The Percentage of Low Serum B12 and Folate Affect Serum Total Homocysteine Level In Egyptian Women With Recurrent Miscarriage

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Abstract

The current study was aimed to find out the percentage of low serum B12 and folate and their interactions with serum total homocysteine level in Egyptian women experiencing unexplained recurrent miscarriage. A total of 50 women were studied for determination of serum B12, folate and total homocysteine, of them 20 women were primary aborters and 15 women were secondary aborters and 15 healthy women who served as controls. Their age ranged from 19-40 years with the mean value 30.5 years . The results of this work showed significant differences between the percentage of those with low serum B12 and folate in primary, secondary and all aborters compared with the control group (p<0.05). Also, the percentage of those with high serum homocysteine showed a significant difference in primary and all aborters but not in secondary aborters when compared with the control group. On the other hand, the mean values of serum B12, folate and homocysteine showed significant differences in all women with recurrent miscarriage compared with the control group (p<0.05). Statistical correlation showed significant negative correlation between serum folate and total homocysteine in primary aborters (r= -0.449 p<0.05).We could conclude that ; there were an interaction between deficiencies of B12 and folate with hyperhomocysteinemia which may contribute to the etiology of unexplained recurrent early pregnancy loss. Identification of those women with hyperhomocysteinemia may be of help in therapeutic normalization and might permit a normal birth.

Introduction

Recurrent early pregnancy loss was defined as two or more spontaneous miscarriages within 16 weeks of menstrual age[1]. Moderate hyperhomocysteinemia , low serum folate and vitamin B12 deficiency are associated with the risk of thrombosis and recurrent early pregnancy loss[2].

Vitamin B12 is involved in the methionine-homocysteine metabolism and its deficiency may interfere in cellular development as shown in megaloblastic anemia or various neurological disorders[3]. One of its biochemical functions in mammals is to maintain normal folate metabolism, which is essential for cell multiplication during pregnancy[4].

The predominating circulating form of folate is 5-methyltetrahydrofolate that participates in the synthesis of S-adenosyl methionine which is used in the folate – dependent remethylation of homocysteine[5].

The interconversion of homocysteine to methionine suggests that, they share common regulatory mechanisms and metabolic functions. Any impairment of the methionine cycle may disrupt this[6].

Aim of the work

The current study was aimed to find out the percentage of those with low serum B12 and folate and their interactions with serum total homocysteine level in Egyptian women experiencing unexplained recurrent miscarriage.

SUBJECTS AND METHODS

Between June 2003 and February 2004, 35 non-pregnant women with a history of recurrent spontaneous abortions defined as three or more consecutive pregnancy losses at less than 22 weeks of gestation were investigated . They were selected from "Benha University Hospitals". Another 15 healthy women participated as controls. The ages of all women ranged from 19-40 years with the mean value 30.5 ±0.8. They were classified into two groups:

Group I (control group) : 15 non- pregnant women with a good obstetric history and had delivered at least two children.

Group II (study group): 35 non- pregnant women . They were subdivided into two groups :

Group II a (primary aborters): 20 non- pregnant women with past history of at least two consecutive early pregnancy losses within 16 week of gestation , following conception from the same partner.

Group II b (secondary aborters) : 15 non- pregnant women with a past history of at least two consecutive early pregnancy losses within 16 weeks of gestation following conception from the same partner and following delivery of at least one live- birth child.

EXCLUSION CRITERIA:

This included patient having general or local genital cause for abortion e.g; trauma, uterine anatomical or pathological abnormalities or incompetent cervix . Also, cases with concurrent infection during pregnancy, women having tonics supplementation within six months before the study, patients with past history of toxaemia , hypertension, ischaemic heart disease, diabetes , oral contraceptives , smoking as well as patients having medications that might interfere with methionine-homocysteine metabolism, renal or liver cell failure.

Methods:

All participants were subjected to the following: Recording obstetric history including last menstrual period, gravidity and parity and previous abortions. Complete general examination including pulse, blood pressure and pallor. Obstetric examination : Bimanual examination for the size of the uterus and vaginal examination for detection of cervical dilatation . Ultrasound examination to exclude uterine anatomical or pathological abnormalities and to exclude cervical incompetence and other local genital causes of abortion . Laboratory investigations including ABO blood grouping , Rh factor, complete blood picture, complete urine analysis, fasting serum glucose[7] , cholesterol[8], creatinine[9], aspartate aminotransferase (AST)[10], alanine aminotransferase (ALT)[10], B12[11] , folate[12] and total homocysteine[13] .

