Association Between Homocysteine Levels and Ovarian Reserve in Subfertile Women

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ABSTRACT

Objective: To evaluate the relationship between homocysteine levels and anti-mullerian hormone (AMH) levels, a biomarker of ovarian reserve, and the effect of high homocysteine levels on ovarian reserve in subfertile women.

Study Design: Observational case-control study.

Place and Duration of the Study: Samsun Training and Research Hospital, Samsun, Turkiye, from October to December 2023.

Methodology: Seventy-nine subfertile women and 35 healthy fertile women were included in this study. AMH, homocysteine, thyroid stimulating hormone (TSH), free T3 (fT3), free T4 (fT4), iron, and ferritin levels of subfertile and fertile women were compared. Logistic regression, receiver operating characteristic, and Kaplan-Meier analyses were performed for homocysteine levels.

Results: AMH, fT4, iron, and ferritin levels were lower in subfertile women than in fertile women ($p < 0.001$). Homocysteine and TSH levels were higher in subfertile women than in fertile women ($p < 0.001$). The sensitivity for homocysteine levels was 94.90% and the specificity was 94.30%. In the Kaplan-Meier analysis, homocysteine levels of 12.90 µmol/L and above were found to be risky in terms of fertility. Homocysteine levels, AMH, and ferritin levels were negatively correlated and TSH levels were positively correlated in subfertile women ($p < 0.001$). However, these correlations were not observed in fertile women ($p > 0.05$).

Conclusion: High homocysteine levels can be considered as a risk factor affecting ovarian reserve in subfertile women.

Key Words: *Anti-mullerian hormone, Female subfertility, Homocysteine, Hyperhomocysteinaemia, Ovarian reserve.*

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INTRODUCTION

Subfertility is a growing health problem in the industrialised countries.[1] Approximately 10-15% of couples worldwide are affected by subfertility. Only half of subfertility cases have a female factor, and ovulation is the primary cause in half of cases.[2] Approximately 12.7% of women of childbearing age in the United States seek subfertility treatment. Approximately 85% of subfertility cases have an identifiable cause, while 15% are referred to as unexplained subfertility. Proper diagnosis and effective treatment can make it easier for couples undergoing subfertility treatment to achieve their fertility goals.[3]

Anti-mullerian hormone (AMH), a reliable marker of ovarian reserve, regulates different steps of folliculogenesis through its neuroendocrine effects. AMH expression is primarily regulated by bone morphogenetic proteins, gonadotropins, and oestrogen.[4]

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AMH has a good predictive value for reproductive lifespan and menopause timing and is superior to day 3 follicle-stimulating hormone (FSH).[5] Physiologically, AMH impairs the aromatisation capacity of granulosa cells in antral follicles. AMH is involved in the regulation of follicular growth initiation and the threshold of FSH sensitivity.[6] In contrast, high serum AMH levels in patients with polycystic ovary syndrome (PCOS) are involved in the main steps of anovulation. In addition, the interactions of AMH with internal and external ovarian factors such as FSH are associated with the pathogenesis and pathophysiological features of PCOS. PCOS is a major cause of anovulatory subfertility.[7]

Homocysteine, an amino acid containing a sulfhydryl group, is an intermediate in the metabolism of the amino acids cysteine and methionine. High homocysteine levels are considered as a predictive risk factor for additional screening for cardiovascular disease, stroke progression, vitamin B12 deficiency, and methionine metabolism.[8] In addition, considering the relationship between high homocysteine levels and fertility, high homocysteine levels are weakly associated with oocyte aneuploidy.[9] Observational studies have associated high homocysteine levels with pregnancy loss, decrease in offspring birthweight, and female fertility.[10] However, lowering homocysteine levels in women with PCOS may improve fertility outcomes.[11]

Among the main causes of subfertility, the most common cause of ovulation disorders and amenorrhoea is PCOS, which affects 70% of women with ovulation disorders. Other causes include health problems such as thyroid disease, pituitary disease, and adrenal hyperplasia. Therefore, in addition to history and physical examination, thyroid panel levels are checked as diagnostic tests for ovulation disorders. Correction of the specific defect in response to abnormal thyroid panel levels may stimulate ovulation.[3] In addition, a study of 156 women diagnosed with PCOS reported that ferritin levels were significantly associated with low AMH levels and decreased ovarian volume.[12] On the other hand, non-protein-bound iron catalyses the formation of homocysteine from methionine, S-adenosyl-L-homocysteine (SAH), and cystathionine.[13]

The aim of this study was to investigate the effect of homocysteine, a high-risk factor for many pathologies, on subfertility using AMH, a reliable indicator of ovarian reserve. In addition, the relationship between these two parameters, the sensitivity and specificity of homocysteine levels on subfertility, and the level of fertility risk were investigated.

