The Role of Homocysteine, Folic Acid, and Vitamin B12 in Primary Unexplained First Trimester Recurrent Pregnancy Loss

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Abstract: Objective: To assess the serum levels of homocysteine (HCY), folic acid and vitamin B12 in cases of primary unexplained first trimester recurrent pregnancy loss. **Patients and Methods:** The current case control study was conducted at Ain Shams University Maternity Hospital during the period between May 2008 and October 2008. Finally, included women were divided into two groups: Group I: (cases) included forty five patients with primary unexplained first trimester repeated pregnancy loss who were admitted for termination of pregnancy due to early pregnancy failure. Group II: (control) included matched apparent healthy pregnant women in the first trimester with no history of bad obstetric outcome and had at least one living newborn. Maternal venous samples were taken for assay of serum levels of HCY, folic acid and vitamin B12. **Results:** A total of 90 women were included in the study. The mean HCY level was 10.8+4 Umol/L in group I ,while in group II was7.9+4.3 Umol/L with statistical significant difference between the 2 groups($p<0.01$).The mean folic acid was 8.7+2.1 ng/ml in group I and 10.8+2.2 ng/ml in group II with statistical significant difference between both groups($p<0.01$).There was a statistical significant difference between the 2 groups regarding the serum level of vitamin B12 (339+296pg/ml in group I versus 374+209pg/ml in group II) ($p<0.05$). The best cut off value for the 3 markers (HCY, folic acid, vitamin B12) is 15 Umol/L, 12 ng/ml, and 360 pg/ml respectively. **Conclusion:** High level of hyperhomocysteinaemia and low level of folate and vitamin B12 may contribute for the etiology of first trimester unexplained recurrent early pregnancy loss.

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Key Words: Homocysteine – folic acid - vitamin B12-recurrent pregnancy loss.

1. Introduction

Recurrent fetal loss is defined as the number of consecutive miscarriages which is not less than 2 occurred within 16 week of gestation and it is a very interesting pathology of pregnancy[1]. Further to thrombophilia, very important causes have been identified, since the damage of the vascular system supporting the placenta may cause a deficiency of placenta functions and development, leading to a loss of the conception product, also in a condition of hyperhomocysteinemia, causing damage to the vascular endothelium. Hyperhomocysteinemia seems to be a risk factor for arterio-venous thrombotic diseases, even not in pregnancy [1]. Vitamins related to HCY metabolism such as folate, vitamin B12 and Vitamin B6 are essential in adequate fetal growth and integrity of healthy pregnancy [2]. An average of 20-30% of pregnant women suffer from any vitamin deficiency, and without prophylaxis, about 75% of these would show a deficit of at least one vitamin. About 25% of pregnant women in developing countries are folate deficient. Folate deficiency may lead to congenital malformations (neural tube damage, orofacial clefts, cardiac anomalies), anaemia , spontaneous abortions, pre-eclampsia, IUGR and abruption placentae. A daily supplemental dose of 400 µg/day of folate is recommended when planning pregnancy [3]. In developing countries diets are generally low in animal products and consequently in vitamin B12 content. An insufficient supply may cause reduced fetal growth. In vegetarian women, supplementation of vitamin B12 may be needed [3].

2. Patients and Methods

The current case control study was conducted at Ain Shams University Maternity Hospital during the period between May 2008 and October 2008. The study was approved by ethical and research committee council of Obstetrics and Gynecology Department, Ain Shams University. The study purpose and procedures were explained to all enrolled women and a written informed consent was obtained from each participant. Included women were divided into two groups : Group I : (cases) included forty five patients with 2 or more consecutive primary unexplained first trimester repeated pregnancy loss(RPL) admitted for medical or surgical termination of pregnancy due to early pregnancy failure. Group II: (control) included matched apparent healthy pregnant women in the first

trimester with no history of bad obstetric outcome and had at least one living newborn .Women in group II attended Ain Shams University Maternity Hospital for routine antenatal care. Maternal venous samples were taken for assay of serum levels of HCY, folic acid and vitamin B12.Gestational age was calculated based on the first day of last menstrual period and or transvaginal sonography. Any patients with past or present medical disorder associated or not associated with pregnancy were excluded from the study. A venous blood sample was collected before giving any medications or intravenous fluids, in a vacutainer tube containing Ethylenediaminetetraacetic acid (EDTA), and was centrifuged within 30 minutes of collection at 2500 rpm for 10 minutes, and then the serum was stored at -20 ^{o}C for measurement of HCY, folic acid and vitamin B12. Assessment of HCY based on the florescence polarization immunoassay (FPIA) technology using (Axsyium kits®, Abbott, Ireland). Vitamin B_{12} and Folic acid assay based on ion capture technology using the same kits.