Sampling:

In all women, testing procedures were routinely performed within 6 months from the end of the last pregnancy and about one week before the expected first day of the next menstrual period .

About 7.0 ml venous blood was taken from each woman after overnight fasting. One ml of the blood sample was taken on dipotasium EDTA for determination of ABO blood group , Rh factor, and blood picture . From the other 6.0 ml, sera were separated and used for determination of fasting glucose, cholesterol, creatinine , aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The remaining sera were kept frozen at $-80^{0}C$ for later determination of vitamin B12, folate and total homocysteine .

Determination of serum vitamin B12 by chemiluminescence (Immulite-1instrument, USA)**:**The kit was supplied by Diagnostic Products Corporation , 5700 West 96th street, Los Angeles, CA 90045-5597,USA.

Sample pretreatment:

200 µl of each adjustor, control and serum were pipetted into the tubes. Then, 1000 µl of the working solution which is formed of Borate-KCN Buffer and Dithiothreitol solutions in a ratio of 50:1 was added to all tubes. All tubes were vortexed, capped, and placed in a covered , boiling water bath (100^0C) for 15-20 minutes. The tubes were removed and cooled in an ambient water bath for 5 minutes. From the treated sample, 350 µl was pipetted to an immulite sample cup. The remaining of the procedure was done according to the National committee for clinical laboratory standards[11].

Determination of serum vitamin B12 by chemiluminescence (Immulite-1 instrument, USA)**:** The kit was supplied by Diagnostic Products Corporation , 5700 West 96th street, Los Angeles, CA 90045-5597,USA:

Sample pretreatment:

200 µl of each adjustor, control and serum were pipetted into the tubes. Then, 1000 µl of the working solution which is formed of Borate-KCN Buffer and ligand-labeled folate and Dithiothreitol solutions in a ratio of 50:1:1 was added to all tubes. All tubes were vortexed, capped, and placed in a covered , boiling water bath (100^0C) for 15-20 minutes. The tubes were removed and cooled in an ambient water bath for 5 minutes. From the treated sample, 350 µl was pipetted to an immulite sample cup. The remaining of the procedure was done according to Jacques et al[12].

Determination of serum total homocysteine by ELISA (Dynatech instrument, Germany): The kit was supplied by IBL-Hamburg, Germany. IBL-cat.-No.:AX51301)

Sample pretreatment:

In a glass tubes, 25 µl of the samples, calibrators and controls were diluted in 500 µl of sample pre-treatment solution which is formed of phosphate buffer, 0.09% NaN_2 plus adenosine / dithiothreitol, citric acid

and bovine S-adenosyl-L-homocysteine hydrolase, tris-buffer/glycerol, methylparabene in a ratio of 18:1:1, respectively. All the tubes were capped and incubated for 30 minutes at 37^0C. At the end of this step and before the tubes were cooled, 500 µl of merthiolate (0.15%) /phosphate buffer was added , mixed well and incubated for 15 minutes at 18-25^0C. Then ,500 µl of adenosine deaminase, phosphate buffer, BSA, 0.09%NaN_3 , phenol-red dye was added to all tubes and incubated for 5 minutes at 18-25^0C. In the SAH – coated microtitre strips, 25µl of diluted calibrator, controls and samples was pipetted into their wells. The remaining of the procedure was done according to the method of Frantzen et al[13].

Statistical analysis:

The results of this work were statistically analyzed using cut-off value (mean value ± one SD), percentage, test of proportion (z-test), student . t- test and correlation coefficient (r). p values > 0.05 were considered non significant (N.S), while p values < 0.05 were considered significant[14].

RESULTS

Table (1) showed the mean values and ±SEM of age , gravidity, parity and number of abortions in different studied groups.

In addition, table (2) showed the cut-off values (mean ± 1 SD) of serum B12(≤657.59 pg/ml) , folate (≤5.09 ng/ml) and total homocysteine (≥10.97µmol/l). There were 12,(60%) out of 20 , 10,(66.7%) out of 15 and 22,(62.9%) out of 35 women with recurrent abortion had low serum B12 in primary, secondary and all aborters, respectively. Also, 13,(65%) out of 20 , 10,(66.7%) out of 15 and 23,(65.7%) out of 35 women with recurrent abortion had low serum folate in primary, secondary and all aborters, respectively. On the other hand, 10,(50%) out of 20, 5, (33.3%) out of 15 and 15, (42.9%) out of 35 women with recurrent abortion had high serum total homocysteine in primary, secondary and all aborters, respectively. Comparing the proportions using the z-test of women with recurrent abortion versus the control group, the percentage of those with low serum B12 and folate showed statistical significant differences in primary aborters (z=2.37 p=0.008 ; z=3.08 p=0.001, respectively) , secondary aborters (z=2.55 p=0.005 ; z=2.97 p=0.001, respectively) and all aborters (z=2.79 p=0.003 ; z=3.44 p=0.0002, respectively). On the other hand, the percentage of those with high serum total homocysteine showed statistical significant differences in primary aborters (z= 2.28 p=0.011) and all aborters (z= 2.05 p=0.020) while there was non-statistical significant difference in secondary aborters (z= 1.30 p=0.097) as compared with the control group.

