

Pentraxin-3 Level Assessment Among Ischemic Stroke Patients with Polycystic Ovarian Syndrome

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Abstract

Background: Pentraxin-3 (PTX3) is an important component of the innate immune system, playing a significant role in the inflammatory process and tissue damage. Assessing its levels in different clinical conditions may offer insights into its diagnostic and prognostic significance.

Objectives: This study aims to evaluate whether PTX3 levels are elevated in ischemic stroke patients with PCOS and whether increased PTX3 is linked to greater stroke risk and worse outcomes.

Patients and Methods: A case-control study was conducted in the neurology and obstetrics & gynecology clinics at Azadi Teaching Hospital in Kirkuk starting from 1st February 2023 to 1st March 2024. Women diagnosed with polycystic ovarian syndrome (PCOS) according to Rotterdam criteria were divided into three groups: the first group had PCOS with stroke, while the second group had PCOS without stroke; and the third group was considered an age matching control group with age ranges between 18 and 50 years. Blood samples were withdrawn in the morning to determine the levels of PTX3, C-reactive protein (CRP), triglyceride/ high density lipoprotein (TG/HDL) ratio, and homeostatic model assessment for insulin resistance (HOMA-IR) and measured in all studied groups.

Results: The level of PTX3 was significantly higher in patients with PCOS with stroke group (5.717 ± 1.172 ng/mL) than those without Stroke (3.665 ± 1.085 ng/mL) and Control (2.377 ± 0.740 ng/mL) groups ($p < 0.001$). Pentraxin3 levels positively correlated with stroke severity ($r = 0.42$, $p < 0.001$), while there was no significant correlation with CRP, TG/HDL Ratio, and HOMA-IR.

Conclusion: Pentraxin-3 levels are markedly higher in PCOS patients who have had an ischemic stroke, and the level is in proportion to the severity of the stroke. This means that PTX3 could potentially be used as a biomarker for risk assessment.

Keywords: Polycystic ovarian syndrome, Ischemic stroke, Pentraxin 3, CRP, PCOS.

Introduction

Stroke is one of the emergent neurological disorders that is common with high morbidity and mortality rates in the world (1). Despite advances in the medical field, the incidence of ischemic stroke is still a major health problem in the world (2). However, the condition may worsen if it occurs in association with other metabolic and inflammatory disorders that increase the severity of stroke and chances of a good outcome (3). Among these conditions is polycystic ovarian syndrome (PCOS), which is one of the endocrine disorders that is more predominant in females of childbearing age and is characterized

by hormonal, inflammatory, and metabolic manifestations (4). However, since inflammatory disorders are one of the constituents of PCOS, their role in ischemic strokes has become an essential topic of this study. Pentraxin-3 (PTX3) is one of the newly discovered acute-phase inflammatory glycoprotein markers that may be useful in the diagnosis and prognosis. PTX3 plays a key role in vascular inflammation and endothelial dysfunction by affecting nitric oxide synthesis, cell proliferation, and matrix metalloproteinase production. It is linked to arterial hypertension and may serve as a potential biomarker (5). Furthermore, it has been identified as a potential biomarker for cardiovascular and cerebrovascular diseases, and further studies were recommended in this direction to improve awareness and treatment of these conditions (6). The role of PTX3 in patients with ischemic stroke focuses on the inflammatory responses to blood vessel damage due to cerebral ischemia. It's a glycoprotein that has been rapidly produced in response to proinflammatory cytokines like TNF- α and IL-1. High PTX3 levels can be seen after cerebrovascular disease like myocardial infarction, signifying its role in the inflammatory responses that occur after tissue injury. Also, PTX3 accelerates the activation of complement pathways and the attraction of immune cells, thus connecting innate immunity to vascular pathology (7). However, the specific role of PTX3 concentrations in ischemic stroke outcomes is still unclear. The various studies are inconsistent in regarding

