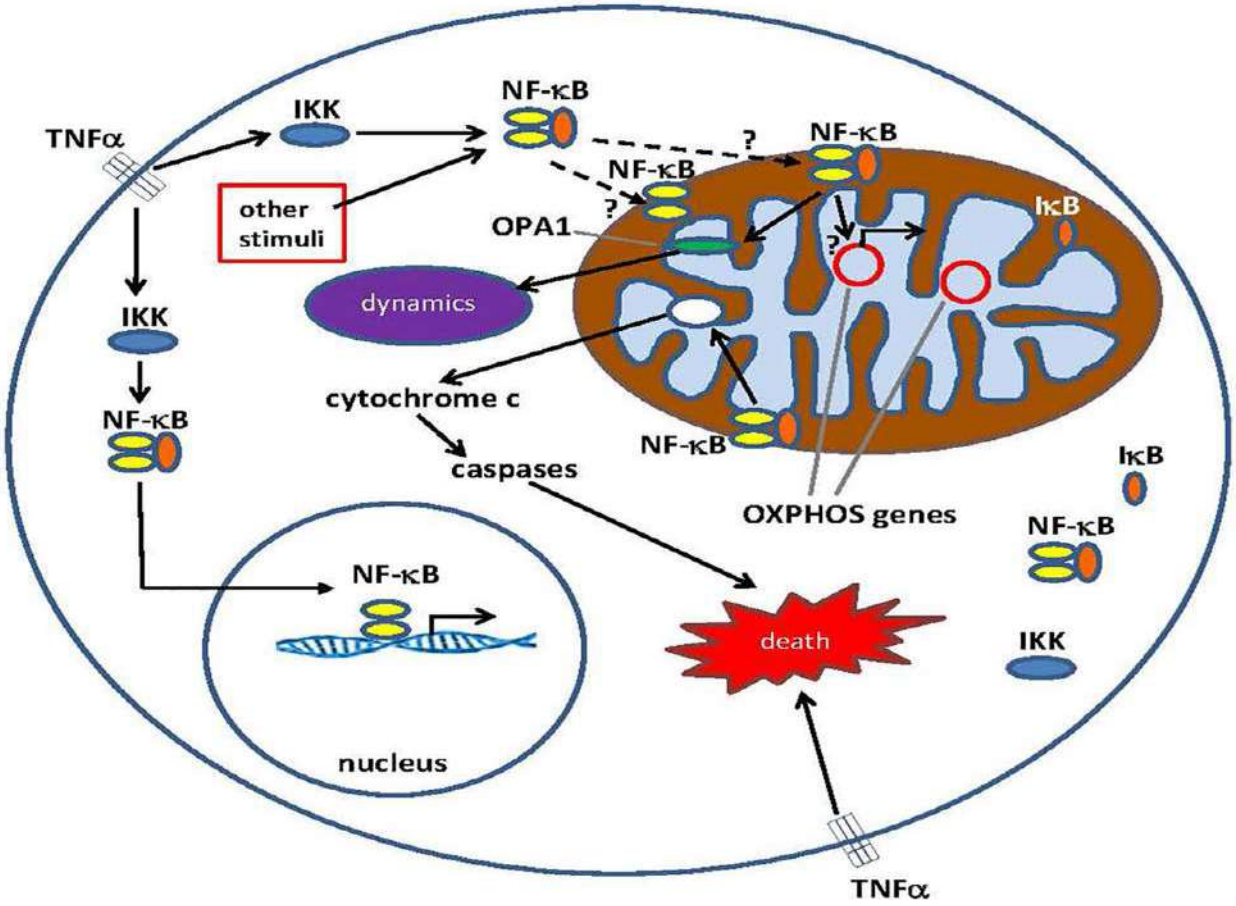

Figure 1: Nuclear factor kappa B pathway

The impact of NF-κB on cell survival is multifaceted, with proinflammatory or neuroprotective effects depending on the type of the cell, developmental stage, and pathology (Qin et al., 2007). NF-κB has several effects on PCOS, including insulin resistance,hyperandrogenemia, cardiovascular illnesses, endometrial dysfunction, and hypothalamus endocrine gonadal axis abnormality (Tan et al., 2023), figure (2).

