The J. of the Egyptian Society of Haematology and Research, Vol. 2, No. 1, March: 51-55, 2006

Hyperhomocysteinemia in Recurrent Miscarriage

KHALED R. GABER, M.D.; MONA K. FARAG, M.D.; SOMAYA E.T. SOLIMAN, M.D.* and MOHAMED A. ABD AL-KADER, M.D.**

*Prenatal Diagnosis & Fetal Medicine Department, National Research Centre; *Radioisotope Department, Nuclear Research Centre; **Gynecology Department, Faculty of Medicine, Cairo University.*

ABSTRACT

Objective: An elevated total plasma homocysteine level has been suggested as a possible risk factor in women suffering from recurrent miscarriage. The current study was undertaken to assess the association between homocysteine, folate, cobalamin (vitamin B12) and the risk of recurrent miscarriage.

Setting: Recurrent Miscarriage Clinic, National Research Centre, in collaboration with the Radioisotope Department, Nuclear Research Centre and the Obstetrics and Gynecology Department, Kasr Al Aini University Hospital.

Design: Case-control study.

Materials and Methods: The study included 57 non-pregnant Egyptian women. They were classified according to their obstetric history into 2 groups: 32 cases with at least two consecutive miscarriages (Study group), and 25 cases with normal obstetric history (Control group). All cases were tested for plasma total homocysteine, serum folate and cobalamin (vitamin B12).

Results: The fasting total homocysteine was significantly higher in the study group as compared to the control group. While the median concentrations for the vitamins studied were significantly lower in women of the study group as compared to the controls. Elevated homocysteine and reduced vitamin B12 can be considered risk factors for recurrent miscarriage with odds ratio (OR) and 95% confidence intervals (95% CI) of 1.839 (1.286, 2.63) and 1.993 (1.346, 2.951) respectively in the group of recurrent miscarriages. The OR (95% CI) in the study population for low serum folate concentrations was 1.23 (0.776, 2.256).

Conclusion: Elevated homocysteine and reduced serum vitamin B12 are risk factors for recurrent miscarriage. Low serum folate did not seem to be a risk factor for recurrent miscarriage. Testing for homocysteine levels in women suffering from unexplained recurrent miscarriage and pre-conceptional supplementation with vitamin B12 might be beneficial to improve pregnancy outcome.

Key Words: *Homocysteine - Miscarriage.*

INTRODUCTION

Miscarriage is the most common adverse pregnancy outcome, affecting between 10 and 15% of clinically recognized pregnancies **[1,2]**. As many as 5% of all couples attempting to conceive have two successive pregnancy losses, and 1% have three or more consecutive losses **[3]**. Recurrent miscarriage is usually defined as the loss of three or more consecutive pregnancies before viability. In many clinical situations, the definition is altered to two or more consecutive spontaneous miscarriages **[4]**.

An increased miscarriage rate has been observed in pregnancies preceding that of fetuses or newborn infants with neural tube defects (NTDs). Carmi et al. **[5]** found a significantly higher miscarriage rate (48%) in pregnancies preceding those of fetuses with NTDs, compared to those with other birth defects (20%).

It has been hypothesized that both forms of reproductive failure could have one factor in common: Hyperhomocysteinemia (HHcy), which interferes with embryonic development, as well as with vascular function **[6]**. The hypothesis that homocysteine (Hcy) might induce vascular disease was originally advanced by Mc Cully **[7]**, based on the observation that thromboembolism and atherosclerosis were features in children with inherited disorders of Hcy metabolism.

The introduction of the antiphospholipid syndrome (APLS), in the early 1980s, as an etiological cause for recurrent pregnancy loss, has substantiated the "thrombosis theory" of repeated fetal loss **[8]**. Hyperhomocysteinemia

belongs among the familial thrombophilias and is a long known vascular disease risk factor. In vitro, Hcy has been shown to directly damage endothelial cells that predispose thrombogenesis and arteriosclerosis **[9]**. In addition, Hcy induces tissue factor (TF) expression in vitro, which is the initiator of blood coagulation in vivo **[10]**.

Homocysteine is a non-protein forming sulfur amino acid, whose metabolism is at the intersection of two metabolic pathways remethylation and transsulfuration. Remethylation requires the cofactors, folate and cobalamin (vitamin B12) **[11]**.