its possibility as a disease severity marker for stroke and its failure as a predictor of stroke events (8, 9). Despite the fact that some research has reported increased PTX3 levels in stroke patients (10, 11), this has raised questions about its clinical significance. The complexity of the issue is amplified when considering patients with PCOS. This condition is also characterized by hyperandrogenism, insulin resistance, and low-grade chronic inflammation, all of which are known to be metabolically associated with cardiovascular disease risk factors, including stroke. Women with PCOS have been noted to have more cardiovascular disease complications and mortality than women without PCOS (12), with significantly increased levels of inflammatory markers such as C-reactive protein (CRP) (13). However, the relationship between the protein PTX3 and ischemic stroke within the PCOS population has not been well explored. Research suggests that the persistent inflammatory state that is characteristic of PCOS may regulate PTX3 production, which in turn may influence the risk of stroke. Thus, the investigation of the role of PTX3 in ischemic stroke in the context of PCOS may open up new possibilities for treatment and risk assessment, given the possible consequences of these associations. This study aims to evaluate whether PTX3 levels are elevated in ischemic stroke patients with PCOS and whether increased PTX3 is linked to greater stroke risk and worse outcomes. The results may help to improve the understanding of the mechanisms of inflammation in ischemic stroke in patients with PCOS and, therefore, to develop more specific approaches to stroke prevention and treatment

Patients and Methods

Study design: A case-control study was conducted at Azadi Teaching Hospital in Kirkuk, starting from 1st February 2023 to 1st March 2024. The study was conducted in the neurology, obstetrics, and gynaecology clinics, and the

women who had polycystic ovarian syndrome (PCOS) with and/or without stroke, as well as healthy women, were included in the study.

Data collection, participants, and clinical assessment: Patients were screened for eligibility according to the relevant diagnostic criteria after obtaining written informed consent from the

participants and were divided into three groups: the first group had PCOS with stroke, while the second group had PCOS without stroke; and the third group was considered an age-matching control group with age ranges between 18 and 50 years.

The inclusion criteria include enrolling cases of PCOS diagnosed according to Rotterdam criteria and ischemic stroke diagnosed by a neurologist, depending on clinical manifestation and radiological imaging studies. The data were collected according to a standardized questionnaire that contains general information, medical history, and drug history. Blood samples were withdrawn in the morning after an overnight fast.

Exclusion criteria : The exclusion criteria included patients with other types of cerebrovascular diseases, malignant conditions, hemorrhagic stroke, severe systemic diseases, autoimmune or other chronic inflammatory diseases, or the use of immunosuppressive or anti-inflammatory drugs.

Laboratory investigations: The levels of Pentraxin-3 (PTX3) and C-reactive protein (CRP) were measured using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique, following the manufacturer's instructions. PTX3 was calculated using the Human Pentraxin 3/TSG-14 Quantikine ELISA Kit (R&D Systems, Catalog # DPTX30). CRP was measured using the Human CRP ELISA Kit (Abcam, Catalog # ab260058). The standard reference ranges are 2-5ng/mL and 0-10mg/dL, respectively. The triglyceride/high-density lipoprotein (TG/HDL) ratio was measured depending on the fasting lipid profile. Fasting blood samples were analyzed for serum triglycerides and HDL-C levels using enzymatic colorimetric methods with an automated chemistry analyzer (Roche Cobas c311), and reagents supplied by Roche (Triglycerides Gen.2, Catalog # 20767107322; HDL-Cholesterol

Gen.4, Catalog # 05168538190). The TG/HDL-C ratio was determined by dividing the triglyceride concentration (mg/dL) by the HDL-C concentration (mg/dL).

Insulin resistance was assessed by calculating the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index using the following formula: homeostatic model assessment for insulin resistance (HOMA-IR) = fasting insulin (μ U/mL) $\times$ fasting glucose (mg/dL) and dividing by 405 (14). Fasting glucose was measured using the Roche Glucose Hexokinase reagent (Catalog # 04404483 190), and fasting insulin was measured using the Human Insulin ELISA Kit (Abcam, Catalog # ab100578). The severity of stroke was assessed by the National Institutes of Health Stroke Scale (NIHSS) (15).