METHODOLOGY

Permission was taken from the Human Research Ethics Committee of the Samsun Training and Research Hospital, Samsun, Turkiye (Committee Decision Number: GOKA/2023-9-2; Approval Date: 14 September 2023). The patient's data were retrospectively scanned and retrieved from the hospital's information management system from October to December 2023. The participants were contacted and given detailed information about the purpose and scope of the study and were included in the study by obtaining their consent if they volunteered.

This study was an observational case-control study. The number of participants planned to be included in the study was determined with G*Power 3.1.9.7, a statistical power analysis programme. As a result of the sample size analysis, there were at least thirty-five participants in each group. Seventy-nine women who followed up with the diagnosis of subfertility were included in the subfertile (case) group. Thirty-five healthy women without any health problems were included in the fertile (control) group. Being older than 45 years, younger than 18 years, having irregular menstruation, and having any health problem were determined as exclusion criteria. Homocysteine levels in both groups were compared. AMH and thyroid stimulating hormone (TSH), free T3 (fT3), free T4 (fT4), iron, and ferritin levels were also compared. Logistic regression, receiver operating characteristic (ROC), and Kaplan-Meier analyses were performed for homocysteine levels. Correlation analysis was performed to determine the relationship between parameters within the groups.

Women in the subfertile group were those who had been diagnosed as subfertile because they could not get pregnant despite having regular sexual intercourse for at least a year. In addition, the women included in this group were determined according to certain criteria. These criteria were regular menstruation, being in a certain age range (18 - 40 years), intact ovaries, both tubes open in hystero-salpingogram, and no other health problems (chronic diseases such as diabetes, hypertension, cardiovascular diseases, neurological diseases, autoimmune disorders, cancer, etc.). The semen analysis of the husbands of the women in this group did not show any obstacle to having biological children and no health problems were observed in terms of fertility.

Individuals in the fertile group were those who had given birth at least once. In addition, the individuals included in this group were determined according to certain criteria. These criteria were regular menstruation, being in the reproductive age range (18 - 40 years), and not having any health problems (chronic diseases such as diabetes, hypertension, cardiovascular diseases, neurological diseases, autoimmune disorders, and cancer, etc.).

Blood samples were collected between 8:00 am and 10:00 am after fasting for 10-12 hours. Homocysteine and AMH levels were analysed using the chemiluminescence immunoassay method on a Cobas 601 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Iron and ferritin levels were analysed using the chemiluminescence immunoassay method on an Immunity 2000 autoanalyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum fT3, fT4, and TSH levels were analysed using the chemiluminescence microparticle immuno-assay method on a Cobas 8000 autoanalyzer (Roche Diagnostics, Mannheim, Germany).

Statistical analyses were performed *via* SPSS version 22 for Windows (IBM, Chicago, USA). The distribution of continuous variables was tested with the Shapiro-Wilk normality test. It was observed that these data did not exhibit a normal distribution. All data were presented as the median (25-75th percentile) for continuous variables. The groups were compared using the Mann-Whitney U test. Binary logistic regression and ROC curve analyses were performed for the homocysteine levels. In addition, Kaplan-Meier analysis was performed to determine the risk levels of homocysteine in terms of fertility. In addition, correlation analysis was performed using the Spearman correlation analysis. A p-value below 0.05 was considered statistically significant.

RESULTS

Seventy-nine women diagnosed with subfertility between the ages of 20 and 45 years were included in the subfertile group, while 35 healthy women who had given birth at least once between the ages of 24 and 37 years were included in the fertile group. The mean age of the fertile group was 30.62 ± 3.75 years, whereas the mean age of the subfertile group was 30.52 ± 5.90 years. There was no significant difference in mean age between the two groups. The laboratory results of these two groups are shown in Table I.