Hyperhomocysteinemia may be caused by genetic defects of the enzymes involved in its metabolism, nutritional deficiencies or absorption deficiencies of the vitamin cofactors of these enzymes, chronic diseases or administration of some drugs **[12]**.

The current study was undertaken to evaluate the prevalence of hyperhomocysteinemia, folate or cobalamin deficiency in non-pregnant women with history of two or more consecutive miscarriages and no known risk factors for such events, in comparison to non pregnant women with normal obstetric outcome and no history of miscarriage.

MATERIAL AND METHODS

The study was conducted in the Recurrent Miscarriage Clinic of the Prenatal Diagnosis and Fetal Medicine Department, National Research Centre, in collaboration with the Radioisotope Department, Nuclear Research Centre, and the Obstetrics and Gynecology Department, Kasr Al Aini University Hospital.

The study included 57 non-pregnant Egyptian women of the same age range. The cases were classified according to their obstetric history into 2 groups. Control group consisted of 25 currently non-pregnant women, with no history of miscarriage, stillborn or intrauterine growth retardation, and having at least one normal living baby. The study group consisted of 32 non pregnant women with a history of at least 2 consecutive miscarriages before the 20th week of gestation.

The exclusion criteria included: Ectopic pregnancy, elective termination of pregnancy, miscarriage associated with anembryonic pregnancy or fetal malformation detected by ultrasound or pathological examination, immunological disorders confirmed by tests for anticardiolipin antibodies and lupus anticoagulant. Severe uterine (anatomical) abnormalities were ruled out by hysterosalpingography or high resolution ultrasonography. The patients were tested at least 10 weeks post-termination.

The patients and the control subjects did not receive oral contraception, vitamin supplementation, or any medication known to influence homocysteine metabolism within at least a period of 6 months. None of the subjects of both groups had a known endocrine dysfunction, or suffered from gastrointestinal, hepatobiliary, renal or vascular diseases.

Blood samples were collected in the morning after an overnight fast. Blood samples for measurement of total Hcy were collected into tubes containing EDTA. Plasma was then separated immediately by centrifugation at 3000xg for 20 minutes and aliquots were stored at -70ºC until analysis. Plasma was separated immediately to avoid artifactual increase due to the synthesis by blood cells in vitro. Blood samples, for measurement of serum folate and vitamin B12, were collected into empty glass tubes and aliquots were stored at -70ºC until analysis.

Total Hcy (free plus protein bound) levels were measured using a commercially available immunoassay kit Axis (Axis-Shield AS, Norway). It is a solid phase enzyme immunoassay based on competition between S-adenosyl-L-Homocysteine (SAH) in the sample and immobilized SAH bound to the wall of the microtitre plate for binding sites on a monoclonal anti-SAH antibody. Homocysteine is reduced by the use of dithiothreitol (DTT) and then enzymatically converted to SAH by the use of SAH hydrolase in a separate procedure prior to the immunoassay.

Serum folate and vitamin B12 were measured simultaneously by radioimmunoassay technique using commercially available kit produced by DPC (Diagnostic Product Corporation). The test is a dual count solid phase, no boil immunoassay for both folic acid and vitamin B12, based on the principle of: Alkaline denaturation of endogenous proteins, competition for purified binder at pH 9.3, then solid

phase separation. The procedure was done following the instruction manual. Calibration range for vitamin B12: 50-2400pg/ml and 0.5-24ng/ml for folic acid. Analytical sensitivity for vitamin B12: 34pg/ml and for folic acid 0.3ng/ml.

Statistical analysis: Data were analyzed using SPSS statistical package version 10. Numerical data were expressed as mean ± SD, or median and range as appropriate. Comparison between cases and controls were done with Mann-Whitney U tests. Probability *p*-value less than 0.05 was considered significant and less than 0.001 highly significant. Odds ratio (OR) and its 95% confidence interval was the test used to estimate risk of cases in relation to controls.

RESULTS

The study group comprised 32 women with history of recurrent miscarriage (median 5; range 2-9). The median age was 28.0 years (range 20-40 years). The control group, consisting of 25 parous women with no history of miscarriage, had a median parity of 2 (range 1-3 babies), and the median age was 26.0 years (range 20-40 years).

Women with recurrent miscarriage had significantly lower median serum folate and vitamin B12 concentrations, and significantly higher median homocysteine concentrations compared with control. The results are presented in Table (1).