Statistical analysis

Data were analyzed using SPSS® for Windows®, version 13.0 (SPSS, Inc, USA). Description of quantitative (numerical) variables was performed in the form of mean, standard deviation (SD). Description of qualitative (categorical) data was performed in the form of number of cases and percent. Analysis of numerical variables was performed by using independent student's t-test. If a normal distribution of data was not found, a Mann-Whitney U test was used instead of Student's t-test. Diagnostic accuracy was assessed using the following terms: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy .ROC (receiver operator characteristic) curve was used to find out the best cut off value of certain variable). Sensitivity: ability of the test to detect positive cases and calculated as true positive cases/true positive cases + false negative cases. Specificity: ability of the test to exclude negative cases and calculated as true negative cases/true negative cases + false positive cases.

PPV is the percentage of true positive cases to all positive (proportion of all individuals with positive tests, who have the disease). NPV is the percentage of true negative cases to all negative (the proportion of all individuals with negative tests, who are non-diseased. While over all Accuracy means true negative +true positive / all cases.

A difference with Probability (*P*) value <0.05 was considered statistically significant.

3-Results

A total of 90 women were included in the study. Figure-1 shows participants flow diagram.

There is no statistical significant difference between the 2 groups regarding maternal age. Among patients in group (I) 60% of patients had twice RPL, 33.3% had 3 RPL and 6.7% had 4 RPL. The mean values of HCY, folic acid and vitamin B12 are shown in table1. The best cut off value for the 3 markers (HCY, folic acid, vitamin B12) is 15 Umol/L, 12 ng/ml and 360 pg/ml respectively. Regarding HCY, 93.3% of cases above the cut off level, while 6.7% of controls above the cutoff level. Concerning folic acid 95.6% of cases and 37.8% of control had a cutoff level below 12 ng/ml. While, regarding vitamin B12 73.3%of cases and33.3%in controls had a cutoff level below360 pg/ml. Table 2 shows the cutoff values, sensitivity, specificity, PPV, NPV and overall accuracy of vitaminB12, folic acid and HCY in prediction of recurrent pregnancy loss. The ROC curves for HCY, folic acid and vitamin B12 are shown in figure 2, 3, and4 respectively.

Table (1): The mean values of HCY, folic acid and vitamin B12 among both groups *

	Group I (N=45)	Group II (N=45)	*P***
HCY (Umol/L)	10.8±4	7.9±4.3	<0.01(S)
Folic acid(ng/ml)	8.7±2.1	10.8±2.2	<0.01(S)
Vitamin B12(pg/ml)	339±296	374±209	<0.05(S)

*Values are expressed as mean± standard deviation ** Analysis using Independent Student's t-Test

Table (2) Cutoff values, sensitivity, specificity, PPV, NPV and accuracy of vitaminB12, folic acid and HCY in prediction of recurrent pregnancy loss*

Variables	Vitamin B12	Folic acid	HCY	Combination
Cutoff value	360(pg/ml)	12(ng/ml)	15(Umol/L)	
Sensitivity	93.3%	96%	73%	98%
Specificity	45%	50%	42%	60%
PPV	51%	57%	50%	61%
NPV	50%	89%	56%	90%
Accuracy	50%	66%	52%	58%

*Values are expressed as numbers and percentage PPV positive predictive value NPV negative predictive value.