Homocysteine and TSH levels were higher in the subfertile group than in the fertile group. AMH, fT4, iron, and ferritin levels were lower in the subfertile group than in the fertile group. fT3, fT4, and TSH levels in both groups were found to be within reference values. In addition, the AMH, iron, and ferritin levels of the subfertile group were below the reference values, and the homocysteine levels were above the reference values (Table I).

Table I: Laboratory findings of fertile and subfertile groups.

Parameter	Reference range	Fertile (n = 35) Median (Q1 - Q3)	Subfertile (n = 79) Median (Q1 - Q3)	p-value*
AMH (ng/mL)	1.52 - 9.95 (20 - 25 years) 1.20 - 9.05 (25 - 30 years) 0.71 - 7.59 (30 - 35 years)	2.20 (1.90 - 2.95)	0.68 (0.31 - 0.81)	<0.001
Homocysteine (µmol/L)	5.00 - 12.00	9.00 (7.55 - 9.15)	12.90 (12.05 - 14.10)	<0.001
TSH (IU/L)	0.27 - 4.20	2.16 (2.07 - 2.30)	3.04 (2.86 - 3.26)	<0.001
fT3 (ng/L)	2.00 - 4.80	3.08 (2.68 - 3.58)	3.31 (3.09 - 3.60)	>0.05
fT4 (ng/dL)	0.93 - 2.00	1.54 (1.43 - 1.64)	1.19 (1.12 - 1.29)	<0.001
Iron (mg/dL)	60 - 180	86.00 (68.50 - 103.00)	34.00 (31.00 - 39.00)	<0.001
Ferritin (µg/L)	30 - 400	28.90 (14.40 - 43.80)	18.30 (13.05 - 22.80)	<0.001

** Mann-Whitney U test; AMH: Anti-Mullerian hormone; TSH: Thyroid stimulating hormone; fT3: free T3; fT4: free T4; Q1: First quarter; Q3: Third quarter.*

Table II: Correlation of homocysteine levels with other parameters.

Parameter		Fertile (n = 35)	Subfertile (n = 79)
AMH	Cc	0.294	-0.443
	P*	>0.05	<0.001
TSH	Cc	0.179	0.466
	P*	>0.05	<0.001
fT3	Cc	0.120	-0.008
	P*	>0.05	>0.05
fT4	Cc	0.283	-0.033
	P*	>0.05	>0.05
Iron	Cc	0.024	-0.076
	P*	>0.05	>0.05
Ferritin	Cc	0.102	-0.416
	P*	>0.05	<0.001

**Spearman correlation analysis; Cc: Correlation coefficient; AMH: Anti-mullerian hormone; TSH: Thyroid stimulating hormone; fT3: free T3; fT4: free T4.*

In the binary logistic regression analysis performed for the homocysteine levels, the Nagelkerke R^2 was determined to be 0.955 and the expected rate was 98.20% ($p < 0.001$).

ROC analysis was performed to assess the sensitivity and specificity of homocysteine levels.

The area under the curve (AUC) for homocysteine levels was determined to be 0.997 (95% CI = 0.992 - 1.000), the cut-off value was 11.25 µmol/L, the sensitivity was 94.90%, and the specificity was 94.30% ($p < 0.001$).

Kaplan-Meier analysis was performed to examine the effect of homocysteine levels on fertility. The median fertility value was 12.90 (95% CI, 12.54 - 13.26) for homocysteine levels according to the results of the analysis. The Kaplan-Meier analysis predicted that homocysteine levels of 12.90 µmol/L or higher are risky for fertility.

The results of the correlation analysis between the groups' homocysteine levels and other parameters are shown in Table II.

There was no correlation between homocysteine levels and other parameters in the fertile group. In the subfertile group, there was a negative correlation between homocysteine levels and AMH and ferritin levels; and a positive correlation with TSH levels (Table II).

DISCUSSION

Understanding the biomarkers of ovarian reserve is crucial for the assessment of female subfertility. AMH levels show a strong correlation with the number of growing follicles and are increasingly used as one of the biomarkers of ovarian reserve.[14] However, the lack of international standardisation of AMH levels is an important issue.[15] Little is known about the endogenous and exogenous factors that may affect AMH.[14] In this study, subfertile women had no health problems other than the diagnosis of subfertility and their AMH levels were lower than fertile women and below the reference range.