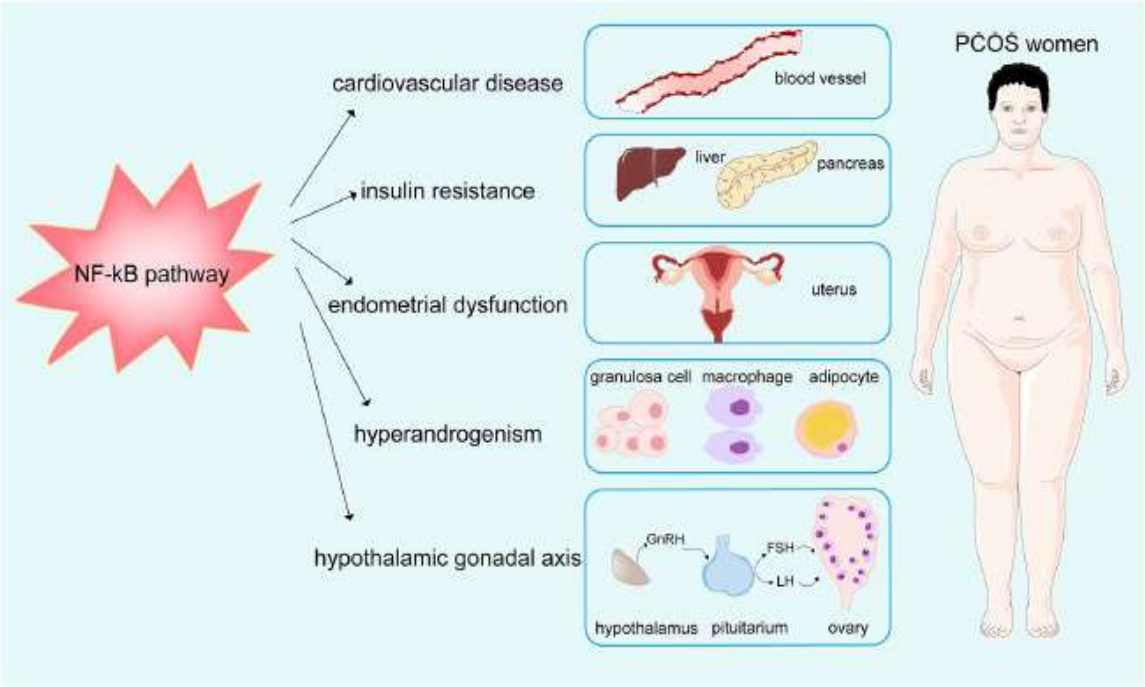

Figure 2:The role of NF-κB in PCOS.

4. **Materials and methods**

In the current investigation, case-control research was undertaken on a group of (120) women, 60 of whom had PCOS, and 60 looked to be in excellent health. The study was conducted between January 2022 and December 2023, with contributors ranging in age from 18 to 40 years. Patients with PCOS were selected from the infertility clinic, the gynecology and obstetrics at the Al-Imamain Alkadhimain Medical City, and outpatient clinic patients. The medical history, clinical and biochemical features, weights, and heights of each patient were documented. Expert gynecologists diagnosed PCOS in all of the patients using the Rotterdam criteria. All research participants had biochemical measures taken throughout the follicular period (2–5 days) of their cycles. Patients were diagnosed with PCOS using the updated Rotterdam criteria, which require two of the following three manifestations: (1) clinical and/or biochemical hyperandrogenism, (2)oligo- or anovulation, and (3) ultrasonography-detected polycystic ovaries. The control group of women who met one of these three criteria was excluded from the research.

The study measured age, body mass index (BMI), insulin,fasting glucose, total testosterone, nuclear factor kappa B (NF-κB), inhibitor of nuclear factor kappa B kinase subunit beta (IKKβ), interleukin 1 receptor-associated kinase 1 (IRAK1), IL-6, serum follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels in both PCOS and control groups. The homeostatic model assessment (HOMA-IR) formula was used to calculate insulin resistance.