Statistical Analysis

Statistical analyses were conducted using SPSS version 26. For descriptive and continuous data, the means and standard deviations were calculated. The normal distribution of the data was measured using the Shapiro-Wilk test. For comparing the biomarker levels between the three groups, analysis of variance (ANOVA) was used, and Tukey's post-hoc test was performed for pairwise comparisons if the ANOVA results were significant. To determine the relationship between PTX3, CRP, TG/HDL ratio, HOMA-IR, and stroke severity as measured by the NIHSS, the Pearson correlation was applied. The set of statistical tests consisted of ANOVA for the comparison of the levels of biomarkers among the three groups, the post-hoc Tukey test for the determination of the specific differences between the pairs of groups, the Pearson correlation coefficient for the assessment of the relationships between the biomarkers and stroke severity, and regression analysis in the evaluation of the predictive value of the biomarkers with respect to stroke severity.

A p-value of less than 0.05 was considered significant.

Results

Study design: The study was carried out, and the participants were divided into three categories according to their medical history and status: the PCOS with Stroke Group, the polycystic ovarian syndrome (PCOS) without Stroke Group, and the healthy control Group.

Demographic and biomarker variables across the studied groups:

There was no apparent statistical disparity in the mean age of the groups. The pentraxin-3 (PTX3) levels were greatly increased in the PCOS with stroke group as compared to the PCOS without Stroke and the healthy control group. The mean PTX3 concentration was quite striking and was seen to be highest at 5.717 ± 1.172 ng/mL in the PCOS stroke group, while those with PCOS without stroke had a mean PTX3 level of 3.665 ± 1.085 ng/mL, and the healthy controls had a mean PTX3 level of 2.377 ± 0.740 ng/mL. This difference was statistically highly significant ($p < 0.001$). When CRP levels were further checked there was an increase in both PCOS groups when compared to the healthy control group. The mean C-reactive protein (CRP) level of the PCOS Stroke group was 8.043 ± 2.102 mg/L while that of the PCOS without Stroke group was 5.448 ± 1.532 mg/L compared to the healthy controls who had a lower mean CRP level of 3.037 ± 0.959 mg/L. However, the CRP values were higher than the normal values in both groups, but the difference in the stroke and non-stroke subgroups was not statistically significant ($p = 0.651$).

The triglyceride/high-density lipoprotein (TG/HDL) ratio and homeostatic model assessment for insulin resistance (HOMA-IR) values were also higher in both groups of patients with PCOS when compared with the healthy control group. Specifically, the TG/HDL ratio was found to be 4.297 ± 0.935 in the PCOS stroke group, while the PCOS without stroke group had

a ratio of 3.719 ± 0.863 . Nonetheless, the mean TG/HDL Ratio of Healthy Controls was considerably lower at 2.203 ± 0.585 . Also, the HOMA-IR values of the groups were 3.547 ± 0.949 for the PCOS stroke group, 3.194 ± 1.034 for the PCOS without Stroke group, and 1.771 ± 0.492 for the healthy controls; however, there was no statistically significant difference in the TG/HDL ratio and HOMA-IR values between the groups, as seen by the p-values of 0.189 and 0.148, respectively.

An analysis of variance (ANOVA) was done to compare the mean biomarker levels over three groups. The results showed a highly significant difference in the mean PTX3 levels, with a p-value of less than 0.001. This meant that the group called PCOS with Stroke had the highest levels and thus was confirmed to be the group with the highest levels. On the other hand, there was no statistically significant difference in the CRP levels ($p = 0.651$). Similarly, there was no statistical significance in the comparison of the TG/HDL ratio and HOMA-IR between the groups, as shown in Table 1.