Hyperhomocysteinemia (fasting Hcy greater than 15µmol/L) was detected in 28.1% (9/32) of cases, compared with 4% (1/25) in the control group, giving an OR (95% CI) of 1.839 (1.286, 2.63). Although there appears to be a marked difference in the median folate concentrations regarding the study group (4.4ng/ml) and the control group (8.5ng/ml), a serum folate deficiency (<3.0ng/ml) was found in 15.6% (5/32) of the study group and 8% (2/25) of the control group, giving an OR (95%CI) of 1.23 (0.776, 2.256) (Table 2).

Low serum vitamin B12 (<200pg/ml) was diagnosed in 43.8% (14/32) of the study group compared with 8.0% (2/25) of the controls, giving an OR (95% CI) of 1.993 (1.346, 2.951) (Table 2).

Table (1): Median levels of homocysteine, serum folate and vitamin B12 in the studied groups.

	Study Group (n=32)	Control Group (n=25)	*p* value
Homocysteine µmol/L	10.0 (3.4-40.0)	6.8 (3.4-19.5)	0.011
Serum folate ng/ml	4.4 (1.9-17.0)	8.5 (2.1-16.0)	0.001
Serum vitamin B12 pg/ml	260 (60-800)	350 (100-700)	0.013

The values are expressed as median (range).

Table (2): Estimated risk of recurrent miscarriage for the studied biochemical markers.

	RM (n)	Control (n)	OR (95% CI)
Homocysteine >15µmol/L	9	1	1.839 (1.286,2.63)
Serum folate <3.0ng/ml	5	2	1.23 (0.776,2.256)
Serum vitamin B12 <200pg/ml	14	2	1.993 (1.346,2.951)

OR: Odds Ratio. CI: Confidence Interval.
RM: Recurrent Miscarriage. n : number of cases.

DISCUSSION

The etiology of recurrent miscarriage is considered to be multifactorial **[13]**. Homocysteine has received increasing attention during the past decade and elevated plasma homocysteine levels have been implicated in a variety of clinical conditions **[14]**. Normal levels of fasting plasma homocysteine are considered to be between 5 and15µmol/L **[15]**. Hyperhomocyteinemia can cause obstetrical diseases that are connected with vascular disorders of pregnancy or the uteroplacental unit **[16,17]**. We found that the incidence of hyperhomocystenemia in the study group was about seven times higher (28.1%) than the control (4.0%). The study by Coumans et al. **[18]** also showed this discrepancy (17.1% vs. 4.5%). Gris et al. **[19]** reported an association between increased levels of Hcy and a first early pregnancy loss. Del Bianco et al. **[20]** found 25% of women with recurrent pregnancy loss to have hyperhomocysteinemia or at least a pathological methionine loading test. Kumar et al. **[21]** reported no sig-

nificant difference in the median fasting total plasma Hcy concentrations between women with recurrent miscarriage and the controls. However, elevated Hcy levels >18μmol/L was considered by the authors to be a risk factor for recurrent pregnancy loss **[21]**. Nelen et al. **[22]** studied women with recurrent miscarriage and found a direct relationship between high levels of Hcy and defective chorionic vascularization.

Thrombophilias are suggested to play a role in recurrent miscarriage **[23]**. Krabbendam et al. **[24]** evaluated the literature of the past ten years regarding the association between thrombophilias and recurrent miscarriage. No relation was found between recurrent miscarriage and the methylenetetrahydrofolate reductase C 667T mutation, the levels of antithrombin, protein C and protein S. They concluded that there is only justification for testing for homocysteine levels, antiphospholipid antibodies and factor V Leiden in women with recurrent miscarriage.

Selhub and coworkers **[25]** suggested that most individuals with increased plasma Hcy concentrations have inadequate concentrations of one or more of the vitamins required for Hcy metabolism. In the study we evaluated the folate and cobalamin serum levels in women with recurrent miscarriage. The levels showed significant differences between the cases and the controls. This result is in contrast with the study conducted by Sutterlin et al. **[26]** where no significant differences in folate and cobalamin serum levels were found between the cases and the controls.

Coumans et al. **[18]** observed a weak significant correlation between the number of previous miscarriages and folate values. In this study, the number of previous miscarriages did not have any significant influence on folate values. This study demonstrated that low serum folate did not seem a risk factor for recurrent miscarriage.

