Correlation of Anxiety and Uterine Artery Doppler Flow in Pregnant Women with High Risk of Down syndrome: A Prospective Cohort Study

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Abstract

Background: Maternal anxiety may lead to unfavorable pregnancy outcomes, but the underlying mechanism is unclear. This study aimed to evaluate the correlation between maternal anxiety and uterine blood flow index in pregnant women with high risk of Down syndrome undergoing amniocentesis.

Methods: This prospective cohort study was conducted at 15-18 weeks of gestational age on 199 pregnant women with high-risk of Down syndrome, candidates for amniocentesis, and 176 pregnant women at low-risk of Down syndrome in the control group from 2017 to 2019. Anxiety state by Spielberger's State-Trait Anxiety Inventory (STAI) and uterine artery blood flow indices were assessed at baseline and two weeks follow-up visits (immediately before amniocentesis and after receiving the karyotype results in the amniocentesis group).

Results: The mean±SD age of participants was 33.11 ± 5.96 years. There were 176 negative and 23 positive results for Down syndrome in the amniocentesis group. State-Trait Anxiety Inventory scores were significantly different between the amniocentesis and control groups at baseline and follow up (p = 0.033 and p=0.003 respectively) and between baseline and follow-up assessments in the amniocentesis-negative group (p = 0.001, with lower follow-up scores). A significant decrease was observed between baseline and follow-up uterine PI in amniocentesis group (p<0.05), and between baseline and follow-up uterine RI in both amniocentesis and control groups (p <0.001). There was a significant but weak correlation between uterine RI and State-Trait Anxiety Inventory scores at follow-up (r=0.137, p=0.008).

Conclusions: Maternal anxiety may decrease uterine artery blood flow in pregnancy.

Trial registration: IR.TUMS.IKHC.REC.1397.174

Background

Stress is a non-specific reaction to psychological, physical, and perceptional stimuli in the environment (1). Although the relationship between stress and various diseases have been confirmed, the underlying mechanisms for this association are not determined (2). Increased blood pressure, peripheral arterial tonometry, microvascular reactivity, pulse wave velocity, and serum catecholamine levels have been observed in stressful situations (2, 3). Anxiety is defined as hyperarousal, fear, and worry at a counterproductive and debilitating level, which is manifested in response to stressors (4).

Pregnancy is a physiologic condition in life but also considered as a stressful life event experienced by women (5). The observed changes in hormones, physical appearance, abilities, and social roles of a pregnant woman may result in increased stress during pregnancy (6). One of the stressful events in pregnancy is undergoing amniocentesis due to abnormal aneuploidy screening test results (7–9). Therefore, prenatal screening tests may be considered as psychological stressors in pregnancy leading to anxiety and discomfort in pregnant women and her relatives.

Various studies have shown that anxiety and stress during pregnancy are associated with unfavorable pregnancy outcomes, including low birth weight, preterm labor, and intrauterine growth restriction (10–12). This may be related to the release of catecholamines, which results in placental hypoperfusion and consequent decreased oxygen and nutrients delivery to the fetus, leading to fetal growth impairment and/or preterm delivery. Due to the similarities in the pathophysiology of intrauterine growth abnormalities and maternal stress and anxiety, it could
be hypothesized that anxiety in pregnancy may result in unfavorable uterine artery blood flow changes and adverse pregnancy outcomes if it continues (13). Fetal growth disturbances are linked to abnormalities in uterine artery blood flow, which is partly due to endothelial dysfunction (14).

In a recent study performed by Shirazi et al, different levels of maternal stress could affect uterine artery blood flow alterations (9). The study did not include healthy control subjects and therefore the relationship between maternal anxiety level and uterine blood flow could not be assessed (9). Regarding the fact that stress is a transient condition, anxiety might have a more significant and persistent effect on circulation (15, 16). Uterine artery blood flow can be assessed non-invasively using Doppler ultrasonography. Although the hypothesis for the underlying effect of maternal anxiety on uterine artery blood flow seems straightforward, the findings of previous studies are controversial (9, 17). This study was conducted to assess the relationship between maternal anxiety and uterine artery blood flow indices among pregnant women candidates for amniocentesis due to abnormal aneuploidy screening test results, as we clinically observed a high level of anxiety among this group (9).