Correlation of markers with stroke severity:

A Pearson correlation analysis was performed to determine the correlation of PTX3, CRP, TG/HDL ratio, and HOMA-IR with the severity of strokes as determined by the NIH Stroke Scale. The results showed a positive correlation between the NIH Stroke Scale scores and the PTX3 levels ($r = 0.42$, $p < 0.001$). It means that there is an association between raised levels of PTX3 and increased severity of stroke. However, the results of the analysis failed to show a correlation between CRP, TG/HDL Ratio, and HOMA-IR with the severity of strokes Table 2.

Table 1. Comparison of demographic and biomarker variables across three distinct studied groups.

Variables	PCOS with Stroke		PCOS without Stroke		Healthy Control		P-Value
	Mean	SD	Mean	SD	Mean	SD	
Age (Years)	33.160	8.931	33.720	9.170	32.893	9.258	0.852†
PTX3 (ng/mL)	5.717	1.172	3.665	1.085	2.377	0.740	<0.001*
CRP (mg/L)	8.043	2.102	5.448	1.532	3.037	0.959	0.651†
TG/HDL Ratio	4.297	0.935	3.719	0.863	2.203	0.585	0.189†
HOMA-IR	3.547	0.949	3.194	1.034	1.771	0.492	0.148†
NIHSS	14.507	7.119	N/A	N/A	N/A	N/A	N/A

† Anova, *Post-hoc Tukey test (PCOS with stroke group as reference), PTX3= Pentraxin-3, HOMA-IR= Homeostatic model assessment for insulin resistance, CRP= C-reactive protein, TG/HDL= Triglyceride/ high density lipoprotein, NIHSS= National Institutes of Health Stroke Scale.

Table 2. Pearson Correlation Coefficients between Biomarkers and Stroke Severity (NIH Stroke Scale).

Variable	Pearson's r	p-value
PTX3 (ng/mL)	0.429	< 0.001
HOMA-IR	-0.115	0.520
CRP (mg/L)	-0.097	0.595
TG/HDL Ratio	-0.030	0.796

PTX3= Pentraxin-3, HOMA-IR= Homeostatic model assessment for insulin resistance, CRP= C-reactive protein, TG/HDL= Triglyceride/ high density lipoprotein.

Relation of various biomarkers with NIH stroke scale severity in PCOS: Figure 1 demonstrates the relationship of 4 biomarkers namely PTX3, CRP, TG/HDL ratio, and HOMA-IR, with stroke severity as measured by the NIH Stroke Scale in patients with PCOS who have had a stroke. Each plot shows a regression line that represents the average trend and a 95% confidence interval that surrounds the line. The relationship between the levels of PTX3 and the severity of stroke is positive and statistically significant. This can be seen from the positively inclined regression line which has a distinct slope. This

finding indicated that increased levels of PTX3 are associated with more severe strokes in PCOS patients, signifying its possibility as a predictor of stroke severity. On the other hand, the scatter plot that associates CRP levels to the NIH Stroke Scale is almost horizontal, indicating a low correlation between CRP and stroke severity. Moreover, the TG/HDL Ratio has a mild increase with the NIH Stroke Scale that indicates a weak relationship.

Finally, the relation between HOMA-IR and stroke scale severity shows weakly negative trend, signifying that HOMA-IR has a poor correlation with stroke severity.

