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# A Study on the Role of Folic Acid and Vitamin D in Pregnant Women with Threatened Abortion

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## ABSTRACT

**Background:** Concentrations of progesterone, folic acid, malondialdehyde (MDA), vitamin D (VD), and ceruloplasmin (Cp) are very important indicators of abortion in pregnant women. Thus, the measurement of correlations between these indicators is very much required for the better understanding of the cause and the pattern of abortion in pregnant women.

**Methods:** Four groups were analyzed for the serum content of folic acid, Vitamin D (VD), progesterone, MDA, and Cp: 25 patients with threatened abortion, 25 patients with inevitable abortion, 25 pregnant women with regular status of pregnancy, and 25 healthy non-pregnant women.

**Result:** In the threatened abortion group, levels of folic acid were considerably lower than in the other groups. Levels of VD also declined in the threatened abortion group, but its level is highest in non-pregnant healthy women. Levels of progesterone were higher in the healthy pregnant women, followed by the threatened abortion group, and lowest in the abortion group. Levels of MDA and Cp were elevated considerably in the threatened abortion group.

**Conclusion:** Deficiencies in folic acid and VD are strongly linked with complications during pregnancy. Adequate monitoring and supplementation are critical in the prevention of threatened abortion and the enhancement of pregnancy outcomes.

## INTRODUCTION

Threatened premature abortion that occurs during the first 12 weeks of pregnancy with a prevalence rate of approximately 15% is a common complication that affects expectant women [1]. A number of factors are the cause of this risk including elevated blood sugar levels, thyroid disorders, harmful lifestyles, chromosome disorders, immunological disorders, and hormonal imbalance. These factors in combination disrupt the tenuous equilibrium that is required for a viable pregnancy.

The vitamins are essential nutrients necessary for the achievement of normal metabolism, physical growth and development, and disease prevention. Observational studies have emphasized the usefulness of vitamin supplementation, particularly folic acid and the B vitamins, to reduce the risk of miscarriage [2]. Moreover, there is evidence that decreased antioxidant defenses and increased susceptibility to infection during pregnancy can lead to complications. Therefore, the supplementation with vitamins can give a protective function against the result of a negative pregnancy and lower the risk of spontaneous miscarriage [3].

The muscle function, bone health, and other vital physiological processes are crucially regulated by VD. It is especially vital during pregnancy, when the physiological changes increase the requirements for such a nutrient within the body. Low status for vitamin D is linked with adverse pregnancy outcomes, including miscarriage. Also, hormones like progesterone and estrogen are vital for the maintenance of pregnancy, and progesterone inadequacy is the predominant cause of early spontaneous pregnancy loss [4-8]. Supplementation with progesterone by the oral or intramuscular routes has been efficaciously utilized for the management of cases of impending miscarriage [9].

The oxidative stress caused by a disproportion between reactive oxygen species (ROS) and antioxidants produces cellular damage and functional impairment. Elevated ROS results in lipid peroxidation and increased content of malondialdehyde (MDA) and is frequently associated with pregnancy disorders such as gestational hypertension, preeclampsia, fetal growth restriction, and recurrent miscarriage [10-14]. Elevated content of MDA in placental tissues and amniotic fluid further highlights the participation of oxidative stress in pregnancy outcome.

Antioxidants also neutralize free radicals to prevent oxidative damage to cells and tissues [15,16]. By supplying electron donation to unstable molecules, antioxidants stop harmful oxidative reactions and rescue cells from damage [17,18].

The levels of folic acid, VD, progesterone, malondialdehyde (MDA), and ceruloplasmin (Cp) in pregnant women are found in the contemporary study, and correlations between these biomarkers are established to find out their role in threatened abortion.

## METHODS

The location for the current study was Al- Shatrah General Hospital, Thi- Qar, and the Biochemistry Laboratory in the College of Education for Girls. The study was conducted from October 15, 2022, to May 25, 2023.

### Participants

The population included 100 women who were divided into four groups:

Threatened abortion group (25 women), Inevitable abortion group (25 women), Healthy pregnant women (25 women), and Healthy non-pregnant control group (25 women).

### Collection and Analysis of Blood Samples

5 mL of venous blood was collected from each person. It was separated after centrifugation at 3000 rpm and assayed either immediately or stored at -20°C.

