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Original Articles

Plasma Homocysteine Levels in Polycystic Ovarian Syndrome with Early Recurrent Pregnancy Loss: A Case-Control Study

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Abstract

Background: Recurrent Pregnancy Loss (RPL) is defined as the occurrence of three (or) more clinically recognised pregnancy losses before 20 weeks of gestation. Spontaneous abortions occur in 15% of clinically recognised pregnancies in reproductive age group women. Hyper homocysteinemia has been associated with clinical vascular thrombosis and this could be the cause for early decidual and chorionic vessel damage that might result in disturbed implantation of the conceptus. **Aim:** To determine the plasma homocysteine levels in patients with polycystic ovarian syndrome (PCOS) having recurrent pregnancy loss (RPL) compared with women with PCOS who had uneventful pregnancies and assess the role of hyper homocysteinemia for RPL in PCOS. **Materials and Methods:** This is a case-control study on 20 PCOS women with RPL and 20 control PCOS women with uneventful pregnancies matched by age, marriage duration and body mass index(BMI). Plasma homocysteine levels were assessed from Feb, 2013 to Feb, 2014. **Results:** The mean plasma homocysteine in the cases and controls were 19.10±1.01 and 16.41±1.24 (p=0.0001). The difference in the homocysteine levels was found to be statistically significant. Conclusion: Risk of RPL in patients with PCOS is increased with hyperhomocysteinemia (p< 0.0001).

Key-words: Plasma homocysteine levels, PCOS, early recurrent pregnancy loss

Introduction

Recurrent Pregnancy Loss (RPL) is defined as the occurrence of three (or) more clinically recognised pregnancy losses before 20 weeks of gestation. Spontaneous abortions occur in around 15% of clinically recognised pregnancies in women of reproductive age group. RPL occurs in about 1-2% of these women.[1] Of the many etiologies polycystic ovarian syndrome (PCOS) is a common endocrine disorder responsible for RPL. Patients with PCOS experience a 30-50% increased rate of early miscarriages.[2,3] PCOS is characterised by hyperandrogenism, chronic anovulation and polycystic ovaries on ultrasonography. Homocysteine is a naturally occurring sulphur containing amino acid, and is an intermediate substance formed during the breakdown of the amino acid methionine. Homocysteine, in turn, is metabolised either into cystathionine or methionine. It is required vitamin B6, Folic Acid and vitamin B12 for metabolism of homocysteine; in the deficiencies of which hyperhomocysteinemia develops and which is also found defects in gene mutation in the enzyme 5-Methyltetrahydrofolate (5-MTHFR).

Hyperhomocysteinemia has been associated with clinical vascular thrombosis and this could be the cause for early decidual and chorionic vessel damage that might result in disturbed implantation of the conceptus.[4,5,6] RPL studies on maternal homocysteine and chronic vascularisation in RPL have concluded that elevated total homocysteine concentrations were associated with defective chorionic villous vascularisation.[7] Saghar and co-autors in their case-control study concluded that patients with PCOS had a significantly higher risk for hyperhomocysteinemia.[8] The present study is done to assess the role of hyperhomocysteinemia in RPL among patients with PCOS.

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Material and Methods

This case-control study was conducted at Owaisi Hospital and Research Centre and Princess Esra Hospital Department of Obstetrics and Gynaecology. The patients for the study selected were-from among the women attending the outpatient department of obstetrics and gynaecology from Feb, 2013 to Feb, 2014.

Inclusion criteria

Twenty PCOS patients (n=20) with early recurrent pregnancy loss (2 or more pregnancy loss of ≤10wks) well matched with 20 PCOS patients (controls(n)=20) with uneventfull pregnancies in terms of age, marriage duration and BMI. PCOS was diagnosed according to Rotterdam criteria 2003. [9,10] (oligo/amenorrhea, hyperandrogenism, PCO on USG).

Exclusion criteria

- Patients with uterine anatomical anomalies
- Other causes of hyperandrogenism(congenital adrenal hypeRPLasia, Cushing syndrome e.t.c),
- Couples with chromosomal abnormalities (evaluated by karyotyping of both partners)
- Other endocrine disorders(hypothyroidism etc)
- Patients with positive tests for Lupus anticoagulant and anticardiolipin antibodies
- Patients with debilitating illness, chronic liver and renal disease, tuberculosis
- Patients with history of Diabetes mellitus
- Pregnancy
- Patients with h/o using folic acid supplementations

An informed consent explaining about the nature and the purpose of the study was obtained from the subjects enrolled into the study. The study was performed with the approval of the Ethics Committee of the institute. Fasting blood samples for FSH, LH, PRL, TSH, Homocysteine, Vit B12 and Serum Folate were collected on the second day of menstrual cycle in serum and EDTA tube from all the subjects by venipuncture. The samples were centrifuged at 3000rpm for 10mins and the Plasma separated. Hormonal profile was measured using ELISA kit. Homocysteine,Vit B12 and Serum folete levels were measured using ADIVA centaur assay by direct chemiluminescent technology. Plasma homocysteine levels of 15umol/l was taken as cut-off limit in the present study.