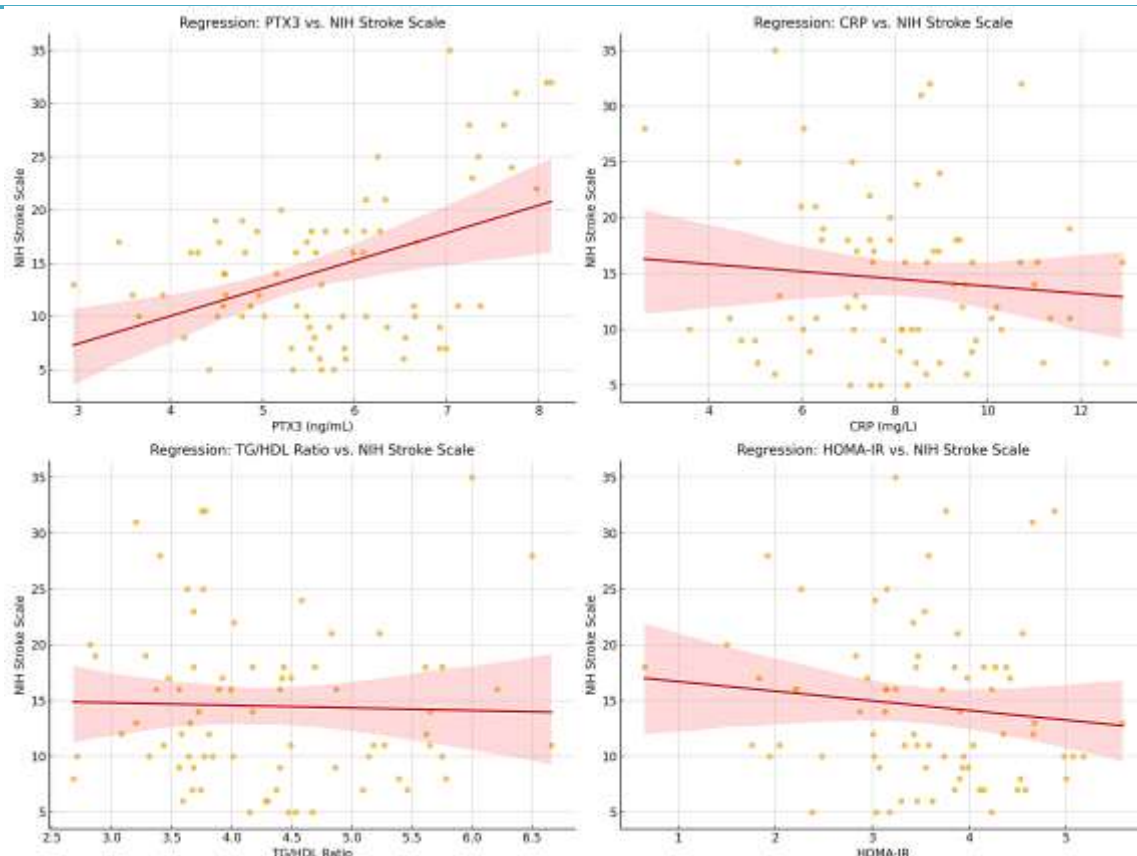


Figure 1. Regression line analysis shows the relation of various biomarkers with NIH stroke scale severity in PCOS.

Discussion

Ischemic stroke is one of the emergent neurological disorders that are common with high morbidity and mortality rate in the world (16). It's important to manage this condition to minimize the morbidity and mortality rate. Currently, biomarkers that are related to inflammatory and metabolic changes are considered as markers of the risk of ischemic stroke especially in people with other diseases like polycystic ovarian syndrome (PCOS). PCOS is defined by a disturbance of hormone secretion, which may involve elevated androgen production and the attenuation of the effectiveness of insulin, as well as lowered levels of inflammation – all of which are known to be the risks for various conditions, including strokes (17). A major problem in managing strokes among PCOS patients is to determine the effect of each biomarker on the severity and progression of the stroke.

The present study examined the biomarkers such as pentraxin-3 (PTX3), C-reactive protein (CRP), triglyceride/high density lipoprotein (TG/HDL) ratio, and homeostatic model assessment for insulin resistance (HOMA-IR) together to capture the interconnected inflammatory and metabolic pathways that may raise ischemic stroke risk in PCOS patients: PTX3 reflects local vascular inflammation, CRP indicates systemic inflammation, the TG/HDL-C ratio signals atherogenic dyslipidemia, and HOMA-IR measures insulin resistance, factors that collectively contribute to vascular dysfunction and stroke development in this population.