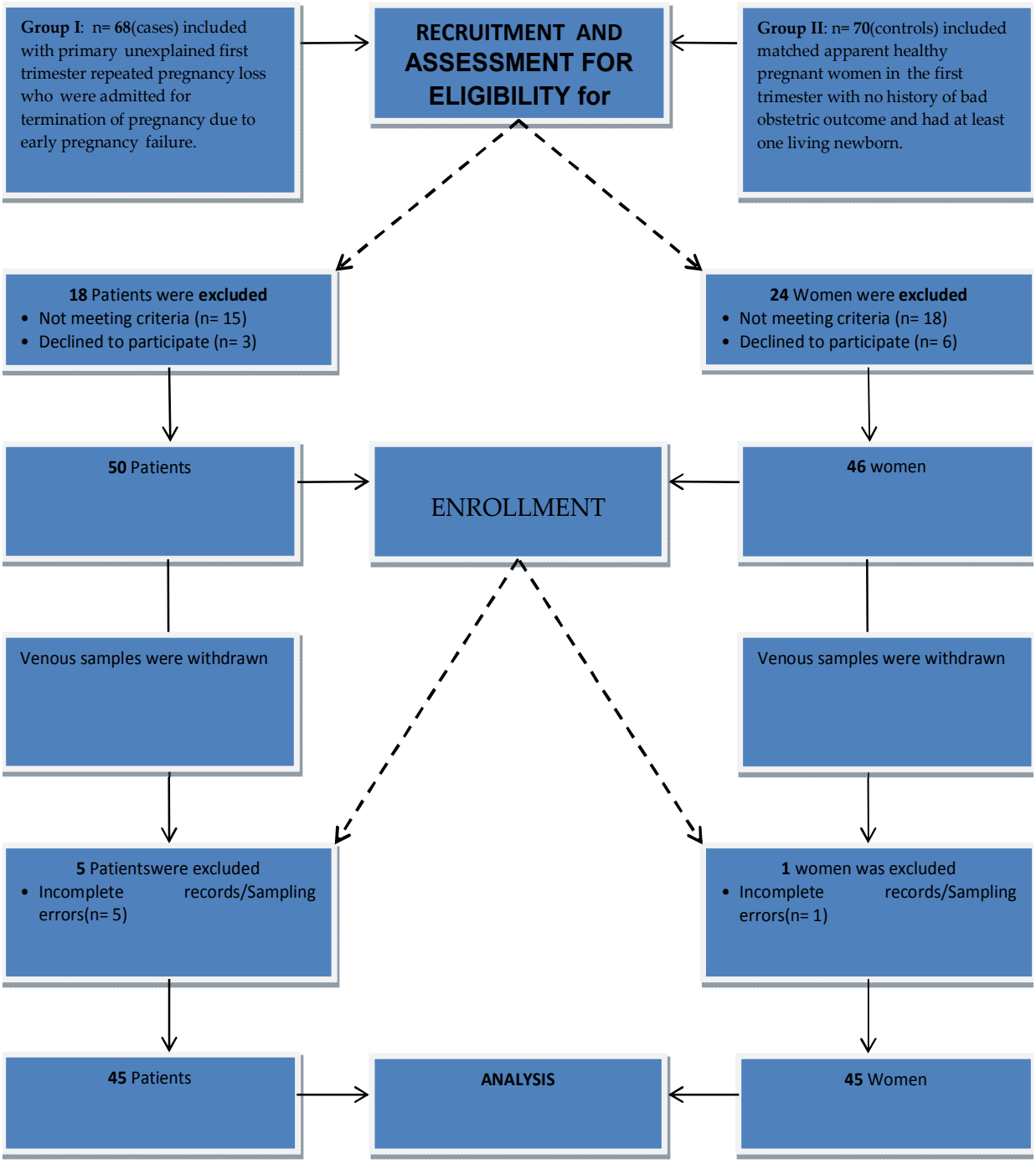

Figure-1 Participants flow diagram.

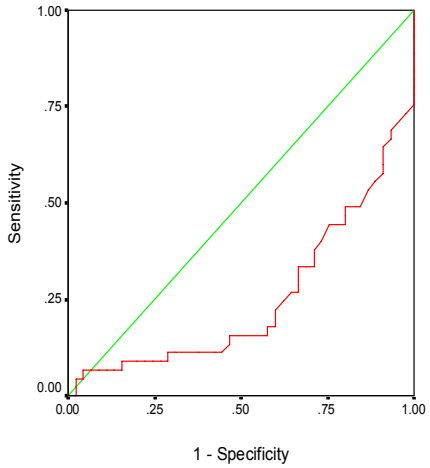

Figure (2) ROC curve for HCY

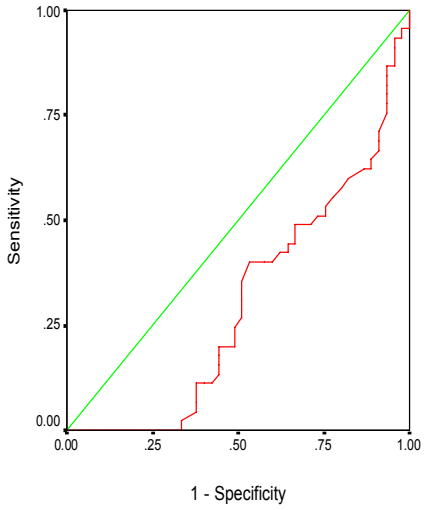

Figure (3) ROC curve for folic acid

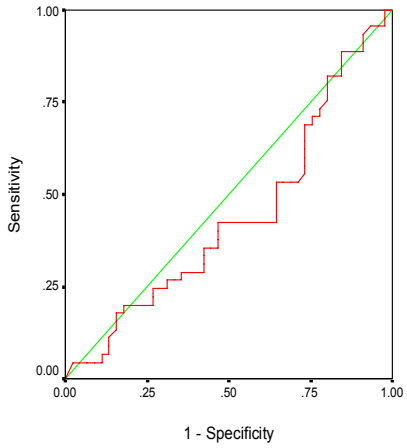

Figure (4) ROC curve for vitamin B12

4. Discussion

In the current study, the level of 15 Umol/L was used as a suitable cut off value for the HCY, with sensitivity of 73% and a specificity of 42%, while PPV of 50% and NPV of 56%, and overall accuracy of 52%. This means that the HCY is a good positive useful test and a less negative useful test.

