Research Article

Evaluation of Malondialdehyde and Homocysteine Levels in Unexplained Infertility Females: A Retrospective, Hospital-Based, Case-Control Study

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Abstract

Malondialdehyde (MDA) and plasma total Homocysteine (tHcy) levels in females who have experienced unexplained-infertility are the focus of this investigation. This present study was a hospital-based, case-control study, where we took fifty unexplained-infertility females and fifty normal healthy woman as controls. MDA and hcy levels were estimated utilizing blood tests taken from the case and control groups. The present study found higher levels of MDA and hcy in the case than in the controls. These results observed the correlation between MDA and hcy levels and female infertility without an obvious cause. These findings could have important implications for future research and the development of infertility treatments.

In conclusion, measuring MDA and plasma total Homocysteine (tHcy) levels may be essential in determining the cause of unexplained female infertility. This study suggests that these substances may be associated with this condition, and their levels could be used to identify and treat infertility in women. Further, more study is needed to determine the meticulous nature of the relationship between MDA, homocysteine, and unexplained-infertility. Still, these findings could be a valuable starting point for future investigations.

Keywords: Unexplained-infertility; Retrospective; plasma total Homocysteine (tHcy); MDA; Prevalence

Introduction

Infertility affects millions of couples worldwide, with an estimated 10%-15% prevalence among reproductive-aged couples [1]. Despite extensive clinical evaluations, approximately 15% of infertility cases remain unexplained. Unexplained female infertility is a complex condition that poses challenges for both patients and clinicians in identifying the underlying causes [2,3].

Oxidative-Stress(OS), which is described by an irregularity between Receptive Oxygen Species(ROS) creation and deficient measure of cell reinforcements, has arisen as a likely supporter of female fruitlessness [4]. However, amplified/high levels of ROS can cause damage to cellular components or biochemical molecules, likely lipids, proteins, and DNA, impairing oocyte quality, disrupting embryo development, and compromising reproductive function [4,5].

Although MDA is a reactive aldehyde generated during lipid peroxidation, that is widely used as a 'Bio-Marker' to assess oxidative stress levels in biological samples [5,6]. However, OS-altered plasma total Homocysteine (tHcy) metabolism has been implicated in female infertility [6]. This is a Sulfur(S)-containing amino acid(aa) that is involved in one-carbon metabolism; this is a critical process for DNA synthesis, methylation, and Cellular Redox Balance(CRB) [7].

Elevated levels of hcy or Hhcy(Hyper-homocysteine) obliterate oocyte quality, diminish undeveloped organism implantation rates, and increase the risk of pregnancy complications/difficulties, for example, pre-eclampsia and recurrent miscarriage [7,8].

Consequently, the need to evaluate the levels of Malondialdehyde (MDA) and plasma total Homocysteine (tHcy) in unexplained-infertility may provide insights into the potential role of OS. Also, altered plasma total Homocysteine (tHcy) metabolism is the pathophysiology of this condition.

Still, there has been a scarcity of research exploring the correlation between M and plasma total Homocysteine (tHcy) levels in cases of unexplained-infertility. Subsequently, compared with a group of fertile (act as a control group) females, we conducted a retrospective clinic-based, case-control study to evaluate the level of MDA and hcy in females encountering unexplained-infertility.

Methodology

Study-design, periods, and number of subjects

This current study was led at a Tertiary-Care Hospital(TCH), and information was collected from the emergency clinic records of patients(who visited the infertility center between November 2020 and December 2021). The study-subjects were separated into Group-I Control and Group-II Patients.

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The patients were selected from the Department of Obstetrics and Gynecology at Hind Medical Science (HIMS), Sitapur, Uttar Pradesh, India; further, all biochemical examinations were performed at the Institute of Medical Sciences, Banaras Hindu University (IMS,BHU), Varanasi, Uttar Pradesh, India. The Institutional Medical Ethical Committee endorsed/granted the present study. Notwithstanding, the patients and controls enlisted after marking their informed consent. The study included one hundred females with infertility complaints, fifty-six healthy and normal volunteer women served as controls, then total enrolled subjects, one hundred fifty-six (total n=156), all subjects having ages between 25 and 30.