The current study showed an increase in PTX3 levels among individuals in the PCOS with Stroke group compared to those in both the PCOS without Stroke and the Healthy Control groups ($p < 0.001$). This finding aligns with the Ye, X., et al. study that

has linked PTX3 levels to vascular inflammatory and ischemic events of cardiovascular disease (18).

The strong connection between PTX3 levels and NIH Stroke Scale ratings (correlation coefficient of 0.429 with a p-value less than 0.001) indicates that PTX3 could serve as an indicator for estimating stroke severity in patients with PCOS. It's crucial to mention that the examination results related to CRP, TG/HDL ratio, and HOMA IR present a more complex scenario. CRP levels were higher in both PCOS groups compared to the healthy controls group; however, the variance between the PCOS with and without Stroke groups did not reach statistical significance ($p=0.651$). This finding challenges the Biya A. study that highlighted CRP as an indicator of stroke severity (19). In our study, the findings did not show a link between factors, possibly due to the nature of inflammation in PCOS conditions. This suggests that CRP alone might not be sensitive enough to identify the precise vascular inflammatory processes linked to strokes. In a way, the TG/HDL ratio and HOMA IR levels in both groups, with PCOS compared to healthy controls, suggest the metabolic issues commonly seen in PCOS cases. There were no variances found in either the TG/HDL ratio or HOMR index between the groups with PCOS Stroke and those without (with p-values of 0.189 for the TG/HDL ratio and 0.148 for the HOMA IR index). This finding differs from studies that suggest high TG/HDL ratios may be indicative of a problem like strokes (20, 21). One way to understand this situation is that dyslipidemia might not affect stroke severity on its own but could also be influenced by factors such as endothelial dysfunction and chronic inflammation, rather than just lipid metabolism (22).

The connection between PTX3 and ischemic stroke has been extensively studied by researchers who believe it could serve as an indicator of prognosis. For instance, Zhu Y et al. study found that higher PTX3 levels are independently associated with increased mortality after ischemic stroke (23). However, conflicting results come from Zhang CY. et al. study, which showed no link between PTX3 and the severity of stroke (24). The variation in results could be due to the variations in the characteristics of patients and their current health conditions. Our research concentrates on patients diagnosed with PCOS, providing a distinct viewpoint on how metabolic and hormonal aspects impact PTX3 expression levels in relation to CRP, TG/HDL ratio, and HOMA-IR. The lack of findings could be attributed to the range of PCOS presentations. Some individuals may exhibit more pronounced metabolic issues than others do. Furthermore, the association between stroke severity and HOMA IR, a metric for insulin resistance, was not found to be statistically significant. This observation aligns with the study conducted by Gu T. et al. In a survey conducted in 2020 (25), it was found that although insulin resistance is recognized, the exact link between it and the seriousness of ischemic stroke remains unclear. Our results indicate that even though insulin resistance is common among those with PCOS, it may not be directly associated with increasing the risk of stroke unless accompanied by other risk factors. The specific roles of the biomarkers studied in this study highlight the process of developing stroke in patients with PCOS. Our research has certain limitations that need to be considered. Firstly, the structure of our study makes it challenging to establish cause-and-effect relationships between biomarkers and stroke severity clearly. Long-term studies are necessary to understand how these biomarkers evolve. Additionally, although our sample size is adequate for statistical analysis, it may not represent all PCOS types. Furthermore, exploring the metabolic

aspects in more detail could illuminate the connection between biomarkers and stroke outcomes.

Conclusion

Pentraxin-3 levels are markedly higher in PCOS patients who have had an ischemic stroke, and the level is in proportion to the severity of the stroke. This means that PTX3 could be potentially used as a biomarker for risk assessment. Nevertheless, other biomarkers such as CRP, the TG/HDL ratio, and HOMA-IR did not show significant differences when compared between stroke and non-stroke patients, which suggests that PTX3 may be a better predictor than the conventional markers. Further work is needed to determine the relationship between PTX3 and stroke-related complications in PCOS and to confirm these results in larger, multicenter studies.

