Association Between Hyperhomocysteinemia and Recurrent Miscarriages: A Cross-Sectional Study Set in Saudi Arabia

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ABSTRACT

Background: Recurrent miscarriage is a condition that causes a lot of distress in couples, and approximately 50% of them are of unknown etiology. Numerous studies have demonstrated the role of hyperhomocysteinemia in vascular occlusions, including occlusion in the blood vessels in the placenta, which has the potential to result in recurrent loss of pregnancy.

Objective: To determine homocysteine levels in Saudi women who had recurrent miscarriages of unknown etiology

Design: A cross-sectional case-control study

Setting: The study was conducted, at the outpatient clinic of Najran University Hospital and Maternity and Child Health Hospital, Najran, Saudi Arabia and this was compared to healthy controls, from 2018 to 2019.

Subject and Methods: A cross-sectional case-control study was conducted to determine homocysteine levels in Saudi women who had recurrent miscarriages of unknown etiology, and this was compared to healthy controls, from 2018 to 2019. All participants were fully examined and evaluated by a consultant obstetrician in advance to exclude known causes of recurrent miscarriage. Plasma homocysteine concentrations were measured using homocysteine enzymatic assay.

Main outcome measures: Plasma homocysteine concentrations

Results: Eighty-eight women were included in this study; 58 had recurrent miscarriages and 30 were healthy controls. Approximately 22% of the patients and 6.6% of the healthy controls showed elevated homocysteine levels. The mean homocysteine concentration in the patients was 8.797±3.29 µmol/l, which was significantly higher than that in the controls, which was 7.17±4.96 µmol/l (P = 0.005).

Conclusion: Hyperhomocysteinemia is a potential risk factor for recurrent miscarriages in Saudi women. Therefore, we recommend the inclusion of this investigation in the diagnostic protocols for recurrent miscarriage and further investigating the cause of the hyperhomocysteinemia.

Limitation: Sample size was small.

Conflict of interest: None

Key words: Recurrent miscarriages, Pregnancy, hyperhomocysteinemia, Saudi Arabia.

INTRODUCTION

Recurrent miscarriage is a complex obstetric condition that affects approximately 1-2% of couples owing to term of definition[1]. It classically defined by two or more failed pregnancies, according to Practice Committee of the American Society for Reproductive Medicine[2]. Various causes that result in recurrent miscarriages have been documented, but approximately 53% of the cases remain unexplained recurrent miscarriages[3,4]. Hyperhomocysteinemia has been suggested as a risk factor for recurrent miscarriages due to its prothrombotic effect. Although certain reviews have discussed the pathogenesis of the prothrombotic effect of hyperhomocysteinemia, the exact mechanism

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remains unclear[5-8]. A high level of homocysteine interferes with the function of fibrinolytic enzymes by causing them to bind to the lysine residue of homocysteine. Moreover, hyperhomocysteinemia might lower the production of beneficial nitric oxide (NO) by endothelial cells and platelets. It is hypothesized that it achieves this by inducing the production of reactive oxygen species, which results in the disturbance of the hemostatic function of both endothelial cells and platelets[9].

Homocysteine is an intermediate derivative of an essential amino acid, methionine, which functions as a methyl transfer and metabolite intermediate of multiple important products, such as homocysteine thiolactone and cysteine[10]. Several biochemical steps are involved in the conversion of methionine to homocysteine. Homocysteine undergoes subsequent remethylation to give rise to methionine, either by the action of betainein the liver or kidney, in which vitamin B6 is an essential co-factor, or by methionine synthase methyltransferase, an enzyme distributed in many tissues called methylenetetrahydrofolate reductase (MTHFR) enzyme within the folate methionine pathway, where B12 and folic acid are co-factors[11]. Homocysteine may also permanently transform to cysteine, a non-dietary essential amino acid, by the action of cystathionine beta-synthase and cystathionine gamma-lyase[12]. Interruption at any level of the homocysteine metabolism pathway due to enzymatic defects or vitamin deficiency results in homocysteine accumulation in plasma[6].

