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The role of maternal stress, cortisol and IL-12 in cases of missed abortion in the first trimester of pregnancy in Saudi women

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ABSTRACT

Background: Missed abortion in early pregnancy is a common gynecological condition and the incidence has been increasing presenting itself as public health problem. This study aimed to examine stress, cortisol concentration, and altered IL-12 levels in women who experienced missed abortions. *Methods:* Women with missed abortions (n = 40) were matched with control women of the same age in the same stage of pregnancy (n = 30). Participants filled out a survey about common stress factors. Venous blood samples were taken from the patients at 7:00 a.m. IL-12 concentrations were detected by ELISA, and serum cortisol was measured by electrochemiluminescence immunoassay. *Results:* Women with missed abortions reported significantly more common stressors, and their serum cortisol and IL-12 concentrations were significantly higher than those of the women with normal pregnancies (all $P < 0.05$). Receiver operating characteristic (ROC) analysis was used to assess the power of the studied parameters to predict women at risk for missed abortion. IL-12 performed the best in predicting women with missed abortions as it had the highest area under the

curve (AUC), followed by cortisol. *Conclusion:* Stress and changes of the immune system may play a role in the etiology of missed abortion in Saudi women in early pregnancy.

Keywords: Stress marker, Cortisol, IL-12, missed abortion, Saudi women, early pregnancy

1. INTRODUCTION

The term "missed abortion" is used when the fetus dies without outside intervention but is not expelled from the uterus (Chen et al., 2015). Missed abortion in early pregnancy is a common gynecological condition and the incidence has been increasing presenting itself as public health problem. Some of the risk factors for missed abortion are infections of the reproductive system, endocrine disease, immune system changes, chromosome fluctuations, oxidative stress, and apoptosis (Bai 2008).

The precise etiology and development of missed abortions remain uncertain despite extensive research in the field. Considering the fast pace of modern life, more researchers have begun to investigate associations between mental and/or physical stress and missed abortion. The existence of such a connection is not universally accepted, however. Some studies, for instance, suggest that as many as 66% of all missed abortions may be attributed to emotional disturbances, particularly severe depression and anxiety. On the other hand, other research has found no adverse effect of physiological stress on obstetric outcomes (Cheng et al., 2009, Xiao and Tao 2008).

As defined by Cohen et al. (Cohen et al., 1995), stress occurs when the demands placed on an individual surpass their ability to adapt, causing biological and psychological changes that may raise the likelihood of disease. Pregnant women commonly experience stress (Woods 2010), which can have long-term effects on their health and the development of their children (Huizink et al., 2003). Several studies have found a correlation between psychosocial stresses - death, divorce, abuse, financial or marital problems, and loss of social support—and the risk of missed abortion among women admitted to hospital or presenting at the emergency room (O'Hare and Creed 1995, Boyles et al., 2000). Other psychological factors affecting pregnancy outcomes can include pressure at work, major change in personal circumstances as well as previous pregnancy loss (Kicia et al., 2015). Retrospective studies have also found an association between higher workplace demands and negative pregnancy outcomes including fetal loss in early pregnancy (Maconochie et al., 2007).

Two neuroendocrine axes are implicated in stress: the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system-adrenal-medullary axis. The HPA axis is considered to have the most direct link to the association between prenatal maternal stress and growth of the fetus (Gao and Yang 2005). Therefore, stress levels are frequently gauged by measuring cortisol concentration.

Prior research has found that T helper 1 (Th1) type cytokines can cause placental damage, disrupt early growth of the embryo, and result in adverse gestational outcomes. They activate natural killer cells, either indirectly or directly, triggering lymphokine-activated killer cells and causing a delayed hypersensitivity response (Han and Yang 2009). The mother's genetics might alter the balance in cytokine profile and thereby affect the predisposition to abortion. Interleukin 12 (IL-12) is the principal cytokine causing T cells to differentiate into Th1 cells and is involved in immuno-inflammatory reactions produced by Th1 type cytokines (Hamza et al., 2010).

