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Homocysteine Levels in Early Spontaneous Abortus

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Abstract: The discovery that hyperhomocysteinemia may be responsible for several pregnacy complications has only recently been made. With this study, it is aimed to determine levels of homocysteine in pregnant women encountered with abortus imminens, abortus incompletus and abortus. In the study in 35 abortus imminens, 36 abortus incompletus, 30 abortus missed and 33 healthy pregnant women, homocysteine levels are specified by making use of competitive immunoassay technique. Homocysteine levels were determined in the abortus imminens group as 8.78±5.29 μmol L^{-1} in the abortus incompletus group as 10.55±4.49 μmol L^{-1}, in abortus missed group as 9.03±4.29 μmol L^{-1} and as 4.87±1.27 μmol L^{-1} in the control group. Homocysteine levels were found to be excessively high with all abortus groups when compared to the control group ($p<0.0001$). The homocysteine levels of pregnant individuals using folic acid (7.354±3.78 μmol L^{-1}) in comparison to those not using (9.58±5.27 μmol L^{-1}) was determined as significantly low ($p<0.01$). As a result of this study, it cannot be explained that all abortus develop in parallel with hyperhomocysteinemia. However, hyperhomocysteinemia is together with an increased risk of abortus and such a risk might possibly be reduced with use of folic acid, it should definitely be recommended to use folic acid.

Key words: Homocysteine, early spontaneous abortus, abortus imminens, abortus incompletus, abortus missed, Istanbul

INTRODUCTION

Spontaneous abortus is the most common complication of pregnancy and might possibly develop with several different causes such as uterine abnormalities, immunological problems, hormonal imbalances, infections as well as genetic reasons (Oral *et al*., 2006). Early spontaneous abortus is found in 15-20% of couples desiring pregnancy. Despite various efforts to find an etiologic factor for early pregnancy loss, more than half of these cases remain unexplained (Lauzikiene *et al*., 2003).

Homocysteine is an amino acid results from the demethylation of the essential amino acid methionine and involved in several key metabolic processes including the methylation and sulphuration pathways (Hague, 2003; Diallo and Teissier, 2000). Blood concentrarions of homocysteine are determined by various dietary factors including folic acid and vitamin B12 by alteration in physiology such as renal impairment and by variation in the activity of enzymes in the various pathways as a result of genetic polymorphisms (Hague, 2003). In healthy pregnancy, plasma homocysteine concentration exhibits a reduction in course of the first trimester, drops to minimum level in the second trimester and towards the end of the pregnancy increases slightly, reaching the value within the first trimester (Walker *et al*., 1999). The mechanism responsible from such reduction in the level of homocysteine concentration is not yet known (Malinow *et al*., 1998). The discovery that hyperhomocysteinemia may be responsible for several pregnacy complications has only recently been made. It neverthless appears clear that hyperhomocysteinemia is associated with the syndromes of habitual abortus, pre-eclampsia, placenta abruptio, thromboembolic events, neural tube defects and perhaps with fetal death-in-utero and intra-uterine growth retardation (Hague, 2003; Diallo and Teissier, 2000; Aubard *et al*., 2000). In such studies where the relation between hyperhomocy-steinemia and pregnancy complications were inquired

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without making any grouping, it is focused on early pregnancy losses (Lauzikiene *et al*., 2003; Theunissen-steegers *et al*., 2004; Vollset *et al*., 2000; Dodds *et al*., 2008) or habitual abortus (Govindaiah *et al*., 2009). In this study, we ignored recurring pregnancy losses, classified spontaneous early abortus according to the clinical courses and targeted to determine homocysteine levels such in pregnant individuals encountering abortus imminens, abortus incompletus and abortus missed.

MATERIALS AND METHODS

Patients: In the study were included such pregnant women in their pregnancy periods between 5 and 12 months applying to Zeynep Kamil Women and Children Diseases Education and Research Hospital, Department of Obstetrics and Gynecology with complaints of vaginal bleeding and/or pain in the belly. Demographic characteristics of all cases (age, gravida, parity, abortus, curettage) most recent date of commencement of menstrual cycle whether or not was subjected to any uterine operation, type of previous births, use of any medications (folic acid, etc.) in the course of pregnancy were duly recorded. All patients were applied with pelvic examination in dorsolitotomy position.

Such factors like cervical carcinoma, ulcers, polyps, erosion, vaginal laseration, vaginitis that could give rise to such a hemorrhage were eliminated.