Methods

Study subjects

This prospective cohort study was conducted on pregnant women referred to Yas Hospital, affiliated to Tehran University of Medical Sciences, Tehran, Iran between October 2017 and April 2019. The study protocol was approved by the Institutional Review Board of Tehran University of Medical Sciences (Registration code: IR.TUMS.IKHC.REC.1397.174). Case and control subjects with a gestational age of 15–18 weeks were included. The case group was selected among patients who were candidates for amniocentesis due to abnormal sequential screening test results while the control group included pregnant women with normal sequential screening test results referred for a routine prenatal visit. Subjects were excluded if they had a recent history of exposure to stressful events, comorbid diseases including hypertension or diabetes, positive aneuploidy culture, history of cigarette or alcohol consumption, or history of major psychological disorders. Written informed consent was obtained from included subjects prior to conducting the trial.

Sample Size

The sample size was calculated based on the findings of a study conducted by Aksoy et al (18), which reported the mean uterine artery PI of 0.95 ± 0.53 among cases and 0.7 ± 0.36 among the control group. Considering the power of 80% and type I error of 0.05 the sample size was calculated as 81 subjects in each of amniocentesis and control groups. Considering the 30% response rate (19) for phone invitation and 20% dropout, the sample size was calculated to be 166 subjects in each of the study groups.

Instruments

The level of the anxiety of the subjects was assessed using the Spielberger's State-Trait Anxiety Inventory (STAI). State anxiety reflects the psychological and physiological transient reactions directly related to adverse situations in a specific moment. In contrast, the term trait anxiety refers to a trait of personality, describing individual differences related to a tendency to present state anxiety (20).
The Spielberger’s State-Trait Anxiety Inventory included 40 questions and rated based on a four-point Likert scale based on the intensity of the feelings of the respondent (21). Scores more than 40 suggested high anxiety and scores that range from 15 to 20 indicate no anxiety. A cut point of 39–40 had been suggested to detect clinically significant symptoms for the scale (22). The Persian version of this questionnaire was validated with a Cronbach’s Alpha coefficient of 0.72 (23).

Doppler ultrasound assessment

All subjects underwent a Doppler ultrasound assessment performed by the same perinatologist using the ACUSON Sequoia 512™ (Siemens Healthcare GmbH, USA) with a convex multi-frequency transducer (3.0 to 5.0 MHz). Uterine artery Doppler assessment was performed trans-abdominally. The uterine artery pulsatility index (PI) and resistance index (RI) were measured for both sides at the origin of the uterine arteries and the mean values for PI and RI were calculated. All follow-up, Doppler assessments were repeated two weeks later.

Study procedure

Subjects in both groups were recruited based on inclusion and exclusion criteria. Subjects in the study group underwent baseline Doppler ultrasound immediately prior to amniocentesis, and the follow-up Doppler assessment was performed two weeks later when they had already received their amniocentesis results. Subjects in this group were categorized into two sub-groups of amniocentesis-positive and amniocentesis-negative according to the positive and negative karyotype results for Down syndrome. In the control group, baseline Doppler study was performed in the first visit between 15–18 weeks, and a follow-up Doppler study was performed two weeks later. In both groups, the Spielberger’s State-Trait Anxiety Inventory questionnaires were filled prior to the baseline and the follow-up Doppler study. All data were collected, recorded, and analyzed among groups and subgroups (Fig. 1).

Statistical analysis

Data were analyzed using the statistical package for social sciences (SPSS) software version 22 (IBM, Inc, Chicago, IL, USA). Mean and standard deviation (SD) were used to present continuous variables while frequency and percentage were used to present categorical variables. The study parameters were compared between amniocentesis and control groups at baseline and follow-up using relevant tests after checking the normality of data. Correlation between study variables was assessed using Pearson correlation. The level of statistical significance was considered as p < 0.05.

Results

A total of 375 subjects (199 cases and 176 controls) participated in the study. The mean ± SD age of the subjects was 33.11 ± 5.96 years. The demographic characteristics of amniocentesis and control groups are presented in Table 1. Although there was a statistically significant difference between case and control groups in terms of age (p = 0.016), this difference was not clinically significant. After receiving the karyotype results for Down syndrome, the mean ± SD age of pregnant women was 34.09 ± 8.19 and 33.77 ± 5.59 years in amniocentesis-positive and amniocentesis-negative groups respectively.
Table 1
Demographic characteristics of study subjects in Amniocentesis and control groups

| Variable       | Case n = 199 | Control n = 176 | p-value |
|----------------|--------------|-----------------|---------|
| Age (years)    | 33.81 ± 5.92 | 32.32 ± 5.91    | 0.016*  |
| BMI (kg/m²)    | 27.52 ± 4.00 | 27.01 ± 4.24    | 0.238*  |
| GA (weeks)     | 16.59 ± 0.8  | 16.77 ± 1.01    | 0.129** |
| Gravida        | 2.48 ± 1.14  | 2.28 ± 1.23     | 0.116*  |
| History of C/S | 85 (42.7%)   | 59 (33.5%)      | 0.068***|

