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Association of Serum Homocysteine Level with Unexplained Recurrent Pregnancy Loss: A Case-Control Study

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Abstract

Background: Recurrent pregnancy loss (RPL) is a very stressful and painful experience for the couple. Recurrent pregnancy loss without apparent causative factor which may be identified in about 50% of cases known as unexplained recurrent pregnancy loss (RPL). Hyperhomocysteinaemia is considered a major risk factor for this problem which is related either to a hereditary defect within the methionine-homocysteine pathway or it might be acquired as a result of deficiencies of vitamin B12 and B9.

Aim of the study: The aim of the study was to evaluate the association of serum homocysteine level with unexplained recurrent pregnancy loss.

Methods: This case-control study was conducted in the outpatient department of Feto-maternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh during the period from May 2020 to April 2021. According to inclusion and exclusion criteria, 34 patients with unexplained recurrent pregnancy loss (RPL) and 34 age and BMI-matched control were selected from OPD during the one-year study period. A 3ml blood sample was collected from the antecubital vein of all 68 participants in a plain test tube and was sent to the Biochemistry Department of BSMMU to assess the serum homocysteine level using direct chemiluminescent technology by the Atellica TM IM analyzer. The results were noted in the questionnaire and the data were analyzed by using SPSS software.

Results: In this study, serum homocysteine levels of the 68 participants of both the case and control groups were measured and analyzed. We found hyperhomocysteinemia among 5.9% of patients in the case group as the abnormal homocysteine level. The mean homocysteine level of case group patients was 8.15±8.11 (µmol/L) which was found as 6.23±1.47 (µmol/L) in the control group. But statistically, we did not find any significant difference between the groups regarding the homocysteine level; the p-value was found as 0.787.

Conclusion: Serum homocysteine level has no significant association with unexplained recurrent pregnancy loss. Even, no significant difference was found when serum homocysteine levels were compared with the control in 1st, 2nd and 3rd-trimester pregnancy loss.

Keywords: Recurrent pregnancy loss (RPL); Homocysteine, Hyperhomocysteinemia; Thrombophilia

Introduction

Recurrent pregnancy loss (RPL) is a major reproductive problem, affecting about 1-5% of couples and is frequently accompanied by considerable

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psychological and social burden for the couple [1]. Recurrent pregnancy loss without apparent causative factor which may be identified in about 50% of cases known as unexplained RPL. The risk of recurrence increases with maternal age and the number of successive losses. Despite intense anatomic, endocrinologic and immunologic screening efforts, up to 50% of RPL remain unexplained [2]. Hyperhomocysteinemia, defined as an elevated level of homocysteine, is described as a risk factor for venous thromboembolism and adverse pregnancy outcomes such as recurrent miscarriages, pre-eclampsia, neural tube defects, placental abruption, intrauterine growth restriction and fetal death [3]. Increased homocysteine has negative pathological effects on vascular endothelium, atherogenesis and the activity of coagulation factors VIII and V, ultimately leads to an increase in the level of thrombin and platelet aggregation and results in venous thrombosis [3]. Maternal hyperhomocysteinemia also interferes with embryonic development through defective chorionic villous vascularization [6]. The complications that are associated with the increased level of homocysteine include recurrent miscarriages, preeclampsia, abortion, neural tube defects, intrauterine growth retardation, fetal death and venous thrombosis [3]. Several other pathologies, not related to pregnancy, also associated with hyperhomocysteinemia are vascular diseases and several age-related pathologies like Alzheimer's disease, Parkinson's disease, stroke and osteoporosis etc [5]. The normal levels of homocysteine range between 5 and 15 µmol/L. Based on the levels of homocysteine, hyperhomocysteinemia has been classified as mild/moderate (15- 30µmol/L), intermediate (30-100µmol/L) and severe (>100µmol/L) [5]. Folic acid and vitamin B12 supplementations can be given for acquired hyperhomocysteinemia. Low-dose aspirin and LMW heparin can be used to treat hyperhomocysteinemia due to inherited causes (MTHFR C677T & A1298C mutations). These treatments have been successfully used in different studies in patients with RPL having hyperhomocysteinemia and/or MTHFR mutation [6-8]. The major objective of this current study was to evaluate the association of serum homocysteine level with unexplained recurrent pregnancy loss.