The imbalance between Th1 and Th2 was found to be associated with a higher risk of missed abortion. Similarly, higher IL-12 levels were seen in women with missed abortions than in women with normal pregnancies at the same gestational age (Paradisi et al., 2003, Cao et al., 2008). Zhang et al. (Zhang et al., 2009) reported that glucocorticoids effect the Th1/Th2 balance through the mechanism underlying the inhibition of IL-2 production.

Changes in cellular immunity and stress are both linked to missed abortion, but IL-12 levels are not always known. The higher percentage of Th1 cells correlates with raised IL-12 concentrations in women with missed abortion, as mentioned in the investigation carried out by Paradisi et al. (2003). There is a lack of data on serum cortisol and IL-12 concentration in women with missed abortion. Therefore, this study aimed to explore the role of maternal stress factors, cortisol concentration, and altered IL-12 levels in this condition commonly seen in early pregnancy.

2. SUBJECTS AND METHODS

This case-control study involved a sample of pregnant women who visited the obstetric/gynecology department for a routine prenatal checkup at King Abdulaziz University Hospital (KAUH), in the Western region of Saudi Arabia from September 2017 to April 2018. Cases were defined as women with missed abortion, whose pregnancy was terminated between 10–11 weeks of gestation

upon the date of the first day of the last normal menses. A total of 40 women ranging in age from 18-40 (average age 29.8) with documented missed abortions who were admitted to KAUH were included. Among the inclusion criteria were gestational age and a positive urine pregnancy test. Ultrasound and pathology were carried out to confirm cases of missed abortion. Sonographic criteria were either a lack of cardiac activity and a crown-rump length (CRL) of ≥6 mm or a lack of embryonic growth and a CRL of ≤6 mm, as determined by a specialist gynecologist. During the study period, normal pregnant women who attended the antenatal outpatient Obstetrics and Gynecology Department at KAUH, matched for maternal and gestational age, were used as controls. The 30 women with normal pregnancies ranging in age from 19-41 (average age 28.1) were placed in the control group based on these criteria: positive urine pregnancy test, 10–11 week gestational age, and sonographic confirmation of normal early pregnancy. Women in both groups had no uterine abnormalities, history of thrombosis, hyperthyroidism, diabetes mellitus, or any conditions that could result in high glucocorticoid concentration.

All the women were informed about the nature of the study and anyone who did not agree to participate was excluded. A full history including personal information, obstetric history, and medical diseases was obtained. Additionally, each subject underwent a complete physical examination including abdominal ultrasound to confirm the normality of early pregnancy. Obstetric vaginal U/S for gestational sac and age as well as calculation of CRL and lack of cardiac activity was obtained to confirm the missed abortion. When missed abortion was diagnosed, the following investigations were also carried out on each participant: complete blood count (CBC) by Sysmex KX-21N (Sysmex, Japan); coagulation profile including prothrombin time (PT), activated partial thromboplastin time (APPT), and fibrinogen, measured by (Dade Behring Kits, Marburg, Germany).

Serum cortisol and IL-12 measurements

The morning after hospital admission, blood samples were taken from women confirmed to have missed abortions. Blood was also drawn in the morning from women in the control group. The blood was allowed to fully clot prior to centrifugation, after which the serum was separated and set aside for testing. Serum cortisol levels were determined by an electrochemiluminescence immunoassay using the COBAS 6000 automated analyzer (Roche, Germany). An enzyme-linked immunosorbent assay (ELISA) kit (Glory Science Co., TX, USA) was used to evaluate IL-12 levels.

Common stress level survey

We evaluated symptoms of stress using a version of the Perceived Stress Scale translated into Arabic (Chaaya et al., 2010). The Arabic version of the stress scale has been shown to be highly valid and reliable, making it useful to measure stress in Arabs (Chaaya et al., 2010). Participants were asked to rate how often they had experienced 15 different stress factors in the previous month. Statements ranged from feelings of not being in control of events to marital issues to specific concerns about the pregnancy and welfare of the unborn baby (the questionnaire is available upon request). Special circumstances that subjects needed to specifically explain like loss of a loved one or divorce were also recorded. The statements were presented on a 5-point Likert scale with descriptors ranging from 0–never to 4–almost always. The responses from the participants ranged between 0 (low stress) and 30 (high stress). A total score of 20 or more was defined as reflecting high stress levels during pregnancy.

