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Comparison of Blood Homocysteine Levels in Women with Unexplained Infertility and Women with Normal fertility

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ABSTRACT

Background: Subfertility affects approximately 15% of couples globally, with unexplained infertility being a common diagnosis. Homocysteine, a sulfur-containing amino acid, has been implicated in reproductive health, but its association with unexplained infertility remains unclear.

Aim: This observational case–control study aimed to compare homocysteine levels among women with unexplained infertility and those with normal fertility.

Methods: Fifty women with unexplained infertility and fifty controls matched for age and BMI were recruited. Homocysteine levels were measured using venous blood samples collected after overnight fasting. Statistical analysis included independent t-tests, ROC (receiver operating characteristic) curve analysis, and odds ratio calculations.

Results: Women with unexplained infertility had significantly higher mean homocysteine levels compared to controls (15.05 ± 2.4 vs. 9.2 ± 2.3 μmol/L, $p < 0.001$). The ROC analysis showed a cutoff value of 12.3 μmol/L, with a sensitivity of 92.0% and a specificity of 99.9%. The odds ratio for unexplained infertility with homocysteine levels >12.3 μmol/L was 180.16 ($p < 0.001$).

Discussion: The findings are consistent with previous studies, suggesting elevated homocysteine levels as a potential biomarker for unexplained infertility. The high discriminatory power of homocysteine levels indicates its potential diagnostic utility in identifying women at risk for unexplained infertility.

Conclusion: Determination of homocysteine levels may be helpful in diagnosing unexplained infertility, providing a cost-effective biomarker to guide treatment strategies and optimize resource utilization in fertility care. Further research could explore interventions that target homocysteine levels to improve fertility outcomes in affected women.

Keywords: Homocysteine, Infertility, Fertility

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INTRODUCTION

Subfertility is the failure to conceive after one year of regular unprotected sexual intercourse. However, infertility has no chance of spontaneous conception because of bilateral salpingectomy or azoospermia[1]. Infertility is the leading reason women of childbearing age visit their general practitioner. Around one in seven heterosexual couples experiences difficulties with conception. Globally, it is estimated that approximately 15% of couples suffer from infertility[2]. Unexplained infertility, a type of infertility not attributed to ovulation issues, poor sperm quality, tubal pathology, or any known cause[3], is among the most common infertility diagnoses, and approximately 30% of all infertile couples worldwide are diagnosed with unexplained infertility[4]. Although the pathogenesis of unexplained infertility has been extensively investigated, the exact etiology remains unclear[5].

Homocysteine is an amino acid that is produced when methionine is metabolized to cysteine[6]. Hyperhomocysteinemia leads to oxidative stress, which in turn leads to elevated oxidation and lipoperoxidation of proteins, nucleic acids, and carbohydrates. These products are known to be involved in cytotoxicity[7]. Previous published literature has shed light on the relationship between homocysteine and recurrent miscarriages in women[8]. For unknown reasons, elevated maternal serum homocysteine levels have been associated with placenta-mediated complications[9]. Homocysteine has been shown to have an inverse relationship with fertility outcomes[10], with lower homocysteine levels linked to a higher likelihood of clinical pregnancy and improved embryo quality in assisted reproductive technology[11]. Therefore, homocysteine can serve as a disease biomarker[12]. Since there are few publications on the relationship between homocysteine concentration and fertility, providing a simple, cost-effective biomarker technique to monitor and guide effective fertility treatment would contribute to the successful use of the health system resources.

Aim of the study

The aim of this study was to illustrate the difference in homocysteine levels between women with unexplained infertility and those with normal fertility.

PATIENTS AND METHODS

This was a case-control observational study conducted from October 1, 2022 to October 1, 2023 at the Department of Obstetrics and Gynecology in Al Imamain Al Kademain Medical City, Baghdad. The study included 50 females aged 20–40 years who, for unexplained reasons, were unable to achieve spontaneous pregnancy (regular unprotected sexual intercourse) within one year of marriage. Unexplained infertility criteria are:

1. Normal semen analysis (using World Health Organization Criteria)[13]: semen volume 1.4(range 1.3–1.5) ml, total sperm number 39 (range 35–40) (10^6 per ejaculate), progressive motility 42% (range 40–43%), total motility 30% (range 29–31%), vitality 54% (range 50–56%) Normal morphology 4% (range 3.9–4%), immotile sperm 20% (range 19–20%).
2. Ovulation adequacy is confirmed with a mid-luteal serum progesterone level above 10 ng/ml, urine tests indicating an LH surge, or serial transvaginal ultrasounds to monitor the growth and rupture of a dominant ovarian follicle.