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Ethical clearance:

The study was conducted ethically and followed the guidelines of the Declaration of Helsinki; ethical clearance was obtained from the Research Ethics Committee of the University Of Kirkuk College Of Medicine (Document no.70, date May 14, 2025). Anonymity and confidentiality were maintained at all times during the study, and written informed consent was obtained from all participants before they were enrolled without any other sources.

Conflict of interest: None.

Use of Artificial Intelligence (AI): The authors state they did not use any generative AI tools for creating or editing the manuscript's language.

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تقييم مستوى البنتراكسين ٣ بين مرضى الجلطة الدماغية الإقفارية المصابين بمتلازمة تكيس المبايض

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الملخص

الخلفية: يُعد البنتراكسين-٣ (Pentraxin 3) مكونًا هامًا من مكونات جهاز المناعة الفطرية، ويلعب دورًا مهمًا في العمليات الالتهابية وتلف الأنسجة. قد يوفر تقييم مستوياته في الحالات السريرية المختلفة رؤى حول أهميته التشخيصية والتنبؤية.

الأهداف: تقييم العلاقة بين مستويات البنتراكسين-٣ و حدوث السكتة الدماغية الإقفارية بين المصابات بمتلازمة تكيس المبايض.

المواد والطرق: تم إجراء دراسة الحالات والشواهد في عيادات الأعصاب والتوليد وأمراض النساء في مستشفى آزادي التعليمي في كركوك، ابتداءً من ١ شباط ٢٠٢٣ وحتى ١ آذار ٢٠٢٤. تم تشخيص النساء المصابات بمتلازمة تكيس المبايض وفقًا لمعايير روتردام، وقُسمن إلى ثلاث مجموعات: المجموعة الأولى ضمت مريضات لديهن تكيس المبايض مع سكتة دماغية، والمجموعة الثانية ضمت مريضات لديهن تكيس المبايض بدون سكتة دماغية، والمجموعة الثالثة كانت مجموعة ضابطة مطابقة بالعمر تتراوح أعمار المشاركات فيها بين ١٨-٥٠ سنة. تم سحب عينات الدم في الصباح لتحديد مستويات PTX3 و CRP ونسبة TG/HDL ومؤشر مقاومة الإنسولين (HOMA-IR) وتم قياسها في جميع المجموعات المدروسة.

النتائج: كانت مستويات PTX3 أعلى بشكل ملحوظ في مجموعة مريضات تكيس المبايض المصابات بالسكتة الدماغية ($1,172 \pm 5,717$ نانوغرام/مل) مقارنة بالمصابات بتكيس المبايض بدون سكتة دماغية ($1,085 \pm 3,665$ نانوغرام/مل)، والمجموعة الضابطة ($2,377 \pm 0,740$ نانوغرام/مل) ($p < 0.001$). أظهرت مستويات البنتراكسين-٣ ارتباطًا إيجابيًا مع شدة السكتة الدماغية ($r = 0.42, p < 0.001$)، بينما لم تكن هناك علاقة ذات دلالة إحصائية مع CRP أو نسبة TG/HDL أو HOMA-IR.

الاستنتاج: مستويات البنتراكسين-٣ مرتفعة بشكل ملحوظ لدى المصابات بمتلازمة تكيس المبايض واللواتي تعرضن لسكتة دماغية إقفارية، وترتبط هذه المستويات بشدة السكتة. مما يشير إلى إمكانية استخدام PTX3 كواسم حيوي لتقييم خطر الإصابة بالسكتة الدماغية.

الكلمات المفتاحية: متلازمة تكيس المبايض؛ السكتة الدماغية الإقفارية؛ البنتراكسين-٣، PCOS، CRP

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