Blood homocysteine levels rise because of cofactor and enzyme dysfunction associated with homocysteine metabolism. Many studies have shown a strong association between high homocysteine levels and various diseases.[16,17] High homocysteine levels have also been reported as a risk factor for congenital birth defects and early pregnancy loss. In addition, high homocysteine levels are recognised as a cause of maternal obstetric complications such as pre-eclampsia.[18] Adults with total homocysteine levels of 10 µmol/L or less are usually safe, but levels of 11µmol/L or more may require treatment. Homocysteine is a guide to disease prevention rather than a disease biomarker.[16] In this study, homocysteine levels in subfertile women were above the reference range and higher than the fertile women, and the cut-off in the ROC analysis was 11.25 µmol/L. In addition, homocysteine levels of 12.90 µmol/L or higher were found to be a risk for fertility.

One of the components of the follicular fluid is homocysteine. Therefore, abnormal homocysteine levels may affect oocyte development. Reduced levels of homocysteine in follicular fluid have been shown to significantly improve oocyte maturation and embryo quality in subfertile women undergoing assisted reproductive therapy.[19] In addition, homocysteine levels and the incidence of hyperhomocysteinaemia have been found to be higher in women with unex-

plained subfertility than in fertile women.[20] The reference range for homocysteine levels varies depending on the method of analysis. When immunoassay methods are evaluated, the reference range is 5.0 - 12.0 µmol/L.[21] In this study, homocysteine levels were analysed using a chemiluminescence immunoassay method. In addition, homocysteine and AMH levels were inversely correlated in subfertile women. However, this was not observed in fertile women.

Severe thyroid dysfunction can lead to subfertility through direct and indirect interactions with the reproductive organs and the hypothalamic-pituitary-ovarian axis. The prevalence of thyroid autoimmunity is high in women with unexplained subfertility.[22] In addition, TSH and AMH levels are among the determinants of live birth in women with unexplained subfertility. However, the advantage of AMH levels over TSH levels is that they vary little over the menstrual cycle.[23] In this study, the TSH levels of the subfertile women were higher than those of the fertile women. In addition, TSH levels were directly correlated with homocysteine levels in subfertile women. However, this was not observed in fertile women.

Low serum ferritin levels may be associated with an increased risk of miscarriage in women with unexplained subfertility.[24] An inverse association between ferritin levels and previous pregnancy loss has been found in women with recurrent pregnancy loss.[25] In this study, the ferritin and iron levels of the subfertile women were lower than those of the fertile women. In addition, the ferritin and iron levels of the subfertile women were below the reference range. Ferritin levels were also inversely correlated with homocysteine levels in subfertile women. However, this correlation was not seen in fertile women.

The limitation of this study is that it was not evaluated according to a specific age range. This is because of the sample size. However, it can form a basis for future research with larger sample sizes and evaluations according to a certain age range.

CONCLUSION

Although homocysteine levels are not associated with AMH levels in fertile women, they are inversely associated in subfertile women. The reason for this is related to the hyperhomocysteinaemia detected in subfertile women. ROC and Kaplan-Meier analyses confirmed this. In the ROC analysis for homocysteine levels, the cut-off value was 11.25 µmol/L and the AUC value was 0.997. Hyperhomocysteinaemia should be considered as one of the causes of unexplained subfertility or low AMH. Homocysteine levels should be analysed in unexplained subfertile women or in subfertile women undergoing assisted reproduction treatment, and if hyperhomocysteinaemia is present, treatment should be designed accordingly.

ETHICAL APPROVAL:
The necessary ethical approval for the study was obtained from the Samsun Education and Research Hospital Human Research Ethics Committee. (Committee Decision Number GOKA/2023-9-2, Approval Date: 14 September 2023).

PATIENTS' CONSENT:
Informed consent was obtained from the participants for the publication of the data.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
AK: Conception and designing of the study, creating the study plan, acquisition, analysis, interpretation of data, and writing of the manuscript.
RA: Data processing, design of the work, and critical revision of the manuscript.
Both authors approved the final version of the manuscript to be published.

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