5. Statistical analysis

Statistical Package for Social Sciences software 21.0 for Windows package software (SPSS) was done to perform data. Comparisons between the study groups were done using an independent samples t-test. ROC curve was performed to find out the diagnostic values of NFKB, IKKβ, and IRAK1discrimination between patients with PCOS and controls.

6. Results

The results are shown in Table 1. There were no differences between the groups in terms of age. (30.58 ± 7.28 vs. 30.79±7.81), while there is a significantly higher BMI of PCOS compared to controls. In addition, Patients with PCOS showed significantly increased levels of NF-κB, IKKβ, IRAK1, LH, FSH, IL-6**,** testosterone, and HOMA-IR compared to the control group. A positive and substantial association was established between testosterone and NF-κB pathway characteristics (NF-κB, IKKβ, and IRAK1). Similarly, serum HOMA-IR showed a substantial positive connection with NF-κB pathway characteristics. (Table 2). The receiver operating characteristic curve (ROC) was used to find out the diagnostic values of NF-κB, IKKβ, and IRAK1discrimination between patients with PCOS and controls. For NF-κB, the area under the curve (AUC) was 1, 95%CI= 1-1, p< 0.001. The sensitivity and specificity of the test at a cut-off value of NF-κB = 13.2 ng/ml were 98% and 100%, respectively. For IKKβ, the AUC was 1, 95%CI= 1-1, p< 0.001. The sensitivity and specificity of the test at the cut-off value of IKKβ = 3.26 ng/ml were 93% and 100%, respectively. For IRAK1, the AUC was 1, 95%CI= 1-1, p< 0.001. The sensitivity and specificity of the test at the cut-off value of IRAK1 = 10.8 ng/ml were 98% and 100%, respectively (Figure 3).

Table 1: Demographic, hormonal, and NF-κB parameters of PCOS and control groups

Parameters	PCOS (60)	Control (60)	*P*
Age	30.58 ± 7.28	30.79±7.81	> 0.05
BMI(kg/m^2)	32.48 ± 4.28	23.82 ± 2.26	< 0.001
NF-κB(ng/ml)	20.3 ± 4.93	7.10 ± 1.48	< 0.001
IKKB(ng/ml)	4.51 ± 1.65	2.13 ± 0.25	< 0.001
IRAK1(ng/ml)	12.94 ± 1.37	6.33 ± 0.69	< 0.001
HOMA-IR	2.37 ± 0.70	0.93 ± 0.40	< 0.001
Testosterone(ng/ml)	1.72 ± 0.56	0.49 ± 0.19	< 0.001
LH (mlU/ml)	9.71 ± 2.25	5.93 ± 1.93	< 0.001
FSH(mlU/ml)	8.25 ± 2.11	7.31 ± 2.21	< 0.05
IL-6 (pg/ml)	**72.75 ± 35.74**	**28.18 ± 14.43**	**< 0.001**

Table 2: Pearson's correlation of NFKB, IKKβ, and IRAK 1 with other variables.

Variables	Testosterone	HOMA-IR
NF-κB	0.708**	0.698**
IKKB	0.515**	0.478**
IRAK1	0.750**	0.705**

