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ORIGINAL ARTICLE

Evaluation of Apoptosis Markers Caspas-8, Cytochrome C Levels in PCOS Patients

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ABSTRACT

Background: Apoptosis is defined as a programmed cell death that eliminates dysfunctional, damaged, and fulfilled cells as a result of a certain stimulus. PCOS, IR, ovarian hyperandrogenism, hyperinsulinemia, and alterations in follicular endocrine signaling may impact the activation, growth, survival, and atresia of follicles. As a result of this impact, polycystic morphology may be observed in the ovaries, follicle development may be inhibited, and anovulation may occur.

Aim: We aimed to investigate the relationship between the apoptosis markers caspase-8 and cytochrome-c levels and PCOS patients.

Methods: Thirty female patients diagnosed with PCOS and 30 healthy volunteers were included in the study as a control group. Caspase-8 and cytochrome-c levels were measured by ELISA method in the sera of patients and healthy volunteers.

Results: 30 PCOS female patients and 30 healthy control group were included in the study. No difference was found between the caspase-8 and cytochrome-c levels of PCOS patients and the caspase-8 and cytochrome-c levels of the healthy control group. ($p < 0.092$, $p < 0.473$). The mean ages of the patient and control groups were similar (mean age of patients: 24, mean age of control: 25). In the patient group, there was no relationship between total testosterone and caspase-8 and cytochrome-c levels ($p < 0.276$, $p < 0.291$).

Conclusion: In polycystic ovary patients, the values of caspase-8, the apoptosis marker involved in the extrinsic pathway, and cytochrome-c, which is involved in the intrinsic pathway, similar to the control group. In order to investigate the relationship between PCOS and apoptosis, we suggest that apoptosis markers should be examined in the follicle fluids of PCOS patients.

Keywords: PCOS; Apoptosis; Caspase-8; Cytochrome-C

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most often syndrome observed among women of the reproductive period.[1] The incidence of this disease is between 8 and 13%. This rate varies according to the populations studied and the diagnostic criteria used.[2] According to the American Institute of Health (NIH) diagnostic criteria, clinical findings of hyperandrogenism and/or biochemical findings of hyperandrogenism and menstrual dysfunction are required to diagnose PCOS,[3] while according to the 2003 Rotterdam diagnostic criteria: Presence of clinical and or biochemical hyperandrogenism, oligo-anovulation or polycystic ovarian morphology must exist. [4]

At its meeting held in 2006, the Androgen Excess Society (AE-PCOS) defined clinical and/or biochemical hyperandrogenism and ovarian dysfunction (oligo/anovulation) and/or polycystic ovarian morphology as diagnostic criteria.[4] Obesity, insulin resistance (IR), hyperandrogenism, abnormal follicular development, and inflammation often accompany PCOS.[5] IR, ovarian hyperandrogenism, hyperinsulinemia, and alterations in follicular endocrine signaling may impact the activation, growth, survival, and atresia of follicles. As a result of this impact, polycystic morphology may be observed in the ovaries, follicle development may be inhibited, and anovulation may occur.[6] The mitochondrion has a significant function in the regulation of oxidative stress.[7] Mitochondrial genes and functional diseases are investigated within the pathogenesis of PCOS. These mitochondrial disorders may explain features such as IR, hyperandrogenism, abnormal follicular development, and inflammation; however, the pathogenesis of mitochondrial dysfunction in PCOS is not clear.[8] The main function of mitochondrion is to be effective in cell energy metabolism and cell apoptosis and to enable signal transduction for cell proliferation.[9] The mitochondrion converts nutrients into energy and leads to the generation of reactive oxygen species (ROS).[10] Excessive generation of ROS causes cell damage. For example, it damages the cell's DNA structure, protein structure, and lipid structure and stimulates mitochondrial-mediated apoptosis.[11] Malondialdehyde (MDA), total antioxidant capacity (TAC), total antioxidant status (TAS), SOD (superoxide dismutase), and GSH (glutathione) are used as markers for the evaluation of oxidative stress level.[12]

Apoptosis is defined as a programmed cell death that ensures the elimination of cells that are dysfunctional, damaged, or fulfilled their function as a result of a certain stimulus.[13] Apoptosis mechanisms are initiated by two pathways called extrinsic and intrinsic.[14] Apoptosis plays a key role in cell balancing and elimination of harmful cells.[15] Insufficient or increased cell death may cause some diseases.[12] In cancer diseases, the balance between cell proliferation and cell death is disrupted, and cells cannot receive death signals to perform apoptosis.[16] The extrinsic pathway of apoptosis is activated by the binding of death ligands to death receptors, while the intrinsic pathway is activated as a result of intracellular signalling.[13]