The median serum antimüllerian hormone (AMH) values according to different age groups of women are as follows:

1. In the age group 20–25 years: 4.23 ng/mL
2. In the age group 26–30 years: 3.48 ng/mL
3. In the age group 31–35 years: 2.43 ng/mL
4. In the age group 36–40 years: 1.28 ng/mL

A normal uterine cavity and open fallopian tubes can be confirmed through hysterosalpingography or laparoscopy.

The first group was age- and BMI-matched with another 50 women (control group II) who had one or more successful pregnancies and without gestational complications such as IUGR stillbirth and no abortion.

The exclusion criteria were as follows:

1. Age <20 years or >40 years
2. BMI <19 kg/m^2 or >25 kg/m^2

3. Currently pregnant
4. Anemia
5. Gross pelvic pathology
6. Women with significant medical disorder: hypertension, diabetes, tuberculosis and any chronic cardiac, hepatic, pulmonary, or renal disorders that require chronic medications
7. Connective tissue or inflammatory disorder
8. Patients with history of pelvic inflammatory disease.
9. Previous arterial and/or venous thrombosis
10. Infertility due to male factors.
11. Patients undergoing drug therapy with S-adenosylmethionine, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azauridinetriacetate, may exhibit falsely elevated homocysteine levels.
12. Patients who received folate, vitamin B6, and /or B12 supplementation within the last three months.

Thus, the total sample size was 100 women. For each participant, a detailed medical history was collected followed by a thorough clinical examination after obtaining legal consents and explaining the aim of the study.

The assessment included the following:

1. General clinical and obstetric examination were performed on all women enrolled in the study.
2. Five milliliters of venous blood samples were collected from women in both groups to measure the level of homocysteine. Blood samples were drawn from the patients' vein after an overnight fast and centrifuged at 2,500 revolutions per minute (rpm) for 5 minutes.
3. Plasma was stored in tubes until serum homocysteine testing was conducted. The tubes were labeled with a serial number so that their identity could not be disclosed nor could the bias occur. These tubes were kept in ice (cold box) and delivered to the designated laboratory.

The patients were instructed on the following sample collection regulations:

1. Avoid protein-rich meals for at least 24 hours before sample collection, as this may affect the results.
2. Samples of FSH, LH, and prolactin were collected on days 2 to 4 of the menstrual cycle, while progesterone and estradiol were collected on day 21 of the cycle.
3. Timing of collection of serum homocysteine was determined with respect to the menstrual cycle (which was performed on day three of the menstrual cycle).

Verbal and written consents were obtained from each participant, all data collected were used for research purposes only, and no divulge of information occurred. An independent t-test was used to determine the difference in homocysteine levels between the two groups. The receiver operating characteristic curve (ROC) of homocysteine levels was calculated to determine the best cutoff value for homocysteine and to illustrate the best discrimination between the two groups. The odds ratio was also documented. A two-tailed p-value of 0.05 or lower was considered significant in the study.

RESULTS

Table 1 shows the demographic characteristics of this group. The average age of women with unexplained infertility was 30.18 ± 5.5 years.

Table 2 shows the demographic characteristics of the controls. The average age of women with normal fertility was 30.8 ± 5.2 years. The mean duration of marriage was 4.7 ± 3.0 years.

The two groups (cases and controls) were age and BMI (body mass index) matched. Therefore, no statistically significant difference was found between the two groups regarding age, weight, height, and BMI. Table 3 shows a statistically obvious difference in mean homocysteine levels between the two studied groups. The mean homocysteine levels were higher in women with unexplained infertility than in women with normal fertility ($p < 0.001$).

Figure 1. shows the ROC of homocysteine levels. The area under the curve (AUC) was 0.951 ($p < 0.001$, 95% CI 0.905-0.996), which showed a significantly high difference between the cases and the controls at a homocysteine level of 12.3 μmol/L with a sensitivity of 92.0% and a specificity of 99.9%.

Table 4 shows the distribution of the cases and controls according to homocysteine levels. Forty-six (93.9%) of the women with unexplained infertility had a homocysteine level greater than 12.3 μmol/L, while only three women (6.1%) with normal fertility had a similar high level of homocysteine

Table 1. Demographic characteristics of the cases.