Reznikoff-Etievant et al. **[27]** was in agreement with our data that vitamin B12 is significantly low in women with recurrent miscarriage. They recommended vitamin B12 assay in women with recurrent miscarriage whether or not hematological abnormalities are present. Candito et al. **[28]** found that vitamin B12 is one of the causes of recurrent pregnancy loss associated with HHcy and that parental B12 therapy led to normal Hcy level within 2 months and to a successful pregnancy.

Although folate deficiency is one of the factors that may lead to alterations in DNA synthesis and chromosome structure in rapidly dividing cells and the serum concentration is a sensitive indicator of the folate available for replicating cells **[29]**, Abir et al. **[30]** found the mean serum concentration of folic acid to be similar in the so called "high risk sera" from women with at least two abortions and in the control sera.

Ronnenberg et al. **[31]** found that the risk of spontaneous miscarriage was four fold high among women with suboptimal plasma concentrations of folate, while Hcy and vitamin B12 status were not associated with spontaneous miscarriage risk. In the current study, we found that the risk of spontaneous miscarriage was two fold high among women with suboptimal plasma concentration of vitamin B12. The apparent inconsistencies can be explained on the basis of the study population, both with respect to genetic background and dietary habits. It should be remembered that certain population, especially Mediterranean population already have adequate intake of folate **[32]**.

In conclusion, elevated homocysteine and reduced serum vitamin B12 are risk factors for recurrent miscarriage. Low serum folate did not seem to be a risk factor for recurrent miscarriage. Testing for homocysteine levels in women suffering from unexplained recurrent miscarriage and pre-conceptional supplementation with vitamin B12 might be beneficial to improve pregnancy outcome.

REFERENCES

1- Wilcox AJ, Weinberg CR, O'Conner JF, Baird DD, Schlatterer JP, Canfield RE, Armstrong EG, Nisula BC. Incidence of early loss of pregnancy. N Engl J Med. 1988, 319 (4): 189-94.

2- Daya S. Recurrent spontaneous early pregnancy loss and low dose aspirin. Minerva Gynecol. 2003, 55 (5): 441-449.

3- Younis JS, Ohel G, Brenner B, et al. Familial thrombophilia-the scientific rationale for thrombophylaxis in recurrent pregnancy loss? Hum Reprod. 1997, 12: 1389-1390.

4- Regan L. Recurrent miscarriage. BMJ. 1991, 302: 543-544.

5- Carmi R, Gohar J, Meizner I, Katz M. Spontaneous abortion-high risk factor for neural tube defects in subsequent pregnancy. Am J Med Genet. 1994, 51: 93-97.

6- Eskes TKAB, Nelen WLDM, van der Molen EF. Reproductive failure and hyperhomocysteinaemia-the role of folic acid. J Fert Res. 1996, 10: 14-17.

7- Mc Cully KS. Vascular pathology of homocysteinemia: Implication for the pathogenesis of arteriosclerosis. Am J Pathol. 1969, 111-128.

8- Silver RM, Branch DW. Recurrent miscarriage: Autoimmune considerations. Clin Obstet Gynecol. 1994, 37: 745-60.

9- de La Calle M, Usandizaga R, Sancha M, Magdaleno F, Herranz A, Cabrillo E. Homocysteine, folic acid and B-group vitamins in obstetrics and gynaecology. Eur J Obstet Gynecol Reprod Biol. 2003, 107 (2): 125-34.

10- Holmes VA. Changes in haemostasis during normal pregnancy: Does homocysteine play a role in maintaining homeostasis? Proc Nutr Soc. 2003, 62 (2): 479-93.

11- Selhub J, Miller JW. The pathogenesis of homocysteinemia: Interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr. 1991, 55: 131.

12- LIevadot J, Blanco Vaca F, Gonzalez F. Determination and utilisation of the plasmatic concentration of homocysteine in clinical practice. Med Clin (Barc). 2005, 124 (14): 544-53.

13- Stirrat GM. Recurrent miscarriage II: Clinical association, causes, and management. Lancet. 1990, 336: 728-733.

14- Cotter AM, Molloy AM, Scott JM, Daly SF. Elevated plasma homocysteine in early pregnancy: A risk for the development of non severe preeclampsia. Am J Obstet Gynecol. 2003, 189: 391-396.