As regard HCY, 93.3% of cases were above the cutoff level while 6.7% where below the cutoff level, while 6.7% of controls where above the cutoff level where 93.3% where below the cutoff level.

Wouters *et al.* showed that moderate hyperhomocysteinaemia is responsible by 14% of primary pregnancy loss while Nelen *et al.,* in their meta-analysis quoted on incidence of 30% of secondary pregnancy loss [4, 5].

Quere *et al.* came to the same conclusion and found that some cases of recurrent miscarriage might be due to hyperhomocysteinaemia as a consequence of an interaction between primary gene defect and nutritional folate deficiency [6].

Ronnenberg *et al.* conducted a similar study to the present work. They assessed the level of HCY, folate and vitamin B status in 49 propagandas against 409 women who maintained a pregnancy that ended in live birth. Their results showed that the risk of spontaneous abortion was 4 fold higher among women with low plasma concentration of both folate and vitamin B 6, while HCY and vitamin B 12 status were not associated with spontaneous abortion risk [7].

HCY, folate, and Vit.B12 with cut off values of 15 Umol/L, 12 ng/ml, and 360 pg/ml respectively, with sensitivity of 98%, specificity of 60% and PPV of 61% while NPV is 90% and accuracy of 58%. This means that the 3 markers are considered better positive than negative with higher sensitivity and accuracy and folate takes the upper hand as regard the highest sensitivity and accuracy.

Kim *et al.* tried to explore the association between the levels of HCY and vitamin B status and their results show clearly a direct relation between an increased level of HCY and deficiency of folic acid, vitamin B6 and vitamin B12 [8]. The association of hyperhomocysteinaemia and vitamin B factors deficiency together with repeated pregnancy loss was investigated by Nelen *et al.,* who compared the level of HCY, folic acid vitamin B6 and B6 and vitamin B12 in 123 women with repeated miscarriages to the levels in 104 healthy controls. They found that women with recurrent pregnancy loss, had a significantly high level of HCY, a significant low level of folate while others levels showed no difference, Nelen *et al.* concluded that high folate concentration might have a protective effect against early pregnancy failure[5].

Nelen *et al.* in another trial studied the effect of daily folic supplementation at a dose of 0.5mg in

patients with recurrent miscarriage they found that dose has significantly dereased total plasma HCY concentration and the effect was profound in patients with high basic HCY level[9].

Nelen *et al.* in study of 362 healthy individuals with normal reproductive performance, quoted a 20% incidence of folate deficiency, then called for randomized controlled studies to investigate the role of folic acid supplementation in prevention of congenital anomalies and repeated miscarriage [10].

Chery *et al.* claims that hyperhomocysteinaemia is related to a decreased blood level of vitamin B 12 in the second and third trimesters of pregnancy [11].

De La Calle *et al.* concluded that hyperhomocysteinaemia might be due to other vitamin B deficiencies than to folic acid alone [12].

Reznikoff-Etievant *et al.* studied Serum B12, folate and homocysteine levels in 110 women with unexplained early recurrent abortion. In case group, low serum B12 and raised Homocysteine were noticed. Among women with low serum B12 level and early recurrent abortion (ERA), 87.5% of the abortions were very early recurrent abortion (VERA) around 5 weeks of amenorrhea. Vitamin B (12) supplementation led to four normal pregnancies in five women who became pregnant again. He concluded that Vitamin B (12) assay should be done in ERA women whether or not hematological abnormalities are present [13].

Khong and Hangue, did not find a significant association between elevated HCY level and spontaneous abortion risk, they suggested that their failure to detect a significant effect of elevated HCY might be due to the relative small number of abortion cases (patients, n=49), also it might be related to the fact that their patients were experiencing their first occurrence of spontaneous abortion [14].

5. Conclusion: High level of hyperhomocysteinaemia and low level of folate and vitamin B12 may contribute for the etiology of first trimester unexplained recurrent early pregnancy loss.

6. Declaration of interest: The authors reported no conflict of interest. All of the authors had substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, drafting and revising the article critically with final approval of the version to be published. The research was funded by the authors.

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