The study has followed a few inclusion criteria-like females diagnosed with unexplained-infertility, based on a comprehensive evaluation that ruled out other causes, including hormonal-imbalances, Tubal-blockage, and including male-infertility factors. However, in this retrospective, hospital-based, case-control study, certain exclusion criteria were applied to ensure the validity and reliability of the findings.Women who met the following criteria were excluded from the present study:

- **Anemia:** Women with a hemoglobin level below the normal reference range for age and gender.
- **Gross pelvic pathology:** Women with known pelvic pathology, such as uterine fibroids and ovarian cysts/ endometriosis, could potentially impact reproductive function.
- **Uncontrolled chronic disease:** Women with uncontrolled chronic diseases, such as diabetes, hypertension, or thyroid disorders.
- **History of the Pelvic Inflammatory Disease(PID);** Women with a history or complain of Pelvic Inflammatory Disease(PID).
- **The malefactor of infertility:** Women whose infertility was solely due to male factors, such as severe male factor infertility or azoospermia.
- **Gestational complications:** Women with a history of gestational complications like-

Intrauterine Growth Restriction (IGR), stillbirth, or abruptio placenta.

- **History of abortion:** Women who had a history of any abortion.

Consequently, applying these exclusion-criteria to achieve the study aimed to minimize potential confounding factors and ensure that the findings are specifically related to unexplained-infertility females. This approach enhances the study's internal validity and increases confidence in the results obtained. The study followed exclusion criteria applied to both the study and control groups to minimize selection bias and ensure that the comparison between the groups was valid. Additionally, the rationale for the exclusion criteria was based on existing literature and clinical expertise. It was carefully considered during the study design to ensure the scientific rigor of the research.

Further, the control group included females who had somewhere around one effective pregnancy and no set of experiences of infertility, including the people who met not many different models with non-attendance of gestational complexities: Females who did not have a history of gestational complications, such as intrauterine growth restriction, stillbirth, or abruptio placenta and females who did not have a history of any abortion were included in the control group.

Blood collection for the estimation of MDA and Hcy

To estimate the serum levels of Malondialdehyde (MDA) and plasma total Homocysteine (tHcy), 2 mL of blood was collected from both group studies. Consequently, the blood sample was centrifugated at 3000 rpm for 5 minutes to isolate the serum. Then, at that point, 100 µL of serum was diluted with 500 µL of distilled water and warmed in a boiling bath for 15 minutes.

A short time later, collected samples were treated with 1 mL of Trichloroacetic Corrosive (TCA)-2-Thiobarbituric Corrosive (TBA)-HCl reagent. Then, subsequent combinations were kept through cooling and centrifugation, empowering the supernatant assortment. The force of the framed pink tone was estimated at 535 nm using spectrophotometry. By plotting the absorbance against a standard diagram, the convergence of MDA is not entirely set in stone. The optical thickness of the pink tone is straightforwardly relative to the convergence of MDA in the serum test, following the strategy-depicted by Buege(1978) and to measure absolute hcy levels in the blood and accessible ELISA units (Protein connected immunosorbent examination) from ENZO Life Sciences were utilized [8].

Data analysis

A comparative statistical analysis evaluated the MDA and hcy levels disparities between Group-I and Group II.

Results

Table 1 shows that females with unexplained-infertility have altogether more elevated levels of both MDA and plasma total Homocysteine (tHcy) ($p<0.001$). Additionally, the results of the relationship analysis demonstrate a positive correlation between the patient's plasma total Homocysteine (tHcy) levels and MDA (relationship coefficient of 0.69), with a significance level of $p<0.001$. MDA levels increment, and homocysteine levels likewise will generally increment in females with unexplained-infertility, and this relationship is statistically significant.

Table 1: Levels of MDA and Hcy in both Group-I and II.