The lack of dietary vitamin B12 and folic acid, or interrupted metabolism are implicated in increased homocysteine levels in the blood[13,14]; therefore, administration of folic acid and vitamin B12 is documented to lower plasma homocysteine levels[5,15,16]. In addition to these acquired causes of hyperhomocysteinemia, single nucleotide polymorphisms (SNPs) of the methylenetetrahydrofolate reductase (MTHFR) gene have attracted the attention of researchers, as these SNPs seem to be the most common genetic causes associated with hyperhomocysteinemia. Mutations in MTHFR C677T and A1298C, are the most common genetic anomalies associated with hyperhomocysteinemia. However, these associations remain uncertain with regard to recurrent miscarriages in patients. A meta-analysis of 16 studies concluded that the C677T mutation in MTHFR was significantly linked with recurrent miscarriage[17]. However, another study excluded this association[18] and a study from Saudi Arabia reported contrasting results[19].

Although many studies have highlighted hyperhomocysteinemia as a risk factor for vascular thrombosis and complications in pregnancy, its role in recurrent miscarriages remains under debate. This conflict may be due to variable genetic polymorphisms in different populations, which are considered the most common genetic cause of hyperhomocysteinemia[20]. Moreover, limited research has been conducted on recurrent miscarriages in Saudi patients. In this study, we focused on measuring the concentration of plasma homocysteine in Saudi patients with recurrent miscarriages and healthy controls to evaluate whether homocysteine levels are related recurrent miscarriages in patients in the Kingdom of Saudi Arabia.

MATERIALS AND METHODS

A cross-sectional case-control study was conducted to determine plasma homocysteine levels in 58 women with recurrent miscarriages of unknown etiology and 30 healthy controls with successful pregnancies (at least two successful pregnancies) between 2018 and 2019. All participants were Saudi nationals.

Consultant obstetricians obtained informed consent from the participants and interviewed, evaluated, and diagnosed them at the outpatient clinic of Najran University Hospital and Maternity and Child Health Hospital, Najran, Saudi Arabia. The study excluded any patients with known risk factors for pregnancy loss, such as uterine malformation, diabetes mellitus, lupus erythematosus, endocrine disorders, hormonal abnormalities, and thrombophilia. The inclusion criteria for the control group in this study were women who experienced at least two successful pregnancies with healthy babies, while excluding any participant with a history of pregnancy losses or pregnancy complications, chronic diseases, or vascular thrombosis.

Venous blood samples were obtained in ethylenediaminetetraacetic acid tubes from patients and controls during routine follow-up in the outpatient clinic. The samples were immediately centrifuged at 2000 RPM for 10 minutes. The plasma was isolated in cryo tubes, stored at -20 degrees C, and underwent a homocysteine assay. The Cobas-C311 homocysteine enzymatic assay (HCYS: ACN 20700) was used to measure homocysteine levels in these samples.
The statistical analysis was based on the following principles: 1) simple data summarization 2) logistic regression (to examine the association

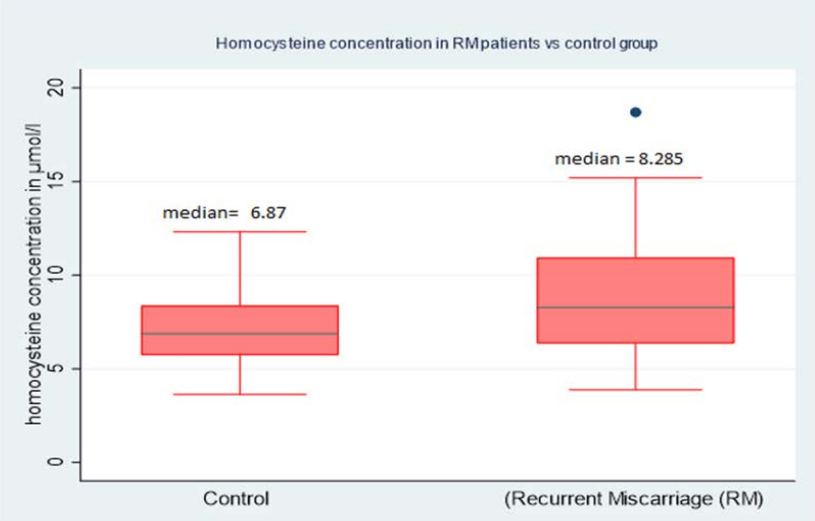

Figure 1: Median of the Homocysteine Concentration in unexplained recurrent miscarriage patients is higher than control group