Numbers are reported as in quantitative and frequency (%) in qualitative variables

BMI: Body Mass Index, GA: Gestational Age, C/S: cesarean section

* Independent t-test
** Mann-Whitney test
*** Chi-Square test

The mean state and trait Anxiety Inventory scores were significantly different between amniocentesis and control group at baseline (p = 0.003 and p = 0.033 respectively). In the amniocentesis group, mean state and trait anxiety scores were > 40 (42.79 ± 11.02 and 41.37 ± 10.8 respectively), indicating high levels of anxiety that mothers experienced (Table 2, Fig. 2).

There was no significant difference at baseline mean uterine artery PI and RI between case and control groups (Table 2.)

Table 2
Baseline uterine artery blood flow indices and anxiety inventory scores in amniocentesis and control groups

| Variable         | Case n = 199 | Control n = 176 | p-value |
|------------------|--------------|-----------------|---------|
| Mean UA.PI       | 1.49 ± 0.62  | 1.65 ± 2.15     | 0.968*  |
| Mean UA.RI       | 0.65 ± 0.14  | 0.66 ± 0.13     | 0.645*  |
| Anxiety trait scores | 41.37 ± 10.8 | 39.11 ± 9.51    | 0.033** |
| Anxiety state scores | 42.79 ± 11.02 | 39.44 ± 10.36  | 0.003** |

UA.PI: uterine artery pulsatility index, UA.RI: uterine artery resistance index

*Mann-Whitney test

**Independent sample t test
There was no difference in anxiety trait scores, anxiety state scores, mean uterine PI, and mean uterine RI among amniocentesis-positive, amniocentesis-negative, and control groups in follow-up assessment ($p = 0.85, p = 0.71, p = 0.7, p = 0.5$ respectively) (Table 3).

### Table 3
Follow-up uterine artery blood flow indices and anxiety inventory scores among amniocentesis-positive, amniocentesis-negative, and control groups

| Variable               | Amniocentesis-positive (n = 176) | Amniocentesis-negative (n = 23) | Control (n = 176) | P-value* |
|------------------------|----------------------------------|---------------------------------|-------------------|----------|
| Mean UA.PI             | 1.23 ± 0.55                      | 1.17 ± 0.4                      | 1.15 ± 0.43       | 0.70     |
| Mean UA.RI             | 0.60 ± 0.16                      | 0.62 ± 0.12                     | 0.60 ± 0.12       | 0.50     |
| Anxiety trait scores   | 38.34 ± 12.76                    | 37.71 ± 10.25                   | 38.31 ± 10.31     | 0.85     |
| Anxiety state scores   | 36.91 ± 11.38                    | 38.81 ± 10.96                   | 38.63 ± 9.91      | 0.71     |

UA.PI: uterine artery pulsatility index, UA.RI: uterine artery resistance index

*ANOVA test

Uterine PI was significantly decreased in the amniocentesis group; there was a significant difference between baseline and follow-up mean uterine artery PI in both amniocentesis-positive ($p < 0.001$) and amniocentesis-negative ($p = 0.04$) groups (case group). No significant difference was observed between baseline and follow-up uterine PI in the control group ($p = 0.4$). On the other hand, RI was decreased in all subjects in amniocentesis and control groups ($p = 0.001$) (Table 4).