**** = p-value < 0.001**

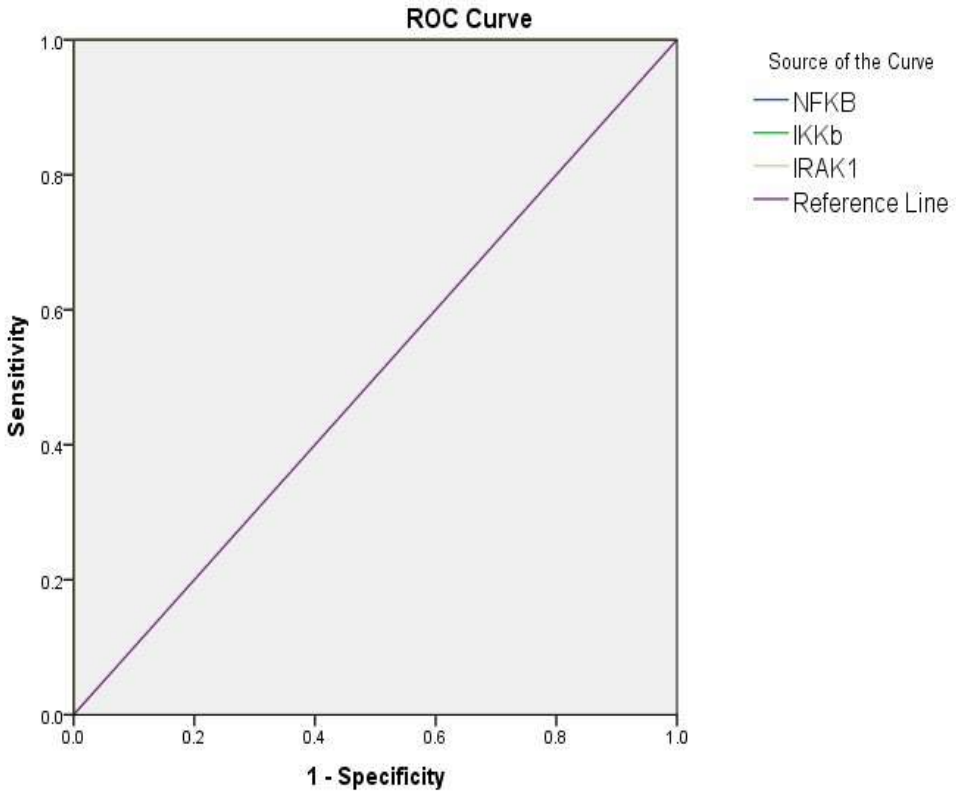

Figure 3: ROC curve of NF-κB, IKKβ, IRAK1

7. Discussion

This study investigates the involvement of the NF-κB pathway in the development of PCOS features, including hyperandrogenism and insulin resistance. The goal is to establish a theoretical basis for treating PCOS diseases.

Systemic inflammation is a significant characteristic of PCOS even though it is not one of the diagnostic criteria (Weiss et al., 2009 & Celik et al., 2019). It has been shown that high levels of androgen in Ishikawa cell cultures prevent estrogen-induced receptivity gene expression, whereas enhanced NF-κB phosphorylation is seen in endothelial cell cultures of PCOS women (Cermic et al., 2003).It has been found that there is a considerable increase in NF-κB expression in the mid-luteal endometrial tissues of PCOS sufferers (Koc et al., 2017). To our knowledge, there are no previous Iraqi studies observed in the literature that deal with the measurement of

NF-κB pathway parameters in the serum of patients with PCOS or any other diseases. In the current study, the results showed that the NF-κB parameters levels significantly increased in PCOS patients compared to the control. This means that this pathway is hyperactive in those patients. These results, in concert with (Liu, et al.,2015 & Ma et al., 2021) discovered that PCOS patients had significantly higher serum NF-κB levels than control participants. The current results are also in agreement with (Ağar et al., 2022). In addition, a study by González et al showed a decreased protein expression of IκB (the inhibitor of NF-κB) in PCOS women, as well as PCOS women show increased p65 protein (one of the five components that form the NF-κB) expression and increased intranuclear NF-κB along with decreased IκB protein expression compared with controls (González et al., 2006).

The NF-κB signaling system is essential for controlling inflammation. Most of the early research on the connection between NF-κB and PCOS concentrates on chronic inflammation. Furthermore, several PCOS-related characteristics have been linked to the NF-κB pathway. For instance, ovarian tissue samples from both healthy and PCOS women showed that the nucleus of granulosa cells had increased levels of NF-κB (Diamanti-Kandarakis et al., 2007). Moreover, aberrant NF-κB activation was noted in endometrial cells, PCOS endometrial tissue, and mononuclear macrophages (Hu et al., 2021). The high levels of NF-κB pathways may be due to the increased inflammatory process secondary to excess body fat accumulation. Indeed, by increasing the release of many bioactive molecules known as adipokines, adiposity induces inflammation(Koc et al., 2017 & Celic et al., 2007). Increased fat accumulation in obese PCOS women may increase the inflammatory events and cause NF-κB levels to be higher than in lean women with PCOS(Ağar et al., 2022).