Methodology

This was a case-control study conducted in the outpatient department of Feto-maternal Medicine, Bangabandhu Sheikh Mujib Medical University Hospital (BSMMU), Dhaka, Bangladesh during the period from May 2020 to April 2021. According to inclusion and exclusion criteria, 34 patients with unexplained recurrent pregnancy loss (RPL) and 34 age and BMI-matched control were selected from OPD during the one-year study period. The study was approved by the ethical committee of the mentioned hospital. Properly written consent was taken from all the participants before data collection. The whole intervention was conducted following the principles of human research specified in the Helsinki Declaration [9] and executed in compliance with currently applicable regulations and the provisions of the General Data Protection Regulation (GDPR) [10]. A convenient sampling technique was applied, according to the availability of the patients as per inclusion and exclusion criteria. As per the inclusion criteria of this study, patients who attended Feto-maternal OPD for periconceptional counseling for RPL and who had a history of consecutive two or more failed clinical pregnancies were included as cases. On the other hand, age and BMI-matched women (patient's attendants) who had at least one successful pregnancy with no history of spontaneous pregnancy loss from both out-patient and inpatient departments of Feto-maternal Medicine, BSMMU were included as control group participants. Women who are diagnosed to have a known cause for RPL, Parental chromosomal abnormalities except for gene mutation, Type-I and Type-II diabetes mellitus, thyroid disorders, chronic renal disease, chronic HTN, PCOS, anatomic defects of the uterus and autoimmune disorders and cases received folate and vit B12 supplementation within previous three months were excluded. A 3ml blood sample was collected from the antecubital vein of all 68 participants in a plain test tube and was sent to the Biochemistry Department of BSMMU to assess the serum homocysteine level using direct chemiluminescent technology by the Atellica ™ IM analyzer. The results were noted in the questionnaire and data were analyzed by using SPSS software version 22.0.

Result

In this study, it was found that the maximum (64.7%) patients in RPL (case) group and 67.7% in the normal (control) group were between 25 to 34 years of age. The mean age of the patients with RPL was (28.44±5.25), whereas the mean of the age of the control group was (29.15±4.72). There was no significant difference between these two groups in terms of age (P= 0.562). An Independent sample t-test was used to compare the mean BMI (Kg/m^2) between case and control groups. The mean difference was not statistically significant (P = 0.208). So, it can be said that age and BMI of the case group (28.44±5.25 and 24.95±3.48) were matched with the control group (29.15±4.72 and 23.69±4.07). In comparison of homocysteine level, 32 (94.1%) patients were found to have normal (<15 µmol/L) level in RPL group and all 34 (100%) participants of control group have normal level of homocysteine. Only 2 (5.9%) have moderate or high (>15 µmol/L) homocysteine level in RPL group and zero percent on control group. The normality of S. homocysteine level data of all participants (case and control) was assessed by Kolmogorov-Smirnov test ($p<0.05$) and was found that the data were not normally distributed. So, Mann-Whitney U test was used to compare the mean S. homocysteine level between RPL group and control group and found that the difference was not statistically significant (P=0.787). So it can be said that mean S. homocysteine level of RPL patients (8.15±8.11)

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was not significantly different from control (6.23±1.47). In this study, it was also evident that there were no significant differences in S. homocysteine levels between patients who had pregnancy loss in 1st trimester, 2nd trimester, or combined 1st and 2nd trimester. Also, no significant difference was found when these were compared with the control.

Table 1 showed that maximum (64.7%) patients in RPL group and 67.7% in control group were between 25 to 34 years of age. The mean age of the patients with RPL was (28.44±5.25), whereas the mean age of the control group was (29.15±4.72). There was no significant difference between these two groups in terms of age (P=0.562).

Independent sample t test was used to compare the mean BMI between RPL and control group in table 2. The mean difference was not statistically significant (P=0.208). So it can be said that BMI of RPL group (24.95±3.48) was matched with the control group (23.69±4.07). The range of BMI of RPL group 20-32.4 (Kg/m^2) and control group 18-31.2 (Kg/m^2).

Table 3 showed that only 2 (5.9%) of the patients in RPL group and none of the participants from control group have hyperhomocystienemia. The majority 32 (94.1%) patients in RPL group and all 34 (100%) participants of control group have normal homocysteine level.

In table 5, Mann-Whitney U test was used to compare the mean S. homocysteine level between RPL and control group. After checking the normality of S. homocysteine level data of all patient with Kolmogorov-Smirnov test ($p<0.05$), it was found that the data set was not normally distributed. The difference was not statistically significant (P=0.787). So it can be said that S. homocysteine level of RPL patients (8.15±8.11) was not significantly different from control (6.23±1.47).

Table 6 showed that there are no significant differences in S. homocysteine level between patients who had pregnancy loss in 1st Trimester, 2nd Trimester or combined 1st and 2nd trimester. Also, no significant difference was found when these compared with the control.