### Biochemical Analysis

Folic Acid was measured using ELISA kits (Roche, Germany). VD was determined via ELISA kits (Bioassay Technology Laboratory, USA). Progesterone was assessed using ELISA kits (GP, China). Cp was measured using Menden et al. (1979) method [20].

### Statistical Analysis

Data analysis was done with the help of the software Microsoft Excel 2010. Values were provided in the form of mean  $\pm$  standard deviation (mean  $\pm$  SD). A one-way analysis of variance (ANOVA) test was applied for the analysis of the difference between the study groups. Pearson's test of correlation was used for analysis of the association between variables under consideration. A value of less than or equal to 0.05 was considered statistically significant.

## RESULTS

### Serum Folic Acid levels

As indicated in Table 1, the concentration of serum folic acid was significantly different in all groups under investigation ( $p \leq 0.05$ ). The threatened abortion group exhibited a significantly lower concentration of serum folic acid compared to the healthy pregnant and healthy non-pregnant control groups, but higher levels than the abortion group ( $p \leq 0.05$ ). Moreover, the concentration of serum folic acid in the abortion group substantially decreased in comparison with that obtained in the positive and negative control groups ( $p \leq 0.05$ ). The negative control group also exhibited a lower concentration of serum folic acid compared to the positive control group ( $p \leq 0.05$ ).

### Serum VD Levels

The abortion group significantly showed lower concentrations of serum VD in Table 2 compared with the threatened abortion, positive control, and negative control groups ( $p \leq 0.05$ ). A substantial decrement in the concentrations of the serum VD was also observed in the threatened abortion group compared with the positive control group ( $p \leq 0.05$ ). No statistically significant difference in the concentrations of the serum VD between the threatened abortion and the negative control groups existed ( $p > 0.05$ ).

### Serum Progesterone Levels

As is evident from Table 3, the serum progesterone concentration was significantly higher in healthy pregnant women compared with the threatened abortion, abortion, and healthy non-pregnant control groups ( $p \leq 0.05$ ). In addition, a much higher serum progesterone concentration in the threatened abortion group compared to the abortion group was also noticed, having a p value of less than or equal to 0.05. The serum progesterone concentration in the healthy pregnant women group was significantly higher than that in the abortion group ( $p \leq 0.05$ ).

### Serum Malondialdehyde (MDA) Levels

Table 4 reveals a statistically significant distinction within the concentrations of the serum MDA in all groups under investigation ( $p \leq 0.05$ ). The threatened abortion group manifested a remarkable elevation in the concentrations of the serum MDA ( $p \leq 0.05$ ). Accordingly, the abortion group exhibited significantly higher concentrations of the MDA than the positive and negative control groups ( $p \leq 0.05$ ). Furthermore, the negative control group exhibited remarkable elevation in concentrations of the serum MDA than the positive control group ( $p \leq 0.05$ ).

### Serum Ceruloplasmin (Cp) Levels

Table 5 reveals a good elevation in serum ceruloplasmin (Cp) levels in the threatened abortion group than all other groups ( $p \leq 0.05$ ). Also, the abortion group displayed significantly higher amounts of the serum Cp than the positive and negative control groups ( $p \leq 0.05$ ). The positive control group exhibited a slightly higher serum Cp concentration than the negative control group.

## Tables

Groups	No.	Folic Acid (ng/mL) (Mean ± SD)
Threatened abortion	25	2.87 ± 0.53 <sup>c</sup>
Abortion	25	2.36 ± 0.73 <sup>d</sup>
Healthy pregnant women	25	4.02 ± 1.14 <sup>a</sup>
Healthy non-pregnant women	25	3.34 ± 1.02 <sup>b</sup>

Table 1: Mean Serum Folic Acid Levels (ng/mL) in Study Groups. This table presents the mean serum folic acid concentrations with standard deviation (Mean ± SD) across different study groups. The values associated with letters indicate statistical differences between groups (a, b, c, d)

Groups	No.	VD (Mean ± SD)
Threatened abortion	25	27.01 ± 6.82 <sup>c</sup>
Abortion	25	23.99 ± 5.95 <sup>d</sup>
Healthy pregnant women	25	28.36 ± 4.89 <sup>b</sup>
Healthy non-pregnant women	25	37.41 ± 3.78 <sup>a</sup>