Statistical analysis

The quantitative data were expressed as mean ± standard deviation (SD) when their distributions were found as parametric and the qualitative data were presented as numbers and percentages. Statistical analyses were carried out using SPSS software, version 17.0. The studied parameters between the two groups of patients were compared using a *t*-test. Non-parametric comparisons were carried out as well for the qualitative data. Receiver operating characteristic (ROC) analysis was used to evaluate the discriminatory power of the studied parameters. P-values of <0.05 were considered statistically significant.

3. RESULTS

Seventy subjects who presented to KAUH at gestational age of 10–11 weeks were recruited. 40 subjects had missed abortions (cases) and 30 subjects had ongoing normal pregnancies (controls). The mean maternal age in the control and case groups was 28.10 ± 6.87 and 29.80 ± 6.77, respectively. No significant difference between maternal ages was found between the two groups. Women with missed abortions had significantly higher cortisol concentrations than women with normal pregnancies (19.31 ± 8.13 vs. 15.7 ± 4.39, P=0.031) (Table 1). Notably, a highly significant difference in IL-12 concentrations between the two groups was observed, with much higher levels seen in the women with missed abortions (30.61 ± 16.19 in case vs. 4.85 ± 1.97 in control, P=0.000) (Table 1). Fibrinogen levels were significantly lower in women with missed abortions than in those with normal pregnancies

(297.95 ± 63.68 vs. 456.20 ± 135.17, P=0.000) (Table 1). Other than cortisol, IL-12, and fibrinogen, the laboratory characteristics of the two groups of women did not differ significantly (Table 1).

Table 1 Subject age and laboratory characteristics of the two groups

	Control (n=30) Mean ±SD	Case (n=40) Mean ±SD	Independent t-test	
			t	*P-value*
Age (years)	28.10 ± 6.87	29.80 ± 6.77	-1.021	0.311
PT (sec)	12.19 ± 0.59	12.49 ± 0.91	-1.566	0.122
APTT (sec)	30.86 ± 4.59	32.56 ± 3.48	-1.770	0.081
Fibrinogen (mg/dL)	456.20 ± 135.17	297.95 ± 63.68	6.514	0.000*
Cortisol (nmol/L)	15.7 ± 4.39	19.31 ± 8.13	2.197	0.031*
IL-12 (pg/mL)	4.85 ± 1.97	30.61 ± 16.19	8.653	0.000*
Fasting sugar (mg/dL)	75.77 ± 11.10	79.23 ± 9.59	-1.395	0.167
Postprandial (mg/dL)	147.30 ± 34.34	151.13 ± 20.73	-0.881	0.382

** Significant difference at P<0.05*

High levels of stress were reported by significantly more women with missed abortions (77.5%) compared with women with normal pregnancies (50%) (P=0.016) (Table 2). More than half (74.1%) of the studied sample with stressors exhibited high stress levels, while 25.9% expressed low stress levels, according to the cutoff recommended by Cohen et al. (1995). Of those reporting stress, 7.5% reported being distressed because of an unexpected event, 5.0% felt they could not control important matters in their lives, 18.6% reported feeling nervous and "stressed," 2.5% had confidence in their ability to deal with personal problems, 2.4% stated that things were going their way, 10.5% reported being unable to cope with everything they had to do, 5.5% reported being able to control frustrations they experienced, 2.5% reported feeling on top of things, 18.9% reported that things outside their control made them angry, 10% felt their babies were not normal, and 19.1% were concerned about having problems with delivery. A non-significant negative correlation was observed between age and stress in pregnant women (r=−0.181, *P*=0.264).

