Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 09, 2024

Original Research Article

Title: Estimation of homocysteine in recurrent pregnancy loss

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ABSTRACT

Background: Recurrent pregnancy loss is considered as two or more miscarriages. Clinical investigation may be initiated after two pregnancy losses. The occurrence of spontaneous miscarriage in India is reported to be around 6.37%, recurrent miscarriage is around 1 to 2%. The risk of miscarriage in subsequent pregnancy is 30% after 2 losses compared to 33% after 3 losses. The prevalence of Hyperhomocysteinemia in recurrent pregnancy loss in India is 38%. **Objective:** to study the levels of maternal serum homocysteine in pregnant women with 2 or more consecutive miscarriages and compare with control group and to determine if hyperhomocysteinemia is associated with RPL **Methods**: 100 patients with history of 2 or more consecutive pregnancy losses are taken as cases and 100 pregnancy lady with alteration one previous live mother are taken as controls. In the study both cases and controls had not taken folic acid in the last 3 months was confirmed by history.

Both cases and control group previous records were examined for laboratory investigation to rule out associated co-morbidities like uterine anomaly on ultrasound, complete blood count for platelets ,coagulation profile , thyroid profile,blood glucose, renal and liver function test , urine albumin ,urine routine and microscopy and serology test for Human

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immune virus, Hepatitis B, syphilis , TORCH infection. **Results:** Out of 100 cases, 17 patients show increased homocysteine levels. Homocysteine group shows P value of 0.001, which is significant at 1% levels.Homocysteine levels more than 10.5 μmol/dl is a risk factor for pregnancy loss. This shows positive correlation between RPL and hyperhomocysteinemia.

Conclusions: This study shows there is positive correlation of socioeconomic class with hyperhomocysteinemia. Pregnant women belonging to Lower class of Kuppuswamy class had higher serum homocysteine level indirectly indicating poor nutrition among this pregnant.

Keywords: Hyperhomocysteinemia, miscarriages, pregnant women

Introduction

According to ASRM (American Society for Reproductive Medicine),

According to the American Society for Reproductive Medicine (ASRM), it is defined as two or more clinical pregnancy losses (documented by ultrasonography or histopathology examination), and non- visualized pregnancy losses are not included[1]. According to European Society for Human Reproductive and Embryology suggested the same inclusion of two or more pregnancy losses but permitted including nonconsecutive pregnancy losses while the other guidelines focus on including only consecutive pregnancy losses. [2,3]

Most of the prospective cohort studies have shown that only two-third of conceptions fail to progress to a live birth in women trying to conceive [4,5,6] 30% of conceptions are lost even before the implantation and 30% are lost post-implantation i.e. in the third and fourth weeks of gestation. These pregnancy losses are classified as preclinical losses [7]. 70% of conceptions are found to be lost before live birth and most of those losses are preclinical. Macklon et al., 2002, and Larsen et al., 2013, have described pregnancy loss iceberg, showing an overview of the outcome of spontaneous human conceptions (on the ‘iceberg’,

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these preclinical losses are shown below the 'waterline') Figure 1.1 [7,8]

15% to 20% of the spontaneous miscarriages happen among healthy couples and of these spontaneous miscarriages 2% to 3% end in recurrent spontaneous pregnancy losses [9]. There is a direct proportional strong correlation between maternal age and the incidence of miscarriages [10] and the underlying cause suggested as the frequency of aneuploidy in the oocytes [11]. Grande et al., 2012, described that the risk of miscarriage increases significantly in older women (35 years) by 9.5% at the age of 24 to 25 years and up to 76% at age³45 years [10]

The prevalence of spontaneous miscarriage in India is reported to be round 6.37% [12] , Recurrent miscarriage is around 1 to 2%[13].The risk of miscarriage in consequent pregnancy is 30% after 2 losses while 33% after 3 losses[5]. In India the prevalence of Hyperhomocysteinemia in recurrent pregnancy loss is 38%[14].