Table 1: The statistical differences of hemocysteine concentration between patients with unexplained recurrent miscarriages and control subjects using t test

Patients group	Patients with miscarriages N= 58	Controls N=30	P value	95% Confidence interval
Hycs concentration Mean ±SD (min–max)	8.8 ±3.29 (3.88-18.7)	7.5± 2.33 (3.64-12.31)	0.04	0.998123-1.386013

N= number of participants, SD; stander deviation, Hycs; homocysteine

Table 2: Distribution of homocysteine concentration above 12 µmol/l in RM and control groups

Study groups	Patients		Control		Odd Ratio	P value	[95% conf. interval]
	N	%	N	%			
Number of participants with high hcys concentration	13	22	2	6	4.2	0.05	0.84-40.40

Table 3: Logistic regression study the relationship between the homocysteine concentration and the number of recurrent miscarriages. Two catagories (≤ 3 RM and ≥4 RM)

Odd Ratio	**P value**	**95% conf interval**
0.93	0.44	0.77-1.12

between the binary outcome variable (RM) and the continuous outcome variable (homocysteine concentration). 3) t-test to see if the homocysteine concentration means of RM patients and the control group differ significantly. When the P-values were less than 0.05, the results were considered statistically significant. STATA version 17.0 was used for the analysis.

RESULTS

The mean age of the patients and controls was 35.16±6.19 years and 31.45 ± 7.19 years, respectively. The mean duration of marriage duration in patients and controls was 8.96 ± 6.44 years and 9.12 ± 7.97 years, respectively. The number of pregnancies lost ranged between 2 and 12, with an average of 3.98±1.85 losses among the participants.

The plasma homocysteine cut-off value was 12 µmol/l, the concentration level higher than these cutoffs was considered as hyperhomocysteinemia[21]. The mean homocysteine concentration in patients was significantly higher than the mean concentration in the healthy controls (P-value = 0.04); (Table 1). Further analysis is shown in Figure 1, which shows that the median homocysteine concentration in unexplained recurrent miscarriage patients is greater than in the control group. Additionally, the proportion of hyperhomocysteinemia among RM patients is significantly much higher than among healthy control (OR=4 P value 0.05) (Table 2).

Moreover, we categorized the number of (RM) in patients in to ≤ 3 RM and ≥4 RM to correlate with homocysteine concentration. Then we use Logistic regression with CI = 95%, we did not recognized any association between homocysteine concentration and number of pregnancy losses based on this categorization (P-value = 0.3416 and correlation coefficient = -0.1271229) (Table 3).

Logistic regression showed no association between plasma homocysteine concentration and frequency of RM.

DISCUSSION

Recurrent miscarriage is a condition that causes a lot of distress in couples, especially when the etiology is unknown, which represents nearly 50% of the cases. Unknown etiology also causes discrepancies in the diagnosis and treatment of recurrent miscarriages at a global level[22]. Therefore, it is necessary to investigate possible causes and related risks that could result in recurrent miscarriages. There is published literature regarding the role of hyperhomocysteinemia in vascular thrombosis[13]. Numerous studies have also examined the association between homocysteine and recurrent miscarriages, but the results remain contradictory. However, some of the aforementioned studies support this association[20].

In our study, we ensured that there was a match between the patients and healthy controls, as all our participants were from the reproductive age group (the mean age of patients and controls was 35.16±6.19, and 31.45±7.19 years, respectively). Both groups had the same ethnic background. A case-control study was performed to allow us to better compare it with similar studies from other parts of the world[5,15,23].