Table 2 Stress distribution of control and case groups

Stress	Control (n=30) N (%)	Case (n=40) N (%)	Chi-square test	
			X^2	P-value
No	15 (50)	9 (22.5)	5.754	0.016*
Yes	15 (50)	31 (77.5)		

** Significant difference at P<0.05*

Subjects with stress in the missed abortion group demonstrated significantly higher cortisol levels than subjects without stress (20.81 ± 8.49 vs. 14.11 ± 3.52, P=0.027) (Table 3). Notably, a highly significant increase in serum IL-12 concentrations between the two groups was also found (34.18 ± 14.57 in subjects with stress vs. 18.32 ± 16.20 in subjects without stress, P=0.008) (Table 3). Subjects with stress demonstrated significantly lower fibrinogen levels than women without stress (285.06 ± 55.44 vs. 339.33 ± 72.45, P=0.021) (Table 3). Other than cortisol, IL-12, and fibrinogen, there were no significant differences in age or other laboratory characteristics between the two groups (Table 3).

Table 3 Comparison between women with stress and those without stress in the case group regarding age and laboratory findings

	No stress (n=9) Mean ±SD	Stress (n=31) Mean ±SD	Independent t-test	
			t	*P-value*
Age (years)	29.11 ± 5.25	30.0 ± 7.21	-0.343	0.734
PT (sec)	12.27 ± 1.03	12.55 ± 0.88	-0.798	0.430
APTT (sec)	31.86 ± 4.07	32.77 ± 3.34	-0.687	0.496
Fibrinogen (mg/dL)	339.33 ± 72.45	285.06 ± 55.44	2.412	0.021*
Cortisol (nmol/L)	14.11 ± 3.52	20.81 ± 8.49	2.295	0.027*
IL-12 (pg/mL)	18.32 ± 16.20	34.18 ± 14.57	2.805	0.008*

Fasting sugar (mg/dL)	81.56 ± 8.37	78.55 ± 9.94	0.825	0.415
Postprandial (mg/dL)	155.22 ± 19.12	152.52 ± 21.43	0.341	0.735

** Significant difference at P<0.05*

IL-12 was found to be the best predictor for differentiation between subjects with normal pregnancy and women with missed abortions with area under the curve (AUC) of 95.4%, followed by fibrinogen level with AUC of 89.6%, and finally cortisol level with AUC of 61.8% (Table 4).

Table 4 Receiver operating characteristic curve (ROC) for the differentiation between control and case group regarding fibrinogen, cortisol, and IL-12

	Cutoff point	AUC	Sensitivity	Specificity	+ PV	–PV
Fibrinogen (mg/dL)	≤ 350	0.896	80.0	86.67	88.9	76.5
Cortisol (nmol/L)	> 19.1	0.618	37.5	86.7	78.9	51.0
IL-12 (pg/mL)	> 8.78	0.954	92.5	100.0	100.0	90.9

Table 5 shows that IL-12 has the highest AUC of 79.2% with a sensitivity of 83.87% and specificity of 77.78%, while cortisol showed AUC of 78.1% with a sensitivity of 80.6% and specificity of 77.8%. Fibrinogen showed AUC of 71.7% with a sensitivity of 74.2% and specificity of 66.7%. IL-12 was found to be the best predictor for stress in the case group, followed by cortisol and finally fibrinogen level (Table 5).

Table 5 Receiver operating characteristic curve (ROC) for the differentiation between subjects with and without stress among case subjects

	Cutoff point	AUC	Sensitivity	Specificity	+ PV	–PV
Fibrinogen (mg/dL)	≤ 315	0.717	74.2	66.7	88.5	42.9
Cortisol (nmol/L)	≥ 14.89	0.781	80.6	77.8	92.6	53.8
IL-12 (pg/mL)	> 20.48	0.792	83.87	77.78	92.9	58.3

4. DISCUSSION

This study is the first investigation of the link between stress and missed abortion in pregnant Saudi women. Stress is linked to 75–90% of human diseases (Zhai and Li 2005). Stress can arise from any sort of physical or psychological stimulus that exceeds a particular point. Not only are the reproductive and endocrine systems critical in a woman's response to stress, but they are also susceptible to stress. In fact, studies have identified stress as an underlying factor in many reproductive and endocrine diseases. Research on animals has demonstrated that gestational maternal stress is associated with adverse conditions like embryonic absorption, malformation, and fetal growth retardation (Xiao and Tao 2008). Research on pregnant women has shown that stress of the mother can affect reproduction, gestation, and growth of the fetus (Tian and Kang 2013).