Table 2: Mean Serum Vitamin D Levels (ng/mL) in Study Groups. This table compares the vitamin D levels (VD) in the blood among different groups of individuals. The table displays the mean and standard deviation of vitamin D levels for each group. The values associated with letters indicate statistical differences between groups (a, b, c, d)

Groups	No.	Progesterone ng/mL (Mean ± SD)
Threatened abortion	25	23.20 ± 3.08 <sup>b</sup>
Abortion	25	1.63 ± 0.66 <sup>c</sup>
Healthy pregnant women	25	41.98 ± 5.96 <sup>a</sup>
Healthy non-pregnant women	25	0.66 ± 0.17 <sup>d</sup>

Table 3: Mean Serum Progesterone Levels (ng/mL) in Study Groups. This table shows a noteworthy surge in level of progesterone in threatened abortion group as compared to abortion group. The values related to letters specify statistical differences between groups (a, b, c, d)

Groups	No.	MDA (µmol/L) (Mean ± SD)
Threatened abortion	25	4.76 ± 1.37 <sup>a</sup>
Abortion	25	3.28 ± 0.98 <sup>b</sup>
Healthy pregnant women	25	2.31 ± 0.68 <sup>c</sup>
Healthy non-pregnant women	25	1.63 ± 0.41 <sup>d</sup>

Table 4: Mean Serum MDA Levels (µmol/L) in Study Groups. The threatened abortion group has a high value of MDA as compared to all other groups. The values correlated with letters stipulate statistical differences between groups (a, b, c, d)

Groups	No.	Cp (g/L) (Mean ± SD)
Threatened abortion	25	3.68 ± 1.03 <sup>a</sup>
Abortion	25	3.22 ± 0.94 <sup>b</sup>
Healthy pregnant women	25	2.61 ± 0.75 <sup>c</sup>
Healthy non-pregnant women	25	2.58 ± 0.84 <sup>d</sup>

Table 5: Mean Serum Ceruloplasmin Levels (g/L) in Study Groups. The value of Cp is higher in threatened abortion group as compared to all studied groups. The values shown in alphabetic letters show statistical difference among groups (a, b, c, d)

## DISCUSSION

A large percentage of women in the childbearing period do not have sufficient folic acid through a regular diet [21–24]. So folate supplementation during pregnancy is routine in the prevention of fetal congenital abnormalities [25,26]. High doses of folic acid (4– 5 mg/ day) are recommended especially for the women having very high risk factors like infertility, habitual pregnancy loss, or a history of neural tube defect in the previous offspring [26]. A low intake of folate is related to different pregnancy complications such as miscarriage, placental abruption, and growth-restricted fetuses [27– 29]. Reduced folate intake affects the developing fetus through decreased cell division, increased inflammatory cytokine production, disruption of methylation reactions, programmed cell death, and increased levels of oxidative stress. Low levels of folate increase neural tube defects (NTDs) in fetuses, and such fetuses are likely to miscarry. The activity of natural folic acid is reduced due to reduced absorption and availability of less active folates for metabolic processes. The absorption of natural folic acid is disrupted by many factors, so the high consumption of synthetic folic acid is the only option and preferable solution [30]. It has also been observed that the degradation and leaching of folic acid occur in cooking, which leads to the absorption of only 50% of the cooked folate, or folic acid supplementation may not lead to active metabolic transformations and may lead to inactive forms of folic acid, which is called the folate trap. This is more evident when there is deficiency in Vitamin B12. The level of folic acid is greatly reduced in the current study, which may have a direct effect on the incidence of abortion and threatened abortion. A decrease in maternal vitamin D during pregnancy has a direct effect on a higher risk of a variety of disturbing obstetric outcomes, including preeclampsia, a higher risk of primary cesarean delivery, and gestational diabetes mellitus (GDM) [31-33]. Furthermore, vitamin D deficiency during pregnancy is associated with intrauterine growth retardation and a range of adverse fetal and neonatal outcomes such as increased preterm delivery [34], miscarriage [35], decreased birth weight [36], and neonatal hypocalcemia [37]. Also noted in the scientific literature is that inadequate vitamin D status is related to impaired placental function, augmented placental inflammation, and other dangerous conditions associated with pregnancy. Vitamin D also plays a role in the regulation of immune and inflammatory responses during pregnancy [38].