### Table 4
Comparison of baseline and follow-up uterine artery blood flow indices among amniocentesis-positive, amniocentesis-negative, and control groups

| Variables               | Amniocentesis-positive | Amniocentesis-negative | Control |
|-------------------------|------------------------|------------------------|---------|
|                         | Baseline | Follow-up | p (within group)* | Baseline | Follow-up | p (within group)* | Baseline | Follow-up | p (within group)* |
| Mean UA.PI              | 1.46 ± 0.59       | 1.25 ± 0.53        | < 0.001  | 1.49 ± 0.63       | 1.17 ± 0.41        | 0.004   | 1.65 ± 2.16       | 1.15 ± 0.43        | 0.409   |
| Mean UA.RI              | 1.30 ± 0.37       | 0.60 ± 0.17        | < 0.001  | 1.31 ± 0.28       | 0.62 ± 0.12        | < 0.001 | 1.33 ± 0.28       | 0.61 ± 0.13        | < 0.001 |
| Anxiety trait scores    | 39.91 ± 12.10     | 35.70 ± 10.75      | 0.017    | 43.18 ± 10.86     | 38.98 ± 11.01      | < 0.001 | 39.44 ± 10.36     | 38.60 ± 9.92       | 0.116   |
| Anxiety state scores    | 39.22 ± 13.06     | 36.96 ± 12.86      | 0.192    | 41.65 ± 10.48     | 37.90 ± 10.23      | < 0.001 | 39.11 ± 9.51      | 38.31 ± 10.31      | 0.180   |

UA.PI: uterine artery pulsatility index, UA.RI: uterine artery resistance index
*Paired-t test

In terms of anxiety trait scores, there was a significant difference between baseline and follow-up in both amniocentesis-positive (p = 0.017) and amniocentesis-negative group (p < 0.001) (Table 4 and Fig. 3). Anxiety state scores were significantly different between baseline and follow-up assessments only in the amniocentesis-negative group (p < 0.001) (Fig. 4).

Pearson correlation test revealed a significant but weak correlation between mean uterine RI at follow-up and both anxiety trait and anxiety state score. There was no more significant correlation between baseline mean uterine RI and PI and anxiety scores. These results were also similar after being controlled for maternal age (Table 5).

Table 5
Partial correlation between baseline and follow-up uterine blood flow indices and anxiety inventory scores

|            | Anxiety trait | Anxiety state | Anxiety trait | Anxiety state |
|------------|---------------|---------------|---------------|---------------|
| Baseline   | Mean UA.PI    | r 0.056       | 0.033         | --------------| --------------|
|            |               | p 0.286       | 0.527         | --------------| --------------|
|            | Mean UA.RI    | r 0.036       | 0.035         | --------------| --------------|
|            |               | p 0.486       | 0.498         | --------------| --------------|
| Follow-up  | Mean UA.PI    | r 0.006       | 0.073         | 0.063          | 0.073          |
|            |               | p 0.226       | 0.161         | 0.226          | 0.161          |
|            | Mean UA.RI    | r 0.137       | 0.137         | 0.137          | 0.137          |
|            |               | p 0.008**     | 0.008**       | 0.008**        | 0.008**        |

UA.PI: uterine artery pulsatility index, UA.RI: uterine artery resistance index

** Significant correlation after controlling for maternal age.

**Discussion**

In this study, the mean age of the amniocentesis-positive group was 34.09 ± 8.1 years. It was previously shown that the risk of Down syndrome significantly increases with an increase in maternal age over 35 years old (24). This relationship might be significant at lower ages in the case of consanguineous marriage (25). This study did not assess the consanguinity of the couples but regarding the high incidence of consanguineous marriage in Iran (26), which may justify the lower age of the amniocentesis-positive group to some extent. This study also found a significant age difference between high-risk and control groups. Although this difference was of statistical significance, the mean difference was nearly one year, which was not of clinical significance.

The findings of this study revealed that the mean anxiety state and trait scores in all participants were higher compared to the normal range (scores less than 39 indicate less anxiety) (9). This finding was in line with the findings of previous studies that indicated a positive antenatal screening test may result in increased anxiety in pregnant women (9, 27–30). On the other hand in our study, the control group also exhibited a higher anxiety level
compared to the normal women in a similar age group. Investigations have shown a higher prevalence of antenatal depression and anxiety disorders in Iranian pregnant women compared to the world average (31–32).

Animal-based studies have shown a significant relationship between stress and fetal growth. In a study, anxiety and chronic stress were found to increase serum cortisol and corticotrophin-releasing hormone in both mother and fetus resulting in dysregulations in the hypothalamic-pituitary-adrenal (HPA) axis in the fetus that in part causes structural abnormalities in the hippocampus, frontal cortex, amygdala, and nucleus accumbens (32). Further animal studies have also revealed a significant decrease in uterine blood flow due to stress (33–35). Human studies have also hypothesized a similar effect for stress and anxiety on uterine blood flow (12, 17). Previous studies have shown that anxiety may be correlated to reduced uterine artery blood flow (9, 17).