This receptor-ligand-adaptor protein complex in the extrinsic pathway is called the death-inducing signaling complex (DISC). This complex causes the formation of caspase-8, the active form of procaspase-8, due to the association of procaspase-8 with the death effector domain (DED) responsible for the transduction of apoptotic signal.[17,18] Caspase-8 leads to cell death by activating procaspase-3, -6, and -7 or cuts the c-terminal region of tBid, which is a proapoptotic member of the Bcl-2 family, and causes the formation of its active form, tBid, and thus the progression of apoptosis to the intrinsic pathway.[18,19] In the intrinsic signaling pathway, death signals induced by DNA damage, growth factor deficiency, and oxidative stress are transmitted to the mitochondrion by two proapoptotic members of the Bcl-2 family (Bax, Bad) and trigger the intrinsic pathway by forming pores on the mitochondrial surface. As a result, the outer mitochondrial membrane is fragmented, and caspase activators, especially cytochrome c, are released.[15] Cytochrome c is an inner mitochondrial protein located in the intermembrane space and involved in the electron transport system for oxidative phosphorylation. In the presence of dATP, cytosolic cytochrome c binds with Apaf-1 and leads to the formation of a multiprotein complex called apoptosome and initiation of the proteolytic caspase death cascade; caspase-9 is activated and effector caspases (caspase-3 and -7) are activated in order. Caspase enzymes activate endonucleases containing DNA fragmentation factor, so that apoptotic cells perform DNA fragmentation.[18,19]

A few ways to evade apoptosis can be listed as follows: decrease of caspase functions, disruption of the receptor signaling pathway, increased expression of inhibitors of apoptosis proteins (IAPs) family molecules.[20]

The reason for the formation of multiple, small ovarian cysts in the ovaries in PCOS has not been fully explained. We measured the caspase 8 and cytochrome c levels, which are markers of apoptosis, in the sera of PCOS patients. We compared these values with serum caspase-8 and cytochrome c levels of the patients without PCOS in the control group and tried to find the difference. In the literature, conflicting results have been found in studies with apoptosis markers in PCOS. In one study, caspase 9 was found to be lower than the control group, but caspase 3 and caspase 7 were similar.[21] In another study evaluating caspase-1 in PCOS patients, the Caspase-1 value was higher in the patient group compared to the control group.[22] Our hypothesis was to investigate the question of whether the apoptosis markers caspase 8 and cytochrome c levels in the patient group would be lower than in the control group due to increased apoptosis in PCOS.Since the results of studies on apoptosis markers in PCOS patients are inconsistent, we aimed to measure the values of caspase 8, which is involved in the extrinsic pathway, and cytochrome c, which is involved in the intrinsic pathway, in PCOS patients.

MATERIAL AND METHODS

Patients: Informed consent form was obtained from the patients participating in the study. PCOS diagnoses based on Rotterdam diagnostic criteria and 2006 Androgen Excess Society criteria. 30 female patients with PCOS and 30 healthy volunteers were included in the study. There was no known chronic disease, malignancy, autoimmune disease, and systemic infectious disease in the patient and control groups.

Caspase-8 and cytochrome c measurements: Blood samples taken from both the patient and control groups were kept at room temperature for 5 minutes and then centrifuged at 4000 rpm for 5 minutes. The serum formed on the upper layer was collected and apportioned into at least two Eppendorf tubes stored at -80˚C until testing. As soon as the required number of patients for this study was reached, all samples were thawed, and then CYCS (cytochrome c) and human caspase-8 assays were run on the ELISA device in a single session with ELISA kits.

Research ethics standards compliance: This study was approved by the Sivas Cumhuriyet University Interventional Human Research Ethics Committee, Sivas, Turkey (Registry No: 2021-06/04, Date: 01.06.2021). The volunteers were informed about how the research would be applied and their possible risks. Then, a written contest form was requested. This study complies with the Principles of the Declaration of Helsinki.

Statistical analysis: SPSS 22.0 program was used for the statistical evaluation of the data. Descriptive analysis was performed on the data. The chi-square test was used. Data were averaged with standard deviation and independent samples t-test was used for comparison. Significance value was accepted as p <0.05. First Author, Second author, et al

RESULTS

30 female patients with PCOS and 30 healthy female volunteers as a control group was included in the study (Figure 1-2).