ROC curve analysis was used to make a comparison of the diagnostic performance of NF-κB, IKKβ, and IRAK1 and organize them according to the AUCthat can occupy and explain if such occupation is significant or not. The optimal cut-off point was calculated for each parameter. Specificity and sensitivity were also calculated to describe the utility of diagnostic tests in clinical cases. In contemporary medicine, evaluating suggestive tests is important not only to establish the presence of a disease but also to rule it out in healthy individuals (Hajian, 2013). ROC plays a dominant role in evaluating the diagnostic capacity of tests to differentiate the true state of subjects, finding the ideal cut-off point, and comparing two alternative indicative tasks when each task is made on the same subject (Hanley and McNeil, 1982 & Kumar et al., 2011). According to the results, NF-κB, IKKβ, and IRAK1 can be used for screening and diagnosis of PCOS.

7.1 NF-κB pathway and insulin resistance in PCOS

The issue of how the NF-κB pathway affects deficits in insulin action arises since it has been demonstrated that activation of the path causes insulin resistance, while inhibition of the process protects against insulin resistance. In the current study, NF-κB, IKKβ, and IRAK1 in the PCOS group were remarkably higher compared to controls, and their levels were positively

correlated with insulin resistance. Similar studies have reported that NF-κB was positively correlated with insulin resistance (Gonzalez et al., 2006 & Tan et al., 2011). The association between insulin action, inflammation, and metabolism is largely dependent on the IKK/NF-kB signaling pathway (Baker et al., 2011& solinas et al., 2010).

PCOS and insulin resistance are tightly related. It was estimated that 75% of PCOS women had insulin resistance (Tosi et al., 2017). Numerous metabolic stressors, such as obesity and high-fat diets, can activate IKKβ and considerably contribute to the onset of insulin resistance, suggesting that the NF-κB pathway may be involved in the pathophysiology of insulin resistance (Arkan et al., 2005 & Cai et al.,2005). Insulin signaling stops gluconeogenesis in hepatocytes and increases glucose glycogen storage. IKKβ expression in the liver led to increased resting levels of free fatty acids and insulin, and impaired insulin signaling led to insulin resistance (Baker et al., 2011). By a variety of mechanisms, IKK family kinases are crucial to the malfunction of insulin metabolism as well as overnutrition (Solinas et al., 2010). This process also drives macrophage recruitment, activation, and differentiation (Baker et al., 2011, solinas et al., 2010, Luo, 2017 & Arkan et al., 2005). Many of the metabolic effects of insulin at target locations are mediated by insulin receptor substrates (IRSs), which are directly phosphorylated by IKKβ with metabolic stress or cytokine-evoked inflammatory alerts. This further impairs insulin receptor communication (Baker et al., 2011, solinas et al., 2010, Goa et al., 2002 & Herschkovitz et al., 2007).When NF-κB signaling is activated, adipose tissue and blood levels of TNF-α and IL-6 rise as a result of an inflammatory cascade that is started.As shown in Table (1), there is a significant variation in IL-6 levels between both groups, with higher concentrations in those with PCOS in comparison with the control group; these outcomes are consistent with (Zhang et al., 2008, Mazloomi et al., 2023). Several investigations have established a relationship between PCOS and low-grade chronic inflammation.Additionally, IL-6 stimulates lipid breakdown in adipose tissue, leading to insulin resistance. According to recent studies, markers of inflammation or their gene are increased in PCOS patients (Xiong et al., 2011, Boulman et al., 2004). Crucially, The inhibitors of NF-κB can enhance the metabolism of lipid and glucose further ameliorating hepatic insulin resistance(He et al., 2021 & Malin et al., 2015). Atypically, overnutrition increases hypothalamic IKKβ expression. Then, the central insulin/leptin signaling and activities are disrupted via activation of the NF-κB pathway (Zhang et al., 2008). Insulin resistance may be exacerbated by pro-inflammatory signaling that IRAK1 mediates via IL-1 receptor/toll-like receptors. MyD88 interacts with IL-1-R and toll-like receptors and stimulates IRAK-4, which activates IRAK-1 by phosphorylation. After interacting with Mitogen-Activated Protein Kinase Kinase 7 (MAP3K7), TNF receptor-associated factor 6 (TRAF6) activates the IKKβ complex, which phosphorylates IκBα before it separates from NF-κB. This process occurs downstream from IRAK1. NF-κB penetrates the nucleus to stimulate the expression of multiple pro-inflammatory genes including for example IL-1 and TNF-α. Insulin receptor substrate 1 (IRS-1) is phosphorylated at Ser24 by IRAK-1, which inhibits IRS-1's ability to bind to phosphatidylinositol 3-kinase (PI3K), This, in turn, affects the metabolic effects of insulin including insulin-stimulated translocation of glucose transporter type 4 (GLUT4)