Table 1: Distribution of participants as per age. (N=68)

Age group (In years)	Case		Control		P value
	(n=34)		(n=34)		
	n	%	n	%	
18-24 yrs.	7	20.6	5	14.7	
25-34 yrs.	22	64.7	23	67.7	
35-40 yrs.	5	14.7	6	17.6	
Mean ± SD	28.44 ± 5.25		29.15 ± 4.72		0.562

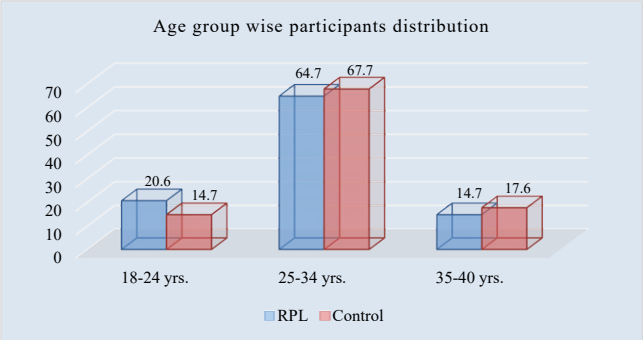

Figure I: Bar chart showed age group wise participants. (N=68)

Table 2: Distribution of participants as per BMI. (N=68)

BMI (Kg/m^2)	RPL		Control		P value
	(n=34)		(n=34)		
	n	%	n	%	
>18.5	0	0	1	2.9	0.208
18.5-24.9	18	52.9	21	61.8	
25-29.9	12	35.3	9	26.5	
≥30	4	11.8	3	8.8	
Mean ± SD	24.95 ± 3.48		23.69 ± 4.07		
Range	20-32.4		18-31.2		

Table 3: Comparison of serum homocysteine level between the groups. (N=68)

S. homocysteine Level	RPL		Control	
	(n=34)		(n=34)	
	n	%	n	%
<15 µmol/L	32	94.1	34	100
>15 µmol/L	2	5.9	0	0

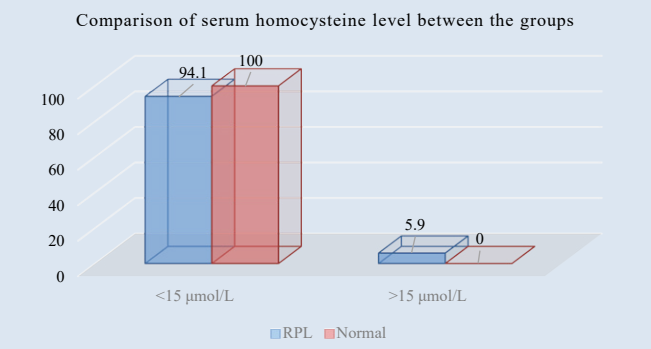

Figure II: Bar chart showed comparison of mean serum homocysteine level between the groups (N=68)

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Table 4: Normality test of continuous variable. (N=68)

Tests of Normality			
	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
S. homocysteine level	0.307	87	0

*Here, 0.000 is not an absolute zero.

Table 5: Mean Serum Homocysteine Level in study population. (N=68)

Parameter	RPL	Normal	P value
	Mean ± SD	Mean ± SD	
S. homocysteine level	8.15 ± 8.11	6.23 ± 1.47	0.787

Table 6: Serum Homocysteine Level between two groups according to trimester. (N=68)

Biochemical parameters	RPL (n=34)			Control (n=34)	P value
	1st	2nd	(1st + 2nd) Trimester combined loss		
	Trimester loss	Trimester loss	Mean ±SD		
	Mean ±SD	Mean ±SD			
S. homocysteine Level	8.37 ± 8.47	7.81 ±4.55	8.16±8.11	6.23±1.47	0.539

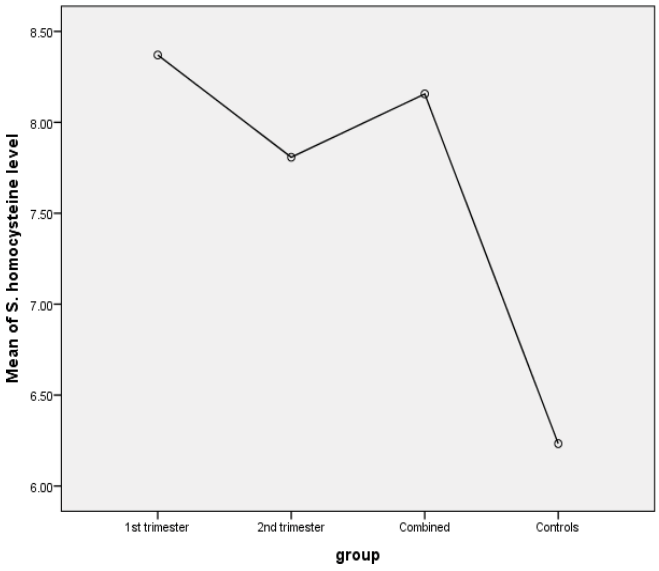

Figure III: Line diagram showed serum homocysteine level according to trimester between two groups. (N=68)