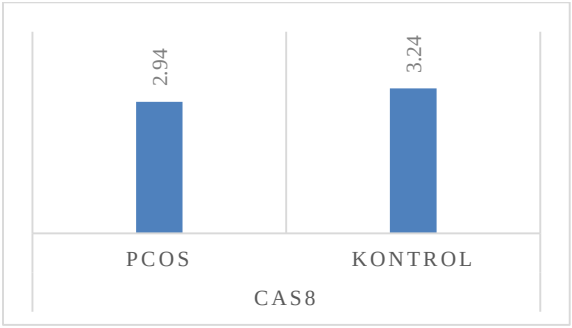

Figure 1: Caspase-8 values in PCOS and control group patients (P<0.092)

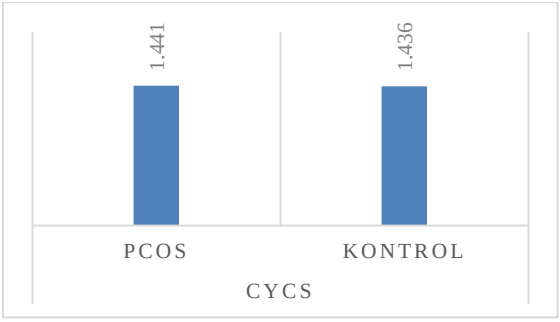

Figure 2: Cytochrome c values in PCOS and control group patients (P<0.473)

No difference was determined between the caspase-8 and cytochrome c levels of PCOS patients and the caspase-8 and cytochrome c levels of the control group (p<0.092, p0.473) (Figure 3). The mean ages of the patient and control groups were similar (mean patient age: 24, mean control age: 25). No correlation was determined between total testosterone and caspase-8 and cytochrome c levels in the patient group (p<0.276, p0.291).