Demographical variables		Mean ± standard deviation
Age (years)		30.1 ± 5.5
Duration of marriage (years)		4.8 ± 2.2
Coital frequency per week		3.3 ± 1.7
BMI (kg/m^2)		21.7 ± 1.5
Weight (kg)		58.1 ± 5.3
Height (m)		16.3 ± 0.5
		Frequency (percentage)
Residency	Urban	42 (84%)
	Rural	8 (16%)
Education	Illiterate	3 (6%)
	Primary	10 (20%)
	Secondary	26 (52%)
	Higher education	11 (22%)
Previous IUI	No	40 (80%)
	Yes	10 (20%)
Previous IVF	No	34 (68%)
	Yes	16 (32%)
Total		50 (100%)

Table 2. Demographic characteristics of the controls.

Demographical variables		Mean ± standard deviation
Age (years)		30.8 ± 5.2
Duration of marriage (years)		4.7 ± 3.0
Coital frequency per week		3.5 ± 1.1
BMI (kg/m^2)		21.5 ± 1.5
Weight (kg)		57.5 ± 5.5
Height (m)		16.2 ± 0.5
		Frequency (percentage)
Residency	Urban	39 (78%)
	Rural	11 (22%)
Education	Illiterate	2 (4%)
	Primary	17 (34%)
	Secondary	24 (48%)
	Higher education	7 (14%)
Gravida	1–2	37 (74%)
	3–4	12 (24%)
	≥ 5	1 (2%)
Parity	1–2	43 (86%)
	3–4	6 (12%)
	≥ 5	1 (2%)
Miscarriages	No	45 (90%)
	Yes	5 (10%)
Total		50 (100%)

Table 3. Distribution of the cases and controls according to homocysteine levels.

Variables	Cases ($n = 50$) Unexplained infertility	Controls ($n = 50$) Normal fertility	p value
Homocysteine level (M ± SD)	15.05 ± 2.4	9.2 ± 2.3	<0.001*

M ± SD: mean ± standard deviation.
*Independent t-test.

($X^2 = 73.990$, df = 1, $p < 0.001$). The odds ratio was 180.16 ($p < 0.001$, 95% CI 38.1-849.8), i.e. the likelihood of unexplained infertility was 180 times higher when homocysteine levels were greater than 12.3 μmol/L.

DISCUSSION

The present study showed that mean homocysteine levels were higher in women with unexplained infertility than in women with normal fertility (15.05 ± 2.4 vs. 9.2 ± 2.3 μmol/L, $p < 0.001$). This result is consistent with a study by Sultana et al., in which the mean homocysteine level was significantly

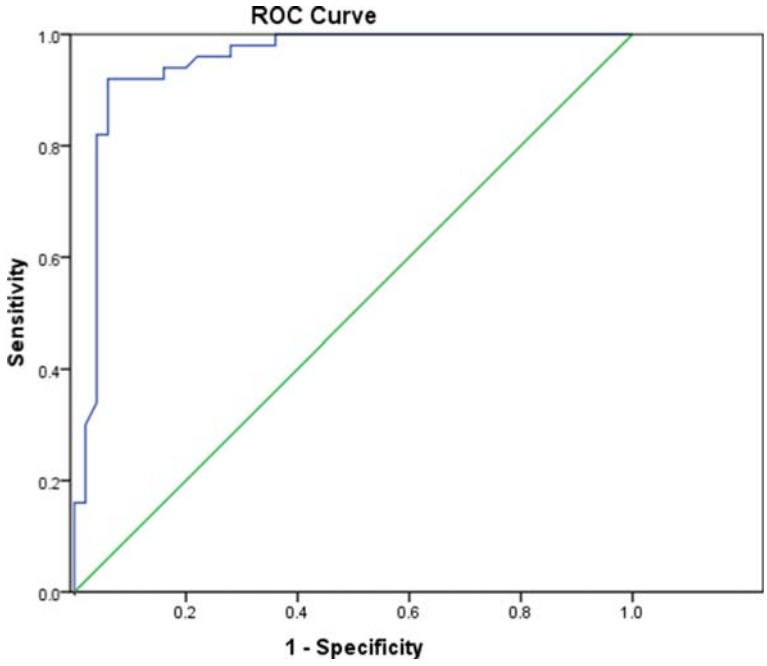

Figure 1. Receiver operating characteristic curve of homocysteine levels.

Table 4. Distribution of the cases and controls according to homocysteine levels.