Moreover, table (3) showed that the mean values of serum B12 in primary aborters (340.25 ±55.30 pglml) , secondary aborters (334.53 ±59.62 pglml) and all aborters (366.49 ±29.09 pglml) were statistically significantly less (p<0.05) as compared with the mean value of serum B12 in the control group (490.27 ±35.71 pglml).

Also, the mean values of serum folate in primary aborters (3.06 ± 0.35 nglml), secondary aborters (2.89 ±0.44 nglml) and all aborters (3.34 ± 0.22 nglml) were statistically significantly less (p<0.05) compared with the mean value of serum folate in the control group (4.03 ±0.25 nglml).

However, the mean value of serum total homocysteine in primary aborters (16.97 ±1.55 μmol/l) , secondary aborters (14.65 ±1.61 μmol/l) and all aborters (15.97 ±1.12 μmol/l) were statistically significantly higher (p<0.05) compared with the mean value of serum homocysteine in the control group (10.35 ±0.12 μmol/l).

Moreover, comparing secondary aborters versus the primary aborters , the mean value of serum B12 (334.53±59.62 pglml) in secondary aborter was statistically insignificant lower (p1>0.05) compared with the mean value of serum B12 in primary aborters (340.25 ±55.30 pglml).

Also, the mean value of serum folate (2.89 ±0.44 nglml) in secondary aborters was statistically insignificant lower (p1>0.05) compared with the mean value of serum folate (3.06 ±0.35 nglml) in primary aborters.

Additionally, the mean value of serum total homocysteine in those with secondary aborters (14.65 ± 1.61 μmol/l) was statistically insignificant lower (p1>0.05) compared with the mean value of serum homocysteine in primary aborters (16.97 ±1.55 μmol/l).

Furthermore, table (4) showed the correlation study in the primary and secondary aborters. Serum folate showed significant negative correlation and non–significant negative correlation with serum total homocysteine in primary aborters (r= -0.449 p<0.05) and secondary aborters, respectively. However , age, number of abortions and serum B12 showed non-significant correlation with serum total homocysteine either in the primary or secondary aborters.

Table (1): Mean values and ±SEM of age, gravidity, parity and number of abortion in the controls, primary , secondary and all aborters .

Studied Parameters / Studied groups	Age (Y)	Gravidity	Parity	Number of abortion
Control group (n = 15)	32.33 ±1.59	3.27 ±0.32	3.27 ±0.32	0.00 ±0.00
Primary aborters (n = 20)	28.65 ±1.20	3.20 ±0.21	0.00 ±0.00	3.20 ±0.21
Secondary aborters (n = 15)	31.07 ±1.55	3.60 ±0.19	1.47 ±0.13	2.20 ±0.11
All aborters (n = 35)	29.69 ±0.96	3.37 ±0.15	0.63 ±0.14	2.77 ±0.15

Table (2): percentage, z-test and p values of low serum B12 , folate and high serum homocysteine in women with recurrent abortion compared with the control group.

Biochemical parameters / Studied groups	Low serum B12 Cut-off value ≤ 657.59 (pg/ml)	Low S.folate Cut-off value ≤ 5.09 (ng/ml)	High S.homocysteine Cut-off value ≥ 10.97 (µmol/l)
Control group (n = 15)	3 (20%)	2 (13.3%)	2 (13.3%)
Primary aborters (n = 20)	12 (60%) 2.37 $p<0.05$	13 (65%) 3.08 $p<0.05$	10 (50%) 2.28 $p<0.05$
Secondary aborters (n = 15)	10 (66.7%) 2.55 $p<0.05$	10 (66.7%) 2.97 $p<0.05$	5 (33.3%) 1.30 N.S
All aborters (n = 35)	22 (62.9%) 2.79 $p<0.05$	23 (65.7%) 3.44 $p<0.05$	15 (42.9%) 2.05 $p<0.05$

Table (3): Mean , ±SEM and p values of serum B12, folate and total homocysteine in primary and secondary aborters compared with the control group.