By means of transvaginal ultrasonographic examination, the week of pregnancy as well as fetal cardiac activity were assessed. Adnex and pathological findings with respect to the uterus (uterine abnormalities and such similar) were recorded. As a result of such pelvic examination and ultrasonographic inquiry, trophoblastic disease, anembryonic pregnancy, pregnancies diagnosed as ectopic pregnancy as well as multiple pregnancies were deleted from the study. A research group was formed by those diagnosed with abortus imminens and abortus incipiens as well as those patients with abortus missed, coming to pregnancy monitoring policlinic for control and accidentally no fetal cardiac beat was monitored. Whereas a control group was constituted of healthy pregnant individuals in their 1st trimester applying to the pregnant monitoring policlinic for their routine antenatal follow-ups.

Laboratory evaluation: Venous blood was taken from all patients after a fasting period of 8 h. Blood samples were centrifuged for 10 min under 1500 revolutions min and were kept under +2-8°C till the date of working on the same. Homocysteine levels were measured by means of the Immulite device and reagents by using competitive immunoassay technique. Normal homocysteine value was taken as 5-12 μmol L^{-1}.

Statistically analysis: Statistical analyses were carried out by such software package GraphPad Prisma V.3. In evaluation of the data, besides identifying statistical methods (mean, standard deviation) in inter-group comparisons single route variance analysis whereas Tukey multi comparison test was used for sub-group comparisons. Results at the level of $p<0.05$ were assessed as being meaningful.

RESULTS

Research groups as well as demographic characteristics of the same are shown on Table 1. No significant difference has been stipulated between the groups with respect to demographic differences. Inter groups homocysteine PT, APTT and Fibrinogen levels are shown on Table 2. It was determined that in terms of homocysteine and APTT levels, there existed an

Table 1: Demographic features of study groups

Demographic features	Study groups: Controls (n = 33)	Abortus imminens (n = 35)	Abortus incompletus (n = 36)	Abortus missed (n = 30)	p-value
Age (year)	27.03±4.99	28.74±5.65	29.11±6.89	29.20±4.80	>0.05
Gravida	1.85±1.00	2.31±1.51	2.67±2.70	2.93±2.10	>0.05
Parity	0.76±0.87	0.83±1.10	1.22±2.19	1.33±1.52	>0.05
Abortus	0.15±0.44	0.51±0.78	0.42±0.69	0.53±0.63	>0.05

Table 2: Homocysteine, PT, APTT and fibrinogen levels of study groups

Groups	Study group: Controls (n = 33)	Abortus imminens (n = 35)	Abortus incompletus (n = 36)	Abortus missed (n = 30)	p-value
Homocysteine (μmol L^{-1})	4.87±1.270	8.78±5.290	10.55±4.490	9.03±4.290	>0.0001
PT (second)	11.24±0.970	11.03±0.800	11.35±1.180	11.48±1.110	>0.05
APTT (second)	36.13±6.000	31.12±3.120	31.70±4.780	32.47±5.810	>0.0001
Fibrinogen (mg dL^{-1})	288.67±78.52	261.58±56.37	284.00±75.18	272.79±68.13	>0.05

Table 3: Homocysteine and APTT levels between abortus groups

Abortus groups	Homocysteine (μmol L^{-1})	APTT (sec)
Abortus imminens-Abortus incompletus	p>0.05	p>0.05
Abortus imminens-Abortus missed	p>0.05	p>0.05
Abortus incompletus-Abortus missed	p>0.05	p>0.05

Table 4: Using of folic acid and homocysteine levels

Groups	Homocysteine (μmol L^{-1})	p-value
Folic acid (+) (n = 61)	7.354±3.78	
Folic acid (-) (n = 73)	9.58±5.27	p<0.01

Table 5: Homocystein levels in healthly ongoing pregnancies and pregnancies resulted with abortus

Groups	Homocysteine (μmol L^{-1})	APTT (sec)
Normal pregnancy (n = 50)	5.44±2.23	34.35±6.03
Abortus (n = 84)	10.08±4.81	31.77±4.15
p-value	<0.0001	<0.05

advanced level of significantly difference between the control group and respective abortus groups. While in abortus groups homocysteine levels were found to be significantly high (p<0.0001) in comparison to the control groups, APTT levels were in abortus groups were found to be significantly low (p<0.0001) in comparison to the control group. Whenever each of such abortus groups were compared with the other abortus groups with respect to homocysteine levels as well as APTT levels, no difference was determined (Table 3).

Whenever the homocysteine levels compared amongst all groups of such pregnant individuals using folic acid and otherwise, it was determined that the homocysteine levels in groups using folic acid were determined as being significantly low (Table 4). Although, loss of pregnancy in the abortus missed and abortus incompletus groups was for certain in the process of our study there happened to occur abortus in the control group as well as healthly ongoing pregnancies within the abortus imminens group.

Whenever the study groups are grouped as pregnancies concluded with abortus and normally ongoing pregnancies, homocysteine and APTT levels are as shown in Table 5. While in pregnancies terminated by abortus homocysteine levels are excessively high (p<0.0001) when compared to normally processing pregnancies whereas APTT values were found to be significantly low (p<0.05).