15- Malinow MR, Bostom AG, Krauss RM. Homocysteine, Diet, and Cardiovascular Diseases. Circulation. 1999, 99: 178-182.

16- Nelen WL, Blom HJ, Steegers EA, den Heijer M, Thomas CM, Eskes TK. Homocysteine and folate levels as risk factors for recurrent early pregnancy loss. Obstet Gynecol. 2000; 95: 519-524.

17- Buemi M, Marino D, Di Pasquale G, Floccari F, Ruello A, Aloisi C, Corica F, Senatore M, Romeo A, Frisina N. Effects of homocysteine on proliferation, necrosis and apoptosis of vascular smooth muscle cells in culture and influence of folic acid. Thromb Res. 2001, 104: 207-213.

18- Coumans ABC, Huijgens PC, Jakobs C, Shats R, de Vries JIP, van Pampus MG, Dekker GA. Haemostatic and metabolic abnormalities in women with unexplained recurrent abortion. Hum Reprod. 1999, 14 (1): 211-214.

19- Gris JC, Perneger TV, Quere I, Mercier E, Fabbro-Peray P, et al. Antiphospholipid / antiprotein antibodies, hemostasis related antibodies, and plasma homocysteine as a risk factor for a first early pregnancy loss: a matched case-control study. Blood. 2003, 102 (10): 3504-3513.

20- Del Bianco A, Maruotti G, Fulgieri AM, Celeste T, Lombardi L, Amato NA, Pietropaolo F. Recurrent spontaneous miscarriages and hyperhomocysteinemia. Minerva Ginecol. 2004, 56 (5): 379-83.

21- Kumar KS, Govindaiah V, Naushad ES, Devi RR, Jyothy A. Plasma homocysteine levels correlated to interactions between folate status and methylene tetrahydrofolate reductase gene mutation in women with unexplained recurrent pregnancy loss. J Obstet Gynaecol. 2003, 23 (1): 55-58.

22- Nelen WL, Bulten J, Steeger EA, Blom HJ, et al. Maternal homocysteine and chorionic vascularization in recurrent early pregnancy loss. Hum Reprod. 2000, 15 (4): 954-960.

23- ACOG Practice Bulletin. Management of recurrent early pregnancy loss. Inter J Obstet Gynecol. 2002, 78: 179-190.

24- Krabbendam I, Franx A, Bots ML, Fijnheer R, Bruinse HW. Thrombophilias and recurrent pregnancy loss: A critical appraisal of the literature. Eur J Obstet Gynecol. Reprod Biol 2005, 118 (2): 143-53.

25- Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. J Am Med Assoc. 1993, 270: 2693-2698.

26- Sütterlin M, Bussen S, Ruppert D, et al. Serum levels of folate and cobalamin in women with recurrent spontaneous abortion. Hum Reprod. 1997, 12: 2292-2296.

27- Reznikoff-Etievant MF, Zittoun J, Vaylet C, Pernet P, Milliez J. Low vitamin B12 level as a risk factor for very early recurrent abortion. Eur J Obstet Gynecol Reprod Biol. 2002, 104 (2): 156-159.

28- Candito M, Magnaldo S, Bayle J, Dor JF, Gillet Y, Bongain A, Van Obberghen E. Clinical B12 deficiency in one case of recurrent spontaneous pregnancy loss. Clin Chem Lab Med. 2003, 41 (8): 1026-1027.

29- Neiger R, Wise C, Contag SA, Tumber MB, Canick JA. First trimester bleeding and pregnancy outcome in gravidas with normal and low folate levels. Am J Perinatol. 2003, 10 (6): 460-462.

30- Abir R, Omoy A, Hur HB, et al. The effect of sera from women with spontaneous abortion on the in vitro development of early somite stage rat embryos. Am J Reprod Immunol. 1994, 32: 73-81.

31- Ronnenberg AG, Goldman MB, Chen D, Aitken IW, Willett WC, Selhub J, Xu X. Preconception folate and vitamin B (6) status and clinical spontaneous abortion in Chinese women. Obstet Gynecol. 2002, 100 (1): 107-113.

32- Caballero B. Fortification, supplementation, and nutrient balance. Eur J Clin Nutr. 2003, 57 (1): 765-785.