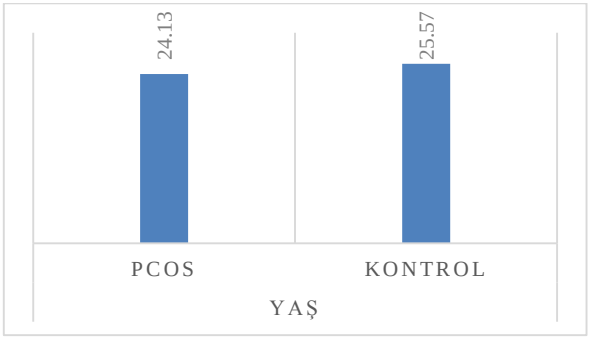

Figure 3: Mean age values in PCOS and control group patients

DISCUSSION

PCOS is the most often syndrome observed among women of the reproductive period.1 PCOS is often accompanied by obesity, insulin resistance (IR), hyperandrogenism, and abnormal follicular development.[5] IR, ovarian hyperandrogenism, hyperinsulinemia, and alterations in follicular endocrine signaling may impact on the activation, growth, and survival of follicles. As a result of this impact, polycystic morphology may be observed in the ovaries, follicle development may be inhibited, and anovulation may occur. Apoptosis is defined as a programmed cell death that ensures the elimination of cells that are dysfunctional, damaged, or fulfilled their function as a result of a certain stimulus.[13] Apoptosis plays a key role in tissue homeostasis and the elimination of harmful cells.15 Apoptosis mechanisms are initiated by two pathways called extrinsic and intrinsic.[14] Caspase-8 is the biomarker involved in the extrinsic apoptotic pathway, while cytochrome c is the marker involved in the intrinsic apoptotic pathway.[20] Many cancer cells develop mechanisms in order to evade apoptosis by increasing the amount of anti-apoptotic molecules or by enabling the inactivation of proapoptotic cell death components.[16] It was determined that caspase-8 undergoes substantial changes in the colon, gastric, and head and neck cancers.[23] In a study conducted with patients with breast cancer, it was determined that caspase-8 expression substantially decreased in malignant tissue compared to adjacent normal tissue.[24] Inflammation is a feature of chronic diseases. It is known that low-grade inflammation is included in the pathogenesis of PCOS.[25] As an indicator of this relation, inflammation markers IL-6, TNF-alpha, C-reactive protein (CRP), interleukin-8 (IL-8), soluble intracellular adhesion molecule-1 (MCP-1), and white blood cells (WBC) were found to be high in PCOS patients.[26] OS and inflammation are closely related conditions, and there is an increase in the production of oxidative stress markers in case of inflammation. In the case of an increase in oxidative stress products, proinflammatory gene expression increases.[27,36] WBC, CRP, and Xanthine oxidase (XO) were higher in PCOS patients compared to the control group. As a result, these studies indicate that the number of inflammatory markers and ROS (reactive oxygen species) increase in PCOS patients.[28] There are also studies expressing the relationship between inflammation and IR and obesity in PCOS patients. It was determined that IL-6, TNF-alpha, and vascular cell adhesion molecule 1 (VCAM-1) levels increase in PCOS patients with IR. These increases accompany mitochondrial dysfunction and cause a decrease in mitochondrial oxygen consumption and an increase in ROS generation.[29] In women, there are mitochondria in the ovaries that contribute to oocyte maturation.[30] A large number of oocytes begin to grow at the beginning of each menstrual cycle. Only one oocyte successfully reaches the meiosis I stage. In the next stage, ROS level increases and antioxidant production decreases. On the other hand, for a successful meiosis II division, antioxidant protection such as CAT (Catalase), SOD (superoxide dismutase), GSH (glutathione) is required.[31] The primary sources of ROS in follicular cells are macrophages, granulocytes, and neutrophils. Follicular ROS induces apoptosis in many follicles. Follicle-stimulating hormone FSH and GSH play an effective role against apoptosis. As a result of an experimental study on rats, it was stated that inhibition of GSH synthesis may increase the rate of follicular atresia.[32] In a study investigating oxidative stress and inflammatory markers in PCOS patients, it was determined that MDA (malondialdehyde) increased in the follicular fluids of PCOS patients compared to the healthy control group and TAC (total antioxidant capacity) and thiol decreased. Again in the same patients, IL-6, IL-8, and TNF-alpha were higher than the control group.[33] This study suggests that OS and inflammation may be the cause of impaired oocyte development in PCOS. In a similar study conducted with PCOS patients, MDA level was higher and GSH level lower in PCOS patients compared to the control group.[34]

In our study, we measured caspase-8 and cytochrome c levels as apoptotic markers. We did not determine any difference in caspase-8 and cytochrome c values compared to the healthy control group. In a study of PCOS patients in the literature, total oxidant status and caspase-9 were lower compared to the control group; however, caspase-3 and caspase-7 values were similar to those in the control group. It was reported that there is a negative relationship between caspase-9 and PCOS.[21] The results of caspase-3 and caspase-7 in this study are similar to the results of our study and support our study. In another study evaluating caspase-1 in PCOS patients, the Caspase-1 value was higher in the patient group compared to the control group.22 In a study investigating the ovarian follicular fluids of PCOS patients with 14 anovulatory cycles and 9 regular ovulatory cycles, Caspase-3 levels were significantly lower in women with PCOS with anovulatory cycles than in women with regular ovulatory cycles.[35] This study supports that apoptosis is relatively more active in patients with anovulatory cycles. As seen in the studies on PCOS patients and our study, in most of the studies on apoptosis markers in blood serum values of PCOS patients, these markers were not decreased. However, in the study conducted with the follicle fluids of PCOS

patients, a decrease in these apoptosis markers is observed.[35] It is thought that the decrease in apoptosis markers caused by the increase in apoptosis in PCOS patients is not reflected in blood serum levels. Therefore, it is recommended to look at these apoptosis markers in the follicle fluids of patients for future studies investigating the relationship between PCOS and apoptosis. The absence of a study in the literature evaluating Caspase-8 and cytochrome c together in PCOS patients renders our study original. The fact that the values of caspase-8, a marker of apoptosis in the extrinsic pathway and cytochrome c values in the intrinsic pathway, are similar to the control group in PCOS patients, it is thought that the decrease in apoptosis markers in PCOS patients is not reflected in blood serum levels.

CONCLUSION

The values of caspase-8, which is a marker of apoptosis in the extrinsic pathway, and cytochrome c values in the intrinsic pathway of PCOS patients were found to be similar to the control group.It is thought that the decrease in apoptosis markers caused by the increase in apoptosis in PCOS patients is not reflected in blood serum levels. Therefore, we suggest that these apoptosis markers should be examined in the follicle fluids of patients for future studies investigating the relationship between PCOS and apoptosis.

Conflict of Interest Statement: "The authors declare that they have no potential conflicts of interest with the contents of this article."

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