Biochemical parameters / Studied groups	Serum B12 (pg/ml)	Serum folate (ng/ml)	Serum total homocysteine (µmol/l)
Control group (n = 15)	490.27 ±35.71	4.03 ±0.25	10.35 ±0.12
Primary aborters (n = 20)	340.25 ±55.30 p<0.05	3.06 ±0.35 p<0.05	16.97 ±1.55 p<0.05
Secondary aborters (n = 15)	334.53 ±59.62 p<0.05 p1: N.S	2.89 ±0.44 p<0.05 p1: N.S	14.65 ±1.61 p<0.05 p1:N.S
All aborters (n = 35)	366.49 ±29.09 p<0.05	3.34 ±0.22 p<0.05	15.97 ±1.12 p<0.05

p<0.05: significant.
p>0.05: Non-significant (N.S).
p   : probability versus control.
p1   : probability versus primary aborter group.

Table (4): correlation coefficient (r) between age , number of abortions, serum B12, serum folate and serum total homocysteine in primary and secondary aborter groups.

Studied groups / Serum homocysteine	Primary aborter group		secondary aborter group	
	(r)	(p)	(r)	(p)
Age	0.098	N.S	0.282	N.S
Number of abortions	0.145	N.S	0.077	N.S
Serum B12	-0.412	N.S	- 0.290	N.S
Serum folate	-0.449	P<0.05	- 0.134	N.S

p<0.05: significant   p>0.05: Non-significant (N.S)

Discussion

Multiple potential etiologies for recurrent early pregnancy loss have been described[15]. Accumulating evidence suggest that the sulfur-containing amino acid homocysteine plays a role in various developmental disorders[16].

Several studies suggested that hyperhomocysteinemia is an independent risk factor for recurrent early pregnancy loss[17-20].

Two main factors affect homocysteine concentration in human: diet (mainly intake of folate and vitamin B12) and polymorphism in genes that encode enzymes or transport proteins involved in folate and vitamin B12-dependent homocysteine metabolism[21].

The present study, showed statistical significant increase of the percentage of women with low serum vitamin B12 , 22,(62.9%) out of 35 compared to 3, (20.0%) out of 15 in the control group (p< 0.05) . Also , the mean values of serum B 12 was statistically significantly lower in all patients with recurrent miscarriage compared with the control group (p< 0.05) .

Bennett.[22], emphasized the implication of vitamin B12 in recurrent early pregnancy loss and infertility. They proposed a hypothesis in order to explain infertility related to Vitamin B12 deficiency: failure of ovulation, abnormal cell division in the fertilized ovum and/or failure of implantation due to megaloblastoid epithelial changes in the endometrium.

Moreover, Rezinkoff-Etiévant et al[3]., reported that it could be possible that ovum and endometrium abnormalities may have been milder than in sever vitamin B12 deficiency, allowing the fertilized ovum to implant and begins to develop, but the embryo's development may stop early.

Many studies on recurrent early pregnancy loss relating deficiency of serum vitamin B12 to have a role in disturbing early pregnancy have been

reviewed. Some of them had similar results to our study[2,3,22]. Other studies showed results that disagree with our study[23,24]. The differences between our results and the other studies may be due to the different sample size , the different serum B 12 assay kits, the different statistical methods used for calculation of the cut-off value of serum B12 and the low socioeconomic status resulting in un-equilibrated diet.

Our results, revealed that the percentage of those with low serum folate was observed in 23,(65.7%) out of 35 women with recurrent early pregnancy loss compared to 2,(13.3%) out of 15 in the control group ($p<0.05$). Also , the mean values of serum folate in primary , secondary and all aborters were lower as compared to the mean value of the control group ($p<0.05$).

Smithells et al[25]., demonstrated that folate had a role in anemia, placental abruption, spontaneous abortion and embryopathy . The increased number of those with folate deficiency in patients who were aborters indicates the importance of serum folate levels in the etiology of recurrent early pregnancy loss. The deficiency of folate might have added to the elevation of serum total homocysteine in some cases.

These results were compatible with that of Wouters et al.[17], Quêré et al.[18], Nelen et al.[19], and Kumar et al.[20], and disagree with the results of SÜtterline et al.[24]

Furthermore, correlation study in primary and secondary aborters ; the data showed that folate had significant negative correlation with serum total homocysteine ($p<0.05$).