(RAJAIE and Athena, 2018).These data suggest that the NF-κB pathway may play a crucial part in insulin resistance.

7.2 NF-κB pathway and Hyperandrogenism

Hyperandrogenism is one of the major features of PCOS. This syndrome, and particularly the related hyperandrogenism, is associated with fertility issues and pregnancy-related risks that PCOS patients may display. In addition, hyperandrogenism also contributes to the altered metabolic profile of these women, thus, worsening reproductive outcomes (Abruzzese et al., 2022). Increasing investigation has revealed that chronic inflammation plays a vital role in PCOS outcomes and the pathophysiology of follicular dysplasia (Rudnicka et al.,2021). It is unclear how hyperandrogenism and PCOS-related inflammatory activation are related. In animal study on mice with PCOS, excess testosterone causes inflammation and pyroptosis activation, which results in ovarian dysfunction and fibrosis (Xiang et al., 2023). Many investigations have demonstrated that chronic inflammation caused by PCOS is linked to elevated androgen levels and insulin resistance. While chronic inflammation in PCOS instances generates androgen rise, rising androgens improve both androgen production and insulin resistance, with a favorable feedback mechanism (Celik et al., 2017, Celik et al., 2019 & Karin and Ben, 2000). Our findings revealed that there was a considerable positive association between NF-κB pathway parameters and testosterone. These findings agree with González et al.,who discovered that intranuclear NF-κB was elevated in PCOS monocyte cells and positively correlated with androgen levels. (González et al., 2009), as well as a study by Koc et al. (Koc et al., 2017).Gonzalez et al. found that oral testosterone administration promotes the cardinal signal of inflammation, NF-κB, in the fasting state, with raised activation after glucose consumption. These identical circumstances also gradually enhance the expression of the NF-κB gene and reduce the protein concentration of IκB, the inhibitor of NF-κB (González et al., 2012), According to Gonzales et al., insulin resistance and hyperandrogenism were linked to a considerable rise in intranuclear NF-κB concentrations in patients with PCOS having hyperglycemia (González et al., 2006). It has been informed that reducing phosphorylation of NF- κB, could decrease hyperandrogenism in an animal sample of PCOS (Shao et al., 2019 & Zuo et al., 2018). Therefore, elevated testosterone levels may elevate NF-κB, indicating a potential correlation between PCOS and moderate chronic inflammation (Liu et al., 2015). Our study findings showing a significant link between NF-κB, insulin levels, and blood testosterone provide compelling evidence of the connection between HOMA-IR, hyperandrogenemia, and elevated pathologic inflammation.

From the above, it can be deduced that androgens can control the NF-κB pathway. Androgens can affectphysiological processes in PCOS patients by activating the NF-κB pathway. Consequently, blocking the NF-κB pathway can serve as a therapeutic option to protect against PCOS development (Tan et al., 2023).

8. Conclusions

The evidence of dysregulated NF-κB signal cascades in PCOS is summarized in this article, which shows that NF-κB influences several features of PCOS, including insulin resistance and hyperandrogenism. Inhibition of the NF-κB might have therapeutic benefits in PCOS. Indeed, NF-κB, IKKB, and IRAK1can be used as new markers for screening and diagnosis of PCOS.

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