Hence this study was conducted to study the levels of maternal serum homocysteine in pregnant women with 2 or more consecutive miscarriages and compare with control group and to determine if hyperhomocysteinemia is associated with RPL.

Materials and Methods: This Case control study was conducted in Vanivilas hospital attached to BMCRI\, Bangalore. Study period was February 2021 to June 2022(18 months)

Inclusion Criteria:

GROUP A-CASES

a. Pregnant women with 2 or more spontaneous consecutive pregnancy losses at the booking visit.
b. Pregnant women in reproductive age group(18 to 39 years)
c. Pregnant women willing to give consent for the study.
d. No other known comorbidites.

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GROUP -B CASES

a. Matched controls with at least one previous live pregnancy outcome.

Exclusion Criteria:

a. Patient not willing to give informed consent.
b. Women with non consecutive pregnancy losses.
c. Patient with known uterine anomalies.
d. Women with known co morbidity like immunological diseases, antiphospholipid antibody syndrome , endocrinology disorder (eg Hypothyroidism) .
e. Women with history of medical termination of pregnancy.
f. Pregnant women with history of folic acid or vitamin B6 supplementation in the last 6month.

Sample Size Calculation:

Minimal sample size is 50.

Sample taken is 100 in each group

Methodology:

After obtaining approval and clearance from the institutional ethics committee , the data is be collected using a prepared Performa by means of personal interview of the patient after taking informed consent and after applying inclusion and exclusion criteria.

a) Thorough history was taken of present pregnancy and previous pregnancy:

Pattern, trimester, and characteristics of prior pregnancy losses.

History of sub fertility or infertility.

Menstrual history.

Prior or current gynecologic or obstetric infections.

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Signs or symptoms of thyroid, glucose tolerance and hyperandrogenic disorders (including polycystic ovarian syndrome).

Personal or familial thrombotic history.

Features associated with the antiphospholipid syndrome (thrombosis, false positive test for syphilis). Other autoimmune disorders.

Use of any Medications.

Environmental exposures, illicit and common drug use particularly caffeine, alcohol, cigarettes, and in utero diethylstilbestrol exposure).

b) Gestational age was based on the participants' last normal menstrual period.

c) Thorough clinical examination including General physical examination, vital , systemic examination .

d) Group A cases, patient who come to the hospital for first Antenatal care visit with history of 2 or more consecutive pregnancy loss with no history of folic acid consumption in the last 6months were taken as study and patients with at least one successful pregnancy were taken as control.

e) Both cases and control group previous records were examined for laboratory investigation to rule out associated co-morbidities like uterine anomaly on ultrasound , complete blood count for platelets ,coagulation profile , thyroid profile,blood glucose, renal and liver function test , urine albumin ,urine routine and microscopy and serology test for Human immune virus, Hepatitis B, syphilis , TORCH infection.

f) Cases and control were advised on first visit to come next morning on empty stomach with fasting for minimum 8hrs and was advised to take the test.

g) A venous blood sample by venipuncture to be used to assess Fasting homocysteine level .Total homocysteine concentration was measured by enzymatic photometric method, after centrifugation and storing. Patient sample Id was taken and confirmed with laboratory.

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h) Hyperhomocysteinemia is defined as homocysteine level more than 95 percentile. In Indian studies, Yajnik et al and Kumar et al have taken cut off as 10.5 micro mol.

STATISTICAL ANALYSIS

Data will be entered in the excel spread sheet. Descriptive statistics will be done which provides the mean, standard deviation and percentages. Inferential statistics like chi-square and unpaired t test will be used for qualitative data and quantitative data respectively using SPSS (statistical Package for Social Sciences) version 20. (IBM SPASS statistics [IBM corp. released 2011]. Any other necessary tests will be dealt at the time of analysis based on data distribution.