The findings of this study revealed a significant reduction in uterine artery RI over time, indicating that regardless of being high-risk or low risk for Down syndrome, the mean RI significantly reduced from baseline through the follow-up assessments, while PI was decreased significantly only in the amniocentesis group. It was previously shown that the uterine artery pressure and resistance decrease as the gestational age increases, which is mainly due to the formation and expansion of placental vascular bed (36). Therefore, one reason for the reduced uterine PI and RI could be the physiological changes in pregnancy. On the other hand, one might attribute the degree of mean uterine artery changes to the level of anxiety in both amniocentesis-positive and amniocentesis-negative groups, as they both were exposed to a stressful event which might have resulted in an increased baseline uterine artery PI and RI. Unfortunately, this hypothesis could not be tested in this study as there were no anxiety-free subjects in this study. The findings of this study also revealed no significant group effects for uterine PI and RI, which indicate that the mean uterine PI and RI changes were not significantly different between amniocentesis and control groups at the baseline and the follow-up assessments.

The findings of this study revealed a significant reduction in trait anxiety scores over time in both amniocentesis-positive and amniocentesis-negative groups while state anxiety score was significantly reduced over time in the amniocentesis-negative group. This indicates that the trait and state anxiety scores changed differently in the amniocentesis-positive and negative groups during the two-week follow-up period. This finding might be due to the reduction of anxiety after observing a negative result or might show that a two-week period could provide an opportunity for mothers to cope with their anxiety. This finding indicates that higher baseline uterine artery PI and RI may be related to higher baseline levels of anxiety in the amniocentesis group. As the anxiety decreased by coping with the condition, the uterine artery PI and RI also decreased in the amniocentesis-positive group. The presence of high baseline levels of anxiety in the control group may justify the lack of significant difference in uterine PI and RI between groups.

The findings of the study revealed high baseline levels of anxiety in both amniocentesis and control groups, which made it impossible to have an anxiety-free arm in our study to assess the effect of anxiety on the uterine blood flow. The other limitation of this study was the short follow-up duration. Two weeks follow-up duration was chosen to decrease the high risk of losses to follow-up as the study was conducted in a tertiary hospital with a substantial number of patients being referred from other cities and provinces. Therefore, longer cohort studies are suggested to screen uterine vascular indices and pregnancy outcomes among pregnancies complicated by anxiety.

**Conclusion**
The findings of this study revealed that high maternal anxiety may result in decreased uterine artery blood flow. It seems necessary to develop stress management training for pregnant women and to provide stronger emotional support, especially for those who have been recognized to be at high risk of Down syndrome by prenatal aneuploidy screening tests.

**Abbreviations**

State-Trait Anxiety Inventory (STAI)

Pulsatility Index (PI)

Resistance Index (RI)

standard deviation (SD)

Body Mass Index (BMI)

Gestational Age (GA)

uterine artery (UA)

cesarean section (C/S)

hypothalamic-pituitary-adrenal (HPA)

**Declarations**

**Ethics approval and consent to participate:** This manuscript was performed in accordance with Helsinki declaration. All patient’s data were kept confidential. This study was approved by Institutional Review Board of Tehran University of Medical Sciences (Registration code: IR.TUMS.IKHC.REC.1397.174) and 96-03-91-36165.

**Consent for publication:** Written consent was signed upon admission by all patients included in this study to use their information in research studies and publication in public (Persian version) and is available for review.

**Availability of data and materials:** The datasets used during the current study are available from the corresponding author on reasonable request.

**Competing interests:** The authors report no conflict of interest

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**Authors' contributions:**

M.S (Mahboobeh): Design of the work

M.T: Drafting the manuscript

M.G: Manuscript editing, Interpretation of data
M.M: Manuscript editing, Interpretation of data

M.S (Mahmoud): Manuscript editing, Interpretation of data

M.E: Drafting the manuscript

LA: Manuscript editing

S.R: Design of the work, Drafting the manuscript

All authors approved the submitted version (and any substantially modified version that involves the author's contribution to the study).

All authors are agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Figures
Figure 1

Consort diagram of the study

Figure 2

Changes in anxiety trait scores during the study duration as per study groups.
Figure 3

Changes in anxiety trait scores during the study duration in amniocentesis subgroups
Figure 4

Changes in anxiety state scores during the study duration as per study subgroups