In our study, 22% of the patients and 6.6% of the healthy controls showed a plasma homocysteine concentration above the cut-off of value, and this result was statistically significant (P-value = 0.05)[21]. Moreover we found also the mean homocysteine concentration in RM patients was significantly higher than healthy controls (P-value = 0.04. This finding is in agreement with multiple studies that linked hyperhomocysteinemia with recurrent miscarriages[5,24-26]; however, it contradicts similar studies that did not support the existence of a relationship[27,28]. We found no association between plasma homocysteine concentration and the number of pregnancies lost (P= 0.3416). The effect of homocysteine on the vascularization of the chorionic villus was investigated by Nelen et al., who demonstrated that vascularization is defective in a state of maternal hyperhomocysteinemia[29]. Nevertheless, mild and moderate hyperhomocysteinemia has been addressed as an independent risk factor for vascular occlusion and cardiovascular disease in pre-existing hypertension[30]. The usual level of homocysteine during normal pregnancy is significantly lower than that in nonpregnant women[31]. Therefore, elevated plasma homocysteine concentration during pregnancy is hypothesized to be linked to placental-associated pregnancy complications[30].

It's worth noting that most RM patients with hyperhomocysteinemia are taking folic acid treatment according to the standard management

guideline[1], which significantly reduces the plasma concentration of homocysteine. However, despite taking folic acid, some patients did not show much improvement[32]. In general, the response to treatment options for hyperhomocysteinemia varies depending on the cause. Only approximately 50% of the patients with hyperhomocysteinemia due to Cystathionine Beta-Synthase CBS deficiency respond to the administration of vitamin B6[33], while an excellent response has been recorded in patients with congenital deficiency of transcobalamin, with weekly vitamin B12 injections. The treatment of patients with defects in MTHFR and methionine synthase have been reported to be refractory[34]. In their clinical trial, Bradley et al. reported that homocysteine-lowering agents (folic acid, vitamin B12, vitamin B6) decreased the level of plasma homocysteine, but could not alleviate the risk of cardiovascular diseases and dementia[35]. MTHFR deficiency is the most common genetic cause of hyperhomocysteinemia[36,37]. There are conflicting data regarding recurrent miscarriages[20] which may be due to the polymorphic nature of MTHFR genes in different ethnicities. Alkhuriji1 et al. studied the role of methylenetetrahydrofolate reductase genes (C677T and A1298C) in Saudi patients with recurrent miscarriages and found that the frequency of the C677T genotype in patients with recurrent miscarriages did not differ from that of controls in the aforementioned study[19]. Their results, however, were not statistically significant, differing from other reports where the frequency of the CC genotype and C allele of A1298C was higher in patients with recurrent miscarriages. The C677TT genotype is most likely associated with hyperhomocysteinemia in a number of regions worldwide. Another study reported that the CC wild-type genotype might have a protective effect against hyperhomocysteinemia[26]. Therefore, the genetic constituents of polymorphic MTHFR and other genetic causes of hyperhomocysteinemia may directly affect homocysteine levels and patients' treatment responses. We recommend that homocysteine measurements be added to the list of investigations in patients with unexplained recurrent miscarriages. If a high concentration is noted, the underlying causes of hyperhomocysteinemia must be determined, which would help in predicting the treatment response. Further epidemiological studies are required to evaluate the nature, causes, and consequences of hyperhomocysteinemia in Saudi Arabia.

CONCLUSION

In our study, we measured plasma homocysteine concentrations in patients with recurrent miscarriages of unknown etiology. We found that these patients had significantly higher homocysteine levels than healthy controls, which is further evidence suggestive of hyperhomocysteinemia as a risk factor associated with recurrent miscarriages. We encourage clinicians to investigate the underlying causes for all cases with hyperhomocysteinemia.

Authorship Contribution: The author did all the effort contribution towards (1) substantial contributions to conception and design, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

Potential Conflicts of Interest: None

Competing Interest: None

Ethical Approval: An ethical approval letter has been acquired from the Research Ethics Committee, Najran University, Kingdom of Saudi Arabia prior to this study. All the participants in this study will kept anonymity.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Acceptance Date: 08 June 2022

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