The progesterone variations are often utilized as indicators during the estimation of the course and stability of pregnancy. A critical decline in the concentrations of progesterone is also known to adversely influence the outcome during pregnancy. Scientific research has been able to uncover an extraordinary association between immunological activity at the reproductive tract level and maternal progesterone, particularly after fetal antigens. These antigenic responses are governed by the interaction at the receptor level at the progesterone-endometrial site. There is also clinical experience that shows the appropriate supplementation with progesterone during early gestation can significantly lower the risk of threatened miscarriage [42]. A decline in progesterone level was observed in the abortion and threatened abortion groups compared with healthy pregnant women. Several studies have proven a relationship between oxidative stress and a wide range of pregnancy complications that can negatively impact fetal growth. These complications are usually caused by a shortage of nutrients and oxygen supply to the fetus and the resultant hypoplasia and placental dysfunction [43]. The placenta plays a vital role in the transfer of nutrients and oxygen from the mother to the embryonic growth. Interference with such transfer can result in hypoxia that is predominantly caused by oxidative stress. Impaired functional performance of the placenta can be caused by various contributing factors that ultimately result in pregnancy complications [44]. Numerous studies have linked such complications with increased oxidative stress that is sourced from placental or maternal tissues [45]. Oxidative stress is also believed to affect major cellular and molecular mechanisms that are vital in the course of disease progression, including abnormal angiogenesis and inflammation responses [46]. Oxidative imbalance results from an overproduction of ROS with an impaired antioxidant protective machinery. Such dysregulation is linked with embryonic developmental defects as well as with unexplained recurrent pregnancy loss and spontaneous abortion [43,47].

Spontaneous abortion is when a mother loses her pregnancy before 20 weeks of gestation and is the cause of an estimated half of miscarriage cases. Others are typically linked with structural abnormalities at birth, uterine abnormalities, infection, maternal disorder, or remain unexplained [45]. Placental tissues from early pregnancy loss have increased MDA and lipid peroxide levels in comparison with normal pregnancy tissues. Earlier research postulated that an overaccumulation of reactive oxygen species (ROS) might catalyze the abnormal and early advancement of the maternal placental blood flow. Additional findings suggest that oxidative pressure generates damaging effects on trophoblastic cells that might cause early pregnancy

loss. The subsequent oxidative imbalance is typically attributed to an insufficient antioxidant defense mechanism that fails to salvage free radicals. Irrespective of inconsistencies within existing literature bases, a potential relationship between elevated ROS concentrations and antioxidant activity loss might form the basis for the miscarriage pathophysiology. Defective placental attachment, which may have its genesis in syncytiotrophoblast dysfunction, is blamed in the case of unexplained recurrent pregnancy loss [45]. Oxidative stress can negatively influence pregnancy by reducing antioxidant defense mechanisms within the body [43,49]. According to research findings, higher antioxidant concentrations can counterbalance excess reactive oxygen species (ROS) in women with recurrent miscarriages. Previous research findings have cited higher concentrations of lipoperoxide and lower values of beta-carotene and vitamin E and A in such women and have talked about the role of oxidative stress in the pathophysiology of recurrent pregnancy loss. Cp is an acute-phase reactant, and its expression has been shown to increase under hypoxic conditions, when compared to healthy subjects [50-52]. There is an increase in placental ceruloplasmin following hypoxia, indicating that placental ceruloplasmin, a protein with antioxidant properties, expression is clearly upregulated in threatened abortion [53].

A limitation of the current study is that the detailed assay description for MDA measurement is not available in the present manuscript version.

This study showed that low levels of folic acid, vitamin D, and progesterone, combined with high oxidative stress and ceruloplasmin levels, were associated with threatened abortion. Therefore, regular monitoring and proper supplementation of folic acid and VD levels are essential for reducing pregnancy complications, and antioxidant therapy will help mitigate oxidative stress and improve pregnancy outcomes.

## CONFLICT OF INTEREST

The authors express no conflict of interest in the publication of this manuscript.

## AUTHOR CONTRIBUTIONS

Shaimaa J. Dohie organized the study, conducted experimentation, collected the data, and wrote the manuscript. Raid M. H. Al-Salih directed the study and approved the final version of the manuscript.

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