DISCUSSION

In this study where we compared the homocysteine levels in the process of the first trimester between normal pregnancies and the abortus in abortus imminens, abortus missed and abortus incompletus groups homocysteine levels were found to be significantly high when compared to those of healthy pregnancies. Lauzikiene *et al.* (2003) found that the homocysteine levels with first early spontaneous pregnancy losses in comparison to normal pregnant individuals, however, they pointed out to no statistically significantly differenc And in the research carried out by Dodds *et al.* (2008) the influence of homocysteine levels at the early stage of pregnancy on development of pregnancy hypertension as well as on preeclampsia were assessed and the effect of hyperhomocysteinemia on such development have been emphasized. Whereas in the study carried out by Govindaiah *et al.* (2009) in habitual abortus, both maternal as well as paternal homocysteine levels were found to be significantly high when compared to the control group. In publications with respect to the relationship between the increase in plasma homocysteine levels and early pregnancy losses including the pathogenetic mechanism of the same, it is being pointed out and emphasized that hyperhomocysteinemia gave rise to damage on desidua and corion arteries as well as disturbed the implantation of pregnancy (Nelen *et al.*, 2000), there occurred a decrease in the intensity of capillary formation with respect to *avascular villus* or vascularized villus (Meegdes *et al.*, 1988) and becoming embryotoxic (Van Aerts *et al.*, 1993). In the study, it was determined solely whether or not there is any relationship between the homocysteine levels and early pregnancy losses but no inquiry either histopathologic or etiologic were made. With respect to such types of abortus classified according to the clinical course the same assume, abortus imminens, abortus missed and abortus incompletus no study where homocysteine levels are investigated has been spotted.

Although, loss of pregnancy in missed abortus and incomplete abortus is for sure as there exists abortus in control pregnancies whereas healthly ongoing pregnancies in the abortus imminens group in the assessments we made the homocysteine levels were found to be meaningfully high in those with pregnancy terminated when compared to those with pregnancies continuing.

According to the study of Walker *et al.* (1999) setting forth the homocysteine levels in normal pregnancy, average homocysteine levels are specified for weeks 8-16 as 5.6 μmol $L.^{-5}$ And we specified in the study group for pregnancies with a normal course between weeks 5 and 12, the average homocysteine levels as 5.44 μmol L^{-1}. Maternal thrombofilies are obstetrically essential pathologies (Gokosmanoglu *et al.*, 2008). In such studies where the relationship between hyperhomocysteinemia and thrombofilia is inquired, it was attempted to be explained in such manner that hyperhomocysteinemia is a risk factor for thrombosis (Den-Heijer *et al.*, 1996;

Boushey *et al*., 1995) and that homocysteine crystals generated a pathological surface on the endothelium cells, thereby activating intrinsic coagulation factors (Dekker *et al*., 1995). We too for this reason planned for the determination of the APTT level amongst those parameters we studied under the working protocol. In healthy pregnant individuals, APPT was found significantly high when compared to abortus groups. Whereas, abortus groups were compared amongst themselves, no difference between APTT values were detected. Also in assessment of pregnancies taking a normal course but ending up with abortus, there likewise were meaningful differences between the same APTT values.

Although, in studies made with respect to folic acid support in hyperhomocysteinemia, it is so opinionated that starting of the use of folic acid in parallel to detection of pregnancy is not sufficient but the common opinion is such that it is necessary for the same is to be commenced to be used prior to pregnancy, there exists no consensus of opinion as to how much in advance to the onset of pregnancy it is to be started and how long it shall be used. Likewise, there is no value as to the recommended doses of folic acid and there is quite a wide range as 0.4-15 mg day^{-1} (Calle *et al*., 2003; Bonette *et al*., 1998). As a result of the recent clinic studies made by Calle *et al*. (2003) it has been reported that administration of a daily dose of 0.4 mg of folic acid starting 4 weeks prior to the onset of pregnancy and used till the 12 week of pregnancy is useful for avoiding early pregnancy losses. We compared our findings only according to the use of folic acid and determined that the homocysteine levels were higher in the group not using folic acid when compared to the group using folic acid. We thereby concluded that folic acid supplements is preventive not only with respect to neural tube defect profilaxy but for early pregnancy losses that might develop secondary to hyperhomocysteinemia as well. Under and by means of this study one might not state that all abortus develop by hyperhomocysteinemia. However, hyperhomocysteinemia is detected in parallel to an increased risk of abortus and as such risk might be reduced by use of folic acid, it should definitely be recommended that pregnant individuals use folic acid.

CONCLUSION

The expectation is as such that in future, pregnancy losses would be reduced to a minimum by several clinic studies to be made to specify the essence of hyperhomocysteinemia and such other factors in gynecology and obstetrics.

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