Statistical Analysis

Data was entered in Microsoft excel and analysis was done using SPSS version 20. Descriptive statistical analysis was done. Results on continuous measurements are presented as Mean and Standard Deviation. Significance is assessed at 5 % level of significance. Student t test (independent, two tailed) has been used to find out the significance of study parameters on a continuous scale between two groups.

Results

The mean age of the women in the RPL group was 26.8±33.14 years and 27.23±3.01 years in the control group (p=0.660 NS). The mean marriage duration in years among the RPL population in the study was 5.21±1.30 and 5.24±1.31 in the controls (p=0.942 NS). The baseline BMI was 26.82±2.31 in the RPL group while it was 26.12±1.30 in the controls (p=2.44 NS).The hormonal profile of Serum folic acid,Vit B12 levels did not show any significant difference in both groups. The plasma homocysteine levels were 19.10±1.01 in the RPL group and 15.41±1.24 in the control group (p=0.0001). Paired comparisons revealed that statistically significant difference was observed in the plasma homocysteine levels of women with PCOS having recurrent pregnancy loss and the control group comprising of PCOS women with uneventful pregnancies

Table 1. Profile of cases and control groups

Variables	Cases (n=20)	Controls (n=20)	*P* value
Age(yrs)	26.8±33.14	27.23±3.01	0.954
BMI	26.82±2.31	26.12±1.30	0.244
TSH	2.04±1.36	2.11±1.01	0.854
LH/FSH Ratio	2.1±0.31	1.8±1.7	0.442
S. PRL	12.14±0.91	11.8±1.71	0.437
Plasma Homocysteine Levels	19.10±1.01	15.41±1.24	0.0001 ***
S. Folic Acid	6.08±1.20	6.01±0.11	0.796
Vit B12	340±33.14	349±30.01	0.373

***p=0.0001

Discussion

Recurrent pregnancy loss is an emotionally disturbing and physically taxing problem for couples who often express their concern regarding the cause for recurrent miscarriages. Role of various etiological factors such as genetic, anatomical, immunological, infectious and endocrine factors have been implicated in RPL. PCOS often is being a common endocrine disorder found in patients with RPL. Patients with PCOS are often obese and have commonly been found to exhibit metabolic disturbances involving insulin resistance. In the recent times another metabolic component, hyperhomocysteinemia has been found to be associated with PCOS. In the present case-control study the cases comprised of 20 PCOS patients with RPL and the controls comprised of 20 PCOS patients with uneventful pregnancies. Their hormonal profile, serum folic acid, Vit B12 levels and plasma homocysteine levels were determined. There was no significant difference observed in their hormonal profile, Vit B12 and Serum folic acid levels, However we found that women with RPL showed (p<0.0001) extremely statistically significant raise in plasma homocysteine levels in comparison to the control group. Hyperhomocysteinemia has often been associated with venous and arterial thrombosis, early miscarriages and congenital malformations. Raised plasma homocysteine levels increase oxidative stress by release of free radicals and reduce endothelial relaxation by impairing nitric oxide production leading to early damage of the decidual and chorionic vessels which may interfere with the implantation of the conceptus.[4] In the recent times Boxmeer et al. (2008) have reported that high homocysteine in follicular fluid reduces embryo quality and has detrimental effects on folliculogenesis.[11] Study Ebisch et al have reported high homocysteine by levels associated with low embryo quality.[12]

Study by Saghar et al. (2011) has shown that plasma homocysteine levels are markedly higher among women with PCOS.[13] Another factor that has been commonly associated with hyperhomocysteinemia in PCOS patients is insulin resistance. Studies have shown that some amount of insulin resistance exists in women with PCOS and this insulin resistance has been found to inhibit beta cystathionine synthase avtivity, an enzyme essential in methionine metabolism.[14,15] Insulin levels were however not determined in the present study and the possibility of insulin resistance causing hyperhomocysteinemia cannot be ruled out. Pratip et al. (2013) in their study on RPL in PCOS in Kolkata had established increase incidence of miscarriage in women with hyperhomocysteinemia and insulin resistance however the cut off level used for hyperhomocysteinemia was 12umol/l.[16]

Conclusions

In conclusion with the above results the risk of RPL in patients with PCOS is increased with hyperhomocysteinemia. Patients with PCOS treated for RPL and infertility should be assessed for homocysteine levels and if necessary corrected for good pregnancy outcome. However further studies with large sample size and randomised trials are required for establishing the role of hyperhomocysteinemia in PCOS patients with RPL.

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