Results: A total of 200 subjects were included in final analysis. Among the study population, 100 (50%) participants were in Cases (RPL) and remaining 100(50%) participants were in control group.

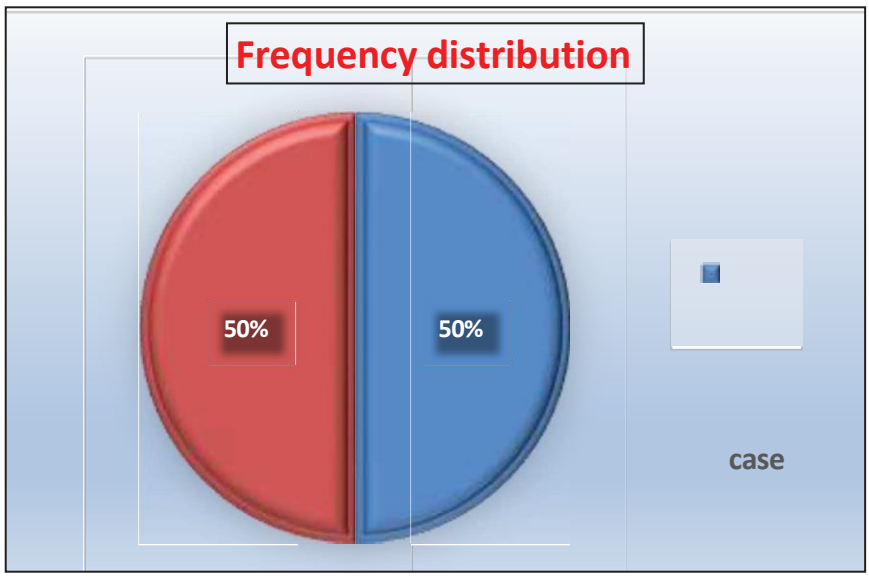

Figure 1: Pie chart of frequency distribution among study population (N=200)

Among the case group,the mean age was 25.66 years (SD+/- 4.699) and it was 23.56 years(SD+/-3.557) in control group. The mean age was higher among patients with

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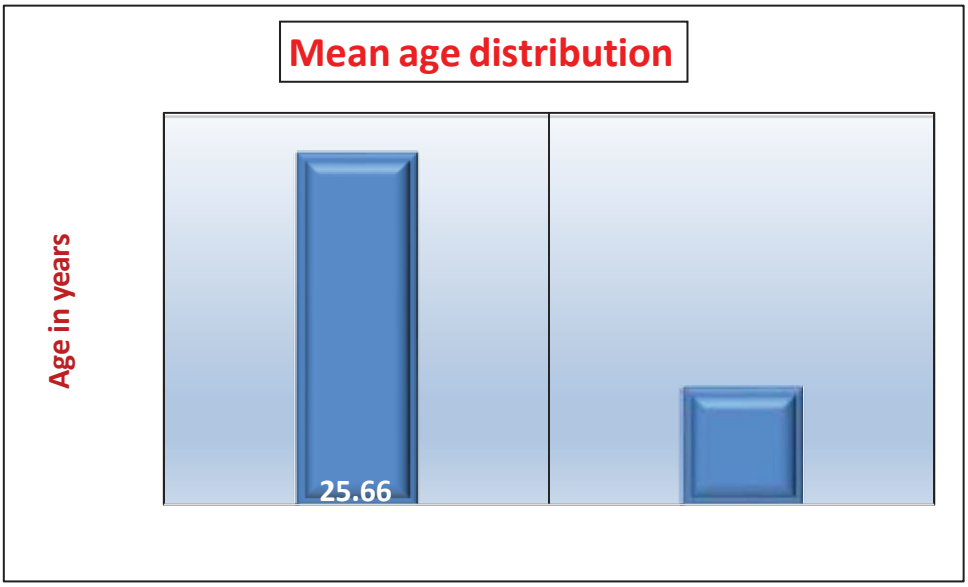
RPL.P VALVE IS <0.0001 which implies that there is significant age distribution among case and control group.