Biochemical-profile	Control(n=56)/Group-I	Unexplained-infertility/ Group-II(n=100)
MDA(nmol/ml)	1.59 ± 1.20	2.38 ± 0.45*
Hcy(µmol/l)	6.89 ± 1.91	13.94 ± 5.29*

This study could add to the ongoing comprehension of the pathophysiology of unexplained infertility in females, and a higher level of MDA might propose expanded oxidative pressure, which could prompt cell harm and unfavorably influence regenerative capability. Modification in homocysteine metabolism might disturb one-carbon metabolism, which assumes a vital part in DNA combination and methylation and may affect oocyte quality and undeveloped organism

improvement. Moreover, the relationship between MDA, homocysteine, and other clinical boundaries might give insights into potential risk elements or indicators for unexplained infertility in females.

Discussion

The total plasma homocysteine is an amino acid containing sulfur (S) that plays a role in methyl (CH3) group transfer within the activated CH3 cycle [9]. During pregnancy, plasma total homocysteine levels typically decrease. However, our study has revealed that females with unexplained infertility exhibit elevated levels of Hcy. This elevation may be attributed to genetic abnormalities in enzymes involved in homocysteine metabolism, such as Methylenetetrahydrofolate Reductase (MTHFR), as well as deficiencies in essential nutrient cofactors like folate and vitamin B12 [9-12].

Higher levels of (tHcy)levels have been aligned with procoagulant impacts alongside expanded pro-oxidant activity and control the degradation of Glutathione Peroxidase(GPO) [13]. Likewise, the bioavailability of Nitric Oxide (NO), and the development of endothelin-1(E-1), lead to the accumulation of Receptive Oxygen Species (ROS), including hydrogen peroxide (H_2O_2), superoxide, and hydroxyl extremists through sulfur autooxidation [13,14]. Prolonged exposure of endothelial cells to high levels of Hcy can contribute to endothelial dysfunction [13].

This current study suggested that the levels of ROS are higher in the case of unexplained-infertility, which lines up with past examination that has found high ROS levels in the peritoneal liquid of females with unexplained-infertility, contrasted with fertile controls (Group-I) going through tubal ligation [15]. ROS acts as a signaling particle in different body physiological cycles. However, an over-abundance of ROS can likewise be involved to doubt or intervene in different neurotic processes [16-19].

Numerous studies have consistently demonstrated that these factors are associated with various clinical mechanisms contributing to the development of unexplained infertility. These mechanisms encompass the initiation of apoptosis, which is linked to embryo fragmentation, implantation failure, and disruptions in the luteal hormones crucial for sustaining pregnancy, ultimately leading to luteal regression. Moreover, they are implicated in DNA damage or impairment in both oocytes and spermatozoa, including the activation of apoptosis, which results in embryo fragmentation and impaired implantation [16-20].

In the context of the present study, our findings indicate that both homocysteine (Hcy) and reactive oxygen species (ROS) may play roles in the pathogenesis of unexplained infertility. However, given the nature of this case-control study and the observed positive correlation between these two factors, our investigation seeks to determine whether Hcy or ROS alone, or their interplay, is solely responsible for infertility [18-19].

Different clinical reports have explained that ROS has caused infertility and has been ensnared in the pathogenesis of different clinical conditions Cardiovascular Disease(CD) [20]. Notwithstanding, plasms(t) he has been portrayed as an independent risk factor for CD [21]. Its consequences for the vasculature incorporate endothelial-cytotoxicity, lipid-peroxidation, expanded platelet attachment, improved initiation of the coagulation framework, and stimulation of vascular smooth muscle cell proliferation

[21-23]. These mechanical components might add to the effect of ROS and Hcy on cardiovascular health.

Conclusion

In conclusion, this retrospective, hospital-based, case-control study provided valuable data on MDA and hcy. Hence, notwithstanding different examinations to decide the reason for unexplained-infertility in females, the examination of biochemical boundaries, such as Hcy and ROS measures, should be considered. Moreover, as Hcy and ROS are modifiable indicators of general mortality and mortality because of cardiovascular causes, the possible role of homocysteine-bringing down treatment and cancer prevention agent supplementation in treating patients with unexplained-infertility ought to be considered and also re-validate the study.

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