The incidence of missed abortion is high. Its many risk factors include chromosomal alterations, endocrine disease, infections of the reproductive tract, immune system changes, oxidative stress, and apoptosis (Bai 2008). Recent research has focused on understanding how health outcomes may be impacted by the psychobiological process of emotional (de)regulation. The body's acute and chronic responses to stress have been linked to health outcomes in several conditions. This occurs as the hypothalamic-pituitary adrenal (HPA) axis is activated as part of the stress-response mechanisms (O'Connor et al., 2000).

This association can be explained by the attachment theory (Robles and Kane 2014), which suggests that the psychobiological attachment system is triggered by stress factors and regulates the physiological and emotional responses to stress. Pregnancy is a stressful condition that can affect a woman's wellbeing and trigger the attachment system (Costa-Martins et al., 2016).

Elevated concentrations of cortisol brought about by stress affect the maternal HPA axis by triggering placental production and excretion of placental-corticotrophin-releasing hormone (Gao and Yang 2005), resulting in an additional rise in the concentration of corticotrophin-releasing hormone (CRH). Elevated CRH concentration plays a major role in lowering the secretion of gonadotrophin-releasing hormone (GnRH) and preventing the synthesis of ovarian estrogen (Li and Guo 2006). Over time, there is a reduction in the level of hormones closely linked to pregnancy such as estrogen, progesterone, follicle-stimulating hormone, and luteinizing hormone (Tian and Kang 2013). Studies indicate that endocrine irregularity is the primary risk factor for missed abortions (Wu 2006).

Reduced progesterone levels, for instance, can lead to decidual dysplasia and affect the implantation and growth of the zygote. In general, normal pregnancy may be at risk if progesterone levels are too low (Wu 2006).

High exposure to stress during pregnancy is well documented. Research on a diverse sample of pregnant women in urban areas found that 78% reported low-to-moderate antenatal psychosocial stress, with 6% reporting high levels (Woods et al., 2010). Worldwide, some of the stress factors affecting pregnant women are burdensome responsibility for family and household, poor working conditions, inadequate material resources, tension in intimate relationships, and complications in pregnancy. As a public health issue, identifying women who experience psychological stress during pregnancy is important because these factors (in addition to biomedical risk factors) may be implicated in complications and adverse outcomes in pregnancy (Littleton 2007).

Stress can lead to inadequate placental blood supply by increasing catecholamine synthesis and secretion and stimulating the sympathetic nerve system. Trophoblasts can then be damaged by insufficient oxygen supply to the placenta, increased reactive oxygen species or reduced antioxidant concentrations or activity, and imbalance of pro-oxidants and antioxidants (Tian and Kang 2013). Our study found that women who had missed abortions had significantly higher serum cortisol concentrations than gestational age-matched women in normal early pregnancy, which is in line with earlier studies. Tian and Kang (2013) reported significantly elevated average serum cortisol concentrations in women with missed abortion compared to those whose pregnancies were normal. Maconochie et al. (2007) found that the likelihood of missed abortion was higher in women who experienced life-changing events or work-related stress during pregnancy. Another investigation found an incidence rate up to 66% of missed abortion combined severe depression and anxiety, among other emotional disorders (Cheng et al., 2009).