However , several studies on folate metabolism relating to recurrent early pregnancy loss suggested a positive association between number of failed pregnancies and dysfunctional folate metabolism[18,19,24,26]. Folic acid on particular has a strong homocysteine lowering effect which has been achieved in women with recurrent early pregnancy loss. This may be due

to methylenetetrahydrofolate reductase (MTRFR) polymorphism which could affect the change in homocysteine and folate concentration resulting from low dose folic acid supplementation[27].

Moreover, the percentage of those with high serum total homocysteine was identified in 15,(42.9%) out of 35 women with recurrent early pregnancy loss compared with 2,(13.3%) out of 15 of the control group (p<0.05).Also, the mean value of serum homocysteine in primary , secondary and all aborters were higher compared with the mean value of serum homocysteine in the control group (p<0.05)..

These results agreed with several case-control studies, that have shown elevated levels of homocysteine to be present in a high proportion of women experiencing recurrent early pregnancy loss, suggesting it as an independent risk factor for recurrent early pregnancy loss like Wouters et al.[17], Quêré et al.[18], Nelen et al.[19], Rezinkoff-Etiévant et al.,[3] and Kumar et al[20]. Other studies showed results that disagree with our study as, Ronnenberge et al.[28], who suggested that their failure to detect a significant effect of elevated homocysteine might be due to the relative small number of abortion.

Homocysteine is found in the blood in several forms and homocysteine thiolactone is though to be among the more damaging forms of this agent[29]. The plasma total homocysteine that is the oxidized (as homocysteine or homocysteine - cysteine mixed disulfide) and protein-bound homocysteine is a continuous biochemical variable, and normality is difficult to define. However , in most studies hyperhomocysteinemia is usually defined as total homocysteine in the 90th or 95th percentile of a control population and this is approximately 10 – 15 µmol/l [30,31].

There are different mechanisms including uterine effect , vascular effect, implantation failure, and embryotoxic effect which could explain

the occurrence of recurrent early pregnancy loss in women with hyperhomocysteinemia .

Firstly, Ayar et al[29]., injected homocysteine in human myometrium in small dose (0.1 mM) and big doses (1.0 and 2.0 mM) . The results showed that; the later doses causes enhancement of spontaneous contraction of the myometrium.

Secondly, The amino acid homocysteine, is of considerable medical importance because it is involved in the etiopathogeny of vascular damage that predisposes thrombogenesis and arteriosclerosis[6]. Homocysteine promote atherosclerosis and abnormal blood clotting through injuring the lining of the arteries and thickening the walls of these arteries[32].It may infer thrombosis by a number of mechanisms, including inhibition of protein C and also, by increasing plasma tissue factor levels[33,34]. So, maternal thrombotic predisposition could interfere with initial development of adequate uteroplacental circulation or may result in the production of microthrombosis of the placental vessels[40,41]. This would reduce fatal blood supply and alter the normal course of pregnancy[6]. Thus, maternal hypercoagulability is a possible cause of miscarriage during the eighth and ninth weeks of pregnancy, when the placenta replaces the yolk sac[37]. Moderate hyperhomocysteinemia was considered to be a risk factor for thromboembolism, seems to influence the thrombotic tendency in patients who were primary or secondary aborters leading to pregnancy failure. Moreover, Homocysteine has been shown to cause vasoconstriction of the umbilical artery by probable interaction with bioavailable nitric oxide[38,39].

Thirdly, the association of vasoconstriction and hypercoagulability due to raised homocysteine levels may lead to fatal loss when vitamin B12 deficiency first develops . A more prolonged deficiency results in infertility by causing changes in ovulation or development of the ovum changes leading to defective implantation[22].

Also, Jerzak et al[40]., suggested that high homocysteine level may negatively influence pregnancy outcome following natural or in vitro fertilization . This is may be due to defective chorionic villous vascularisation during early stages of gestations.

Finally, Vital cellular process such as proliferation and differentiation are dependent on folate and vitamin B12-mediated one carbon metabolism. These effects may be specially pronounced early in embryogenesis when the cells undergo rapid proliferation and differentiation[41] .

More recently, Zetterberg[42], concluded that vitamin B12, folate, and homocysteine play several fundamental roles in growing cells and thus in developing embryo. It is possible that homocysteine by itself induces some of the developmental disorders previously attributed to folate and/or vitamin B12 deficiency .