Figure 2: Bar chart of comparison of age among study population(N=200).



There is significant age difference is cases and control groups.

SOCIOECONOMIC CLASSES: Kuppuswamy classification was used to assess socioeconomic class of both groups.TheSES was comparable in both the groups. In the lower socioeconomic status, 66 (66%) participants in cases and 54(54%) participants were in control group. Out of 200 participants 120(60%) belonged to lower class of Kuppuswamy classification.

TABLE 1: Mean homocysteine level in case and control groups. (N=200)

		N	Mean	SD	t statistic	p-value
HOMOCYSTEINE	Case	100	8.43	4.971	4.863	<0.0001
	control	100	5.77	2.282		

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The mean homocysteine in case group is 8.43 and control group is 5.77.The above table show

p< 0.0001 which means that there is statistically significant difference in mean homocysteine levels in cases and control groups.

The table shows 17% of cases and 5% of control had hyperhomocysteinemia. There is significant statistical difference in number of participant with increased homocysteine levels in case and control groups.

This table shows homocysteinemia is more common in primary aborters. Hyperhomocysteinemia was thrice more common in Primary aborters when compared to secondary aborters.

Discussion:

Several pathophysiological hypotheses including impaired cell proliferation, increased oxidative stress, apoptosis, reduced extra-embryonic vascular development are responsible for recurrent pregnancy loss in hyperhomocysteinemia[15].It is not clear whether hyperhomocysteinemia is a causative factor or marker of RPL. Lowering homocysteine concentration by B-vitamin supplementation has shown to have a positive effect in several case reports[16] with spontaneous pregnancies occurring after a few months of treatment in patients who had previously experienced early spontaneous abortion between 4 qt 12 weeks. Increasing evidences are available for the relationship between hyperhomocysteinemia and MTHFR C677T gene polymorphism and unexplained recurrent pregnancy loss[17,18].

In this study we evaluated homocysteine levels in women showing RPL. During pregnancy, the levels of plasma homocysteine tend to fall due to increased methionine requirement by fetus, hemodilution and increased renal clearance of homocysteine. Hence the value tends to fall from15 μmol to 10.5μmol[19] .Hence values more than 10.5 taken as hyperhomocysteinemia. In our study out of 100 cases with RPL 17 cases had

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hyperhomocysteinemia which is statistically significant.

The women with RPL show significant higher mean homocysteine concentrations than controls, correlating with Nisha et al study.

In our study, hyperhomocysteinemia is more common in primary aborters while Comans et al and Wouters et al found Hyperhomocysteinemia more common in secondary aborters .The mechanism could be an intrinsic genetic metabolic disorder in primary aborters rather than dietary and environmental factors that could have a role in secondary aborters.

Hyperhomocysteinemia also found to be a risk factor for recurrent pregnancy loss as per this study. Daily supplementation with vitamin B12 and folic acid- reduce homocysteine concentration. Regarding MTHFR mutation, treatment is dietary intervention and supplementation with folic acid and vitamin B group[20,21].

Hence we can infer from present study that there is a statistically significant association of Hyperhomocysteinemia with complications likes early pregnancy loss. Many unknown causes remain regarding the impact of hyperhomocysteinemia on pregnancy. Large scale of study and control groups needed to define relation between homocysteine, folic acid and pregnancy loss.

Conclusion:

Our study comprises 100 recurrent pregnancy loss cases and 100 controls. Out of 100 cases, 17 patients show increased homocysteine levels. Homocysteine group shows P value of 0.001, which is significant at 1% levels. Homocysteine levels more than 10.5 μmol/dl is a risk factor for pregnancy loss.

This study shows there is positive correlation of socioeconomic class with hyperhomocysteinemia. Pregnant women belonging to Lower class of Kuppuswamy class had higher serum homocysteine level indirectly indicating poor nutrition among this pregnant.

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