Variable Homocysteine level	Cases (*n*=50) Unexplained infertility	Controls (*n*=50) Normal fertility	*p* value
> 12.3 μmol/L	46(93.9%)	3 (6.1%)	< 0.001
≤ 12.3 μmol/L	4(7.8%)	47(92.2%)	< 0.001

(Chi square test)

higher in the cases compared to the controls (13.67 ± 4.80 vs. 9.87 ± 4.84 μmol/L, p = 0.003)[14]. The present finding is also consistent with that of Rawat et al.,[3] in which homocysteine levels were higher in cases of infertility without an apparent cause than in controls (13.94 ± 5.27 vs. 6.99 ± 1.85)[15]. The result was also in a similar direction to that reported in India by Dubey et al., in which the mean serum homocysteine level was significantly higher in the unexplained infertility group compared to the normal fertility group (20.5 μmol/l vs. 10.9 μmol/l)[16].

Several studies are consistent with the present finding, such as the results of Liu et al.[4] in China (11 ± 4.46 μmol/L vs. 7.12 ± 4.42 μmol/L)[17], Ohiemim et al.[5] in Nigeria (19.2 ± 6.55 μmol/ml vs. 14.0 ± 5.31 μmol/ml)[18], and Bibi et al. in Pakistan (12.8 ± 5.1 μmol/ml vs. 9.7 ± 1.7 μmol/ml)[19]. However, a study by Das et al. clearly showed no difference in homocysteine levels between controls and cases, and the mean homocysteine levels for women in three infertile groups were comparable within each group (Group I: 16.21 ± 3.39 μmol/L, Group II: 16.36 ± 3.56 μmol/L, Group III: 16.98 ± 3.14 μmol/L) and with the fertile group (15.85 ± 9.3 μmol/L), showing no statistically significant difference. This difference can be related to the inclusion criteria[20]. Although previous literature considered a homocysteine level of 15 μmol/L as the upper limit for homocysteine cut-off [14, 17), in the present study, a homocysteine level of 12.3 μmol/L showed a significant difference between the cases and the controls with a sensitivity of 92% and a specificity of 99%. The present result is comparable to the finding of Elagab et al.[6] in Saudi Arabia, in which the plasma homocysteine cut-off value was given as 12 μmol/l[21].

Furthermore, the present findings can also be compared with those of Michels et al.,[7] in which they reported 12.9 μmol/L as the upper limit for homocysteine levels across all observations of all cycles[22]. However, a study by Dubey et al. reported a cut-off value of 13.5 μmol/L, with a maximum sensitivity of 70% and a specificity of 76.7%[16].

The reference range for homocysteine accepted in apparently healthy subjects with different ages and lifestyles was 5-15 μmol/L. However, a study by Jung et al. in South Korea showed that a cut-off level of 9.45 μmol/L[23] indicates adverse health effects, and another study suggested a cut-off level of 11.6 μmol/L, exceeding which would require more attention and physician concern[24]. The present study showed that 93.9% of women with unexplained infertility had high homocysteine levels ($p < 0.001$), which agrees with the results reported by Sultana et al. in Bangladesh, showing that the frequency of hyperhomocysteinemia was significantly greater in the cases compared to the controls (46.7 vs.16.7%, $p = 0.012$)[14], and a study by La Vecchia et al. showed that 69% of women with infertility disorders had abnormal homocysteine levels[25]. Dubey et al. reported that among the patients with unexplained infertility, 22 (73.3%) were found to have levels above the normal healthy range[16]. Similarly, a study by Ménézo et al. reported that 3.4% of women referred for fertility evaluation had homocysteine levels above the critical value[26]. Elevated homocysteine levels are linked to vascular dysfunction, which can reduce blood flow to reproductive organs, potentially impacting endometrial quality and implantation. Topical nitroglycerin releases nitric oxide, which directly improves blood vessel dilation and increases blood flow. Nitroglycerine has been previously used by cardiac surgeons following coronary artery bypass graft surgery for its vasodilatory effect. It has long been used to treat angina pectoralis[27].

In the context of unexplained infertility, it may enhance blood supply to the uterus and endometrium, supporting a more favorable environment for implantation. Further studies may consider a follow-up trial aimed at reducing homocysteinemia in women with unexplained infertility and documenting the percentage of successful pregnancies.

CONCLUSIONS

Homocysteine levels should be included in investigations to determine the cause of unexplained infertility in women.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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