In normal pregnancy, Th2-driven humoral immunity is seen, with the Th1/Th2 cell ratio changing to Th2 cells; however, elevated Th1/Th2 ratios are observed in missed abortion (Cao et al., 2008). Other studies have found that T helper 1 (Th1) type cytokines can result in placental and fetal damage, disrupt early embryonic growth, and lead to adverse outcomes in pregnancy. They activate natural killer cells, either indirectly or directly, and triggering lymphokine-activated killer cells and causing a delayed hypersensitivity response (Han and Yang 2009). IL-12 is the foremost cytokine involved in Th1 cell differentiation and Th1-mediated immuno-inflammatory reaction (Hamza et al., 2010). The number of Th1 cells is proportional to IL-12 levels (Liu and Xia 2009) due to an elevated Th1/Th2 ratio, so we posit that women with missed abortion will have higher IL-12 levels than women in normal early pregnancy at the same gestational age, as reported by Paradisi et al. (2003). Our data backs up this hypothesis, with significantly elevated IL-12 concentrations observed in the case women than in the gestational age-matched controls. However, some previous studies reported findings in contrast to ours, with no significant increase in IL-12 levels seen in women with missed abortion or normal pregnancies (Tian and Kang 2013, Ostojic et al., 2007). Recent studies suggest that glucocorticoids play a role in limiting Th1-type cytokine production and stimulating Th2-type cytokine production, indicating that glucocorticoids impact the Th1/Th2 balance primarily by inhibiting IL-12 production (Zhang et al., 2009). Therefore, when the dual risk factors for missed abortion—stress and cellular immunity—are present, and the dampening effect of stress-related high glucocorticoid concentrations on IL-12 result of the Th1/Th2 imbalance, noticeably elevated cortisol and IL-12 concentrations are expected in women with missed abortion in comparison to those in normal early pregnancy, as demonstrated in this study.

The effect of pregnancy on clotting factors can be detected from as early as the first trimester. Many gynecological units have a policy of performing routine coagulation tests in cases of missed abortion. There is clearly a link between abnormal coagulation or fibrinolysis and recurrent abortions. Screening for such abnormalities is feasible in any laboratory, so any predictive role would have an important impact. Plasma fibrinogen concentrations gradually rise during pregnancy, reaching between 400-600 mg in late pregnancy and delivery. Fibrinogen is important in coagulation and pregnancy maintenance. It also helps in placental adhesion to the uterus, so it is critical in the maintenance of pregnancy. In our study, a highly significant decrease in fibrinogen was found in the case group (297.95 ± 63.68) compared to the controls (456.20 ± 135.17) (P=0.000). Further comparison between subjects with stress and those without stress in the case group revealed the same results (P=0.000). Our results are consistent with previous studies (Kanchana and Girijavani 2017, Mollamahmutoğlu et al., 2011). Kanchana and Girijavani (2017) found decreased fibrinogen plasma levels in 25 cases of missed abortion compared to women with normal pregnancy. Mollamahmutoğlu et al. (2011) evaluated serum concentrations of fibrinogen in women with missed abortion and normal pregnancy and found that fibrinogen was decreased in the missed abortion group.

There are several limitations to this study. We assessed stress using the self-reporting PSS scale rather than evaluation by someone specialized in mental health care. The cutoff point we used to categorize women as having high stress levels (20 or above) was one recommended by Cohen et al. (1995). Our findings show associations, but no causal conclusions can be drawn and so must be interpreted with caution. The findings of this study may only be representative of pregnant Saudi women visiting the KAUH obstetrics/gynecology department for a routine antenatal checkup. Further investigations are needed to measure stress levels in

Saudi women with missed abortion both during and after pregnancy. In addition, previous studies have suggested that abortion can be induced by psychological stress factors, so an investigation of serum cortisol and IL-12 levels women with threatened fetal loss in early pregnancy would be valuable.

5. CONCLUSION

This study indicates that, along with the reported Th1/Th2 imbalance, stress levels are also implicated in the occurrence of missed abortion. While further research is needed to determine the underlying mechanism, stress management may offer a new approach to the prevention of missed abortion. This study highlights the need for increased support from families and the community for women of reproductive age to lower their stress levels by limiting or avoiding exposure to stress factors, thus resulting in fewer cases of missed abortion.

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Conflict of Interest

The author declares conflict of interest.

Ethics approval

The study protocol was approved by the Biomedical Ethics Research Committee at the Faculty of Medicine, King Abdulaziz University (Reference No 235-16).

Informed consent

Informed consent forms were signed by all the participants who agreed to participate in the current study.

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