Discussion

This study aimed to evaluate the association of serum homocysteine level with unexplained recurrent pregnancy loss. Hyperhomocysteinemia is considered one of the major parts of thrombotic risk factors and many studies have investigated their potential association with RPL with inconclusive and controversial results. In this study, the mean age was 28.44±5.25 years in the case group and 29.15±4.72 years in the control group. The difference is not statistically significant (P=0.562) between the two groups. In another study, Xu et al. (2019) [11] found the mean age was 31.82 and 31.16 years for RPL and controls respectively (P=0.109). There were no statistically significant differences between the case and control groups in terms of age in both of the studies, which were similar to the current study. In our study, the mean BMI of RPL patients was 24.95 ± 3.48 Kg/m^2 and that of control was 23.69 ± 4.07 Kg/m^2 (P=0.208). So, the BMI of the RPL group was not significantly different from that of the controls which reflects perfect matching of BMI between the two groups. Creus et al. (2013) [12] also found a statistically non-significant difference in BMI between the case and control groups, which were 23.9±3.4 Kg/m^2 and 24.7±3.9 Kg/m^2 respectively. In Yousefian et al., (2014) [13] BMI of RPL patients and controls were found to be 24.2±2.5 and 25.4±2.7 Kg/m^2 respectively (p=0.23) which was also not significant. Hyperhomocysteinemia was present in 5.9% (2 patients) of the study population among the RPL group, whereas no one had this in the control group. So, the current study found that it may be a cause of unexplained RPL in a few patients. Among the two patients, one had intermediate hyperhomocysteinemia (S. Hcy level was 50 µmol/l). Mukhopadhyay et al., (2017) [8] found hyperhomocysteinemia in 32% of RPL patients in their prospective study, they defined hyperhomocysteinemia when S. Hcy level≥12 µmol/l. The study conducted by Puri et al. (2013) [14] found that hyperhomocysteinemia (considered level ≥13 µmol/l) was present among 65.06% of the cases as compared to only 20.94% of the controls and the difference was statistically significant with a P-value < 0.0001. The odds ratio also revealed a 7.02-fold increased risk of RPL (OR=7.02; CI=3.8558-12.8094, P < 0.0001) depicting hyperhomocysteinemia as an independent risk factor for RPL. It was observed in this study that the mean homocysteine level of RPL patients was 8.15±8.11 µmol/l was not significantly different from the control group (6.23±1.47 µmol/l) with a P-value 0.878. S. homocysteine level was also divided according to different trimester loss and compared with the control which was also found not significant (P-value=0.539). Creus et al., (2013) [12] also found in their study that the mean homocysteine level in the case group was not significantly different from the control (7.7±2.3 vs. 8.1±3.3 µmol/l). In Zarfeshan Fard et al., (2019) [3], the mean homocysteine level was also not different significantly between the case group and control (6.58±1.84 vs. 5.50±0.81 µmol/l). The study of Govindaiah et al., (2009)

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[15] found that hyperhomocysteinemia increased the risk of RPL and the mean homocysteine level in the case group was higher significantly than the control (mean: 11.6±5.0 versus 8.6±4.2 µmol/l, OR=4.48). The review article titled "A Novel Review of Homocysteine and pregnancy complications" by Dai et al., (2021) [16] reviewed 7 articles that analyzed the association of serum homocysteine and RPL and found that 5 articles supported and 2 articles rejected the association between homocysteine levels and RPL. The association between serum homocysteine level and RPL susceptibility has been widely researched, with contradictory results.

Limitation of the Study

The study population was only recruited from one selected hospital in Dhaka city. So, the results of the study may not reflect the exact scenario of the country. The present study was conducted over a very short period and the study was conducted on a relatively small sample size, which was also a limitation.

Conclusion and Recommendation

As per the findings of this current study, we can conclude that serum homocysteine level has not any significant association with unexplained recurrent pregnancy loss. Even, not any significant difference is found when serum homocysteine levels are compared with the control in 1st, 2nd and 3rd-trimester pregnancy loss. All the findings of this current study may be helpful in further similar studies.

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