There are three studies envisage a direct embryo toxic effect of homocysteine. Rosenquist et al[43]., found that exposure of chick embryos to homocysteine resulted in defects both of the heart and the neural tube while , Vanaerts et al[44]., and Greene et al[45]., found that exposure of mouse and rat embryos to homocysteine resulted in growth retardation and abnormalities of somite development but not in neurulation defect or other teratogenic effects.

The precise mechanism of homocysteine toxicity remains elusive but there are several hypothesis. Some of which have been tested experimentally. The toxic effect of homocysteine in developing rat embryos may result from increased formation of S-adenosyl homocysteine that could inhibit critical methylation reactions[44]. Elevated homocysteine concentration could also inhibit *de novo* synthesis of deoxythymidylate (dTMP). Exposure of proliferating B-lymphoid Raji cells to excess homocysteine or methionine increase the uptake of exogenous thymydine

owing to inhibition of thymydilate synthase-catalyzed reaction in which deoxyurydilate (dUMP) is converted to dTMP[45]. It is though that 5,10-methylenetetrahydrofolate, a cofactor in this reaction is depleted in the presence of excess homocysteine due to increased demand for 5-methyltetrahydrofolate to remove homocysteine by remethylation. This might induce DNA damage through increased misincorporation of dUMP in place of dTMP in DNA followed by excision-repair reactions, DNA strands breaks, cell cycle arrest and ultimately apoptosis[42].

We could conclude that hyperhomocysteinemia and deficiencies of folate and vitamin B12 may contribute for the etiology of unexplained recurrent early pregnancy loss. However, identification of women with hyperhomocysteinemia specially who had vitamin B12 and folate deficiency may be of help in therapeutic normalization and might permit a normal birth.

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النسبة المئوية لنقص فيتامين ب12 وحمض الفوليك بمصل الدم يؤثر على مستوى الهوموسيستين بمصل الدم فى النساء المصريات اللاتى يعانين من الإجهاض المتكرر

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يعرف الإجهاض المتكرر بأنه فقدان تلقائى للجنين لمرتين أو أكثر فى خلال 16 أسبوع من إنتهاء الدورة الشهرية. وقد وجد أن زيادة مستوى الحامض الأمينى" الهوموسيستين" و نقص مستوى فيتامين ب12 وحمض الفوليك يكون مصاحبا لحدوث الجلطة الدموية. فإذا حدثت هـذه الجلطات فى المشيمة فإنه يحدث ضمور بها مما يؤدى إلى قصور فى الدورة الدموية وعمليات التغذية للجنين وهذا بدوره يسبب الإجهاض المتكرر والتلقائى لدى هؤلاء السيدات.

يهدف هذا البحث إلى دراسة النسبة المئوية لنقص فيتامين ب12 وحمض الفوليك بـين المرضى اللواتى تعانين من الإجهاض المتكرر والغير معروف سببه وكذا تركيزهما فى مصل الدم ومدى تأثيرهما على مستوى الحامض الأمينى" الهوموسيستين" بمصل الدم.

تم تصنيف هؤلاء المرضى إلى مجموعتين ، إشتملت المجموعة الأولى على 20 سـيدة تعانين من الإجهاض المتكرر وليس لديهن أطفال أما المجموعة الثانية فاشتملت على 15 سيدة تعانين من الإجهاض المتكرر بعد ولادة سليمة لطفل واحد على الأقل. بالإضافة إلى مجموعـة ضابطة مكونة من 15 سيدة و يتراوح أعمار المرضى والمجموعة الـضابطة بـين 19 و 40 سنة.

تشير نتائج هذا البحث إلى زيادة النسبة المئوية لمن تعانين من نقـص فيتـامين ب12 وحمض الفوليك وكذلك زيادة النسبة المئوية للحامض الأمينى" الهوموسيستين" بين المرضـى اللواتى تعانين من الإجهاض المتكرر وهذه الزيادة ذات دلالة إحصائية معنوية. كذلك وجـد أن نقص تركيز فيتامين ب12 وحمض الفوليك بمصل الدم يكون مصاحبا لزيادة تركيز الحـامض الأمينى" الهوموسيستين" بمصل الدم أيضا وهذه الزيادة ذات قيمة إحصائية معنوية عند مقارنتها بالمجموعة الضابطة.

يخلص هذا البحث إلى أن هناك تداخلا مابين نقص فيتامين ب12 وحمـض الفوليـك بمصل الدم والذى يؤدى إلى زيادة مستوى الحامض الأمينى" الهوموسيستين" بمصل الدم والذى يزيد بدوره من إحتمال حدوث الإجهاض المتكرر.