

Association between Maternal Serum Inhibin A and Chromosomal Abnormalities as a Diagnostic Markers in First Trimester of Threatened Abortion

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Abstract

Background: Threatened abortion is defined as a pregnancy complicated by bleeding before 20 weeks' gestation. **Objective:** Assessment of the relation between the concentration of serum Inhibin A and chromosomal abnormalities in a trial to find marker for the threatened abortion. **Patients and Methods:** The present study included 40 pregnant women in the first-trimester, 20 pregnant women suffering from pregnancy complications (threatened aborted) and 20 pregnant women for control. Blood samples were collected from El Hussain Hospital, Cairo, Egypt, under the clinical supervision. Serum is prepared for measurement Inhibin A hormone using Enzyme-Linked Immuno-Sorbent Assay (ELISA), while heparinized blood processed for cell culture to determine chromosomal aberrations. **Results:** Cytogenetic analysis revealed that structural chromosomal abnormalities were recorded in 35 from 600 maternal metaphase (5.83%), compared to (2.6%) that were detected in the examined metaphases of control group; there was no statistical significance between the control and threatened aborted cases ($\chi^2 = 0.85$, $P > 0.05$), while concentration of Inhibin A hormone in the studied group shows Mean of 16.7 pg/ml but in control group shows concentration of 38.7 pg/ml, there was a significant difference between the two groups in serum level of inhibin A ($t = 12.9$, $P < 0.05$). On the other hand we found no correlation between chromosomal anomalies and inhibin A level in our cases ($r = -0.296$, $p = 0.204$). **Conclusion:** Serum inhibin A measured during the first trimester of pregnancy might be useful for prediction of threatened abortion.

Key word: Threatened abortion, Inhibin A, chromosomal abnormalities.

Introduction

Threatened abortion is a condition in which vaginal bleeding is less than in inevitable abortion and the cervix is not dilated, and abortion may or may not occur, (1). First-trimester vaginal bleeding is one of the most common complications in pregnancy with an incidence of 15–25%. About half of these will end in miscarriage within 20 weeks of gestation (2,3) and those women who remain pregnant have an increased risk of developing other complications later in pregnancy (4). Maternal age is an independent risk

factor for threatened miscarriage. Women between the ages of 25 and 30 years had the lowest risk of spontaneous abortion, whereas those with the highest risk of spontaneous abortion were at the extremes of age. It was found that a chromosomal abnormality occurs in 49 percent of spontaneous abortions (5). On the other hand inhibins are glycoproteins produced by the granulosa and theca cells of the ovary and by the Sertoli cells of the testis. Inhibin is a dimeric disulfide-linked glycoprotein molecule, consisting of α and subunits β (6). All inhibins share a common subunit (18–20 kDa), but depending on the type of subunit [A (13 kDa) and B (15 kDa)], inhibin is classified as inhibin A or B. Apart from inhibin A or B, these subunits are also present in circulation in

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large dimeric or monomeric forms (7).

During pregnancy, serum inhibin A can be measured in maternal serum in significant concentration during pregnancy and the level increases as gestational age advances because inhibin A is produced from human placenta and fetal membranes (8, 9). Also, previous studies have revealed that progesterone is the most powerful single predictor of pregnancy outcome in natural conception (10). Although the precise spectrum of functions of these hormones in pregnancy has not been fully delineated, abnormal levels have been noted in association with maternal complications of pregnancy, such as, preeclampsia, preterm labor, fetal abnormality, Down syndrome and threatened miscarriage (11, 12). So, Inhibin A, and karyotyping may be helpful as predictors for threatened abortion ; however, these tests may not be useful in primary care settings (13).

Subjects and Methods

The present study was including 40 pregnant women in the first-trimester according to their last menstrual period (LMP). 20 pregnant women suffering from pregnancy complications (e.g., vaginal bleeding and vaginal pain) and 20 pregnant women for control. Samples were collected from El Hussain Hospital, Cairo, Egypt, under the clinical supervision.

Under the clinical supervision, serum and heparinized venous blood taken from cases by vacutainer collecting tubes (14). Serum is used for detecting Inhibin A hormone using enzyme-linked immuno-sorbent assay (ELISA) technique but samples may be stored at 2–8°C for up to 24 hours, or frozen at –20°C or lower for up to 30 days. While, heparinized blood must be freshly processed for cell culture to determine any chromosomal aberration.

The blood cell karyotyping method was developed to provide information about chromosomal abnormalities. Lymphocyte cells do not normally undergo subsequent cell divisions. In the presence of a mitogen, lymphocytes are stimulated to enter into mitosis by DNA replication. After 48–72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities (15).

Statistical Analysis

Differences between cases and controls were statistically analyzed using the Student's t test for normally distributed continuous variables, the χ^2 test for categorical variables, and Pearson correlation test,

P values < 0.05 were considered significant. All statistical analyses were performed using SPSS version 13.

Results

Forty women were subjected or conducted , with written consent, into this study, the Inclusion criteria for selected cases were: (1) maternal age between 20 and 35 years old, (2) History of early vaginal bleeding, (3) Participants with intrauterine gestation sac with positive fetal cardiac activity, (4) No uterine abnormalities e.g. Septate uterine, (5) No cervical lesion e.g. Erosione, (6) No history for chronic medical diseases; Thyroid disorders, Hypertension, Diabetes Mellitus (DM) or other Autoimmune diseases.

The Exclusion criteria were: (1) maternal age of 35 years or older, (2) uterine abnormalities, (3) cervical lesions, (4) chronic diseases as Diabetes Mellitus or Hypertension. These cases were classified into two main groups, the first were 20 healthy pregnant women and delivered healthy babies, their mean age was (25 years old), this group represented as control group. The second were 20 women who were diagnosed as first trimestric threatened abortions; their mean age was (29 years old). The mean gestational age of was (7 weeks from the last menstrual period), this group represent the studied group.

Cytogenetic analysis revealed that structural chromosomal abnormalities were recorded in 35 from 600 maternal metaphase (5.83%), and these structural chromosomal abnormalities were picked out in 19 of studied cases for maternal metaphases was only (3.1%), compared to (2.6%) that were detected in the examined metaphases of control group (Table 1); therefore using Chi square test, there was no statistical significance between the control and threatened aborted cases ($\chi^2 = 0.85$, $P > 0.05$) (Figure 1).

Cytogenetic analysis using G-banding technique within four metaphases per case shows no evidence for numerical chromosomal abnormalities either in the control group or in the threatened aborted cases according to the Ideogram of the human chromosome (22 pairs of chromosomes and xx) for both two groups.

On the other side according to inhibin A hormone, data analysis was done using GraphPad Prism program. Data of each group are presented in the form of mean $\pm$ SD (Table 2 and Table 3). Students't test was for comparison of concentration of inhibin A between females with threatened aborted females with bleeding in the 1st trimester (group 2) and normal pregnancy (group 1) as shown in Figure 2.

Table 1. Differentiation between threatened aborted/studied group (SG) and control group (CG) according to the structural chromosomal abnormalities.

Chromosomal Abnormalities		Chromatid Gap	IsoChromatid Gap	Break	Deletion	Diradial	Centromeric Separation	Chromotid Exchange	Centric Fusion	Acentric Fragment	Total
Subjects	#	2	1	4	2	2	2	1	2	3	19
	%	0.33	0.16	0.66	0.33	0.33	0.33	0.16	0.33	0.50	3.1
Control	#	2	1	3	1	1	2	1	2	3	16
	%	0.33	0.16	0.50	0.16	0.16	0.33	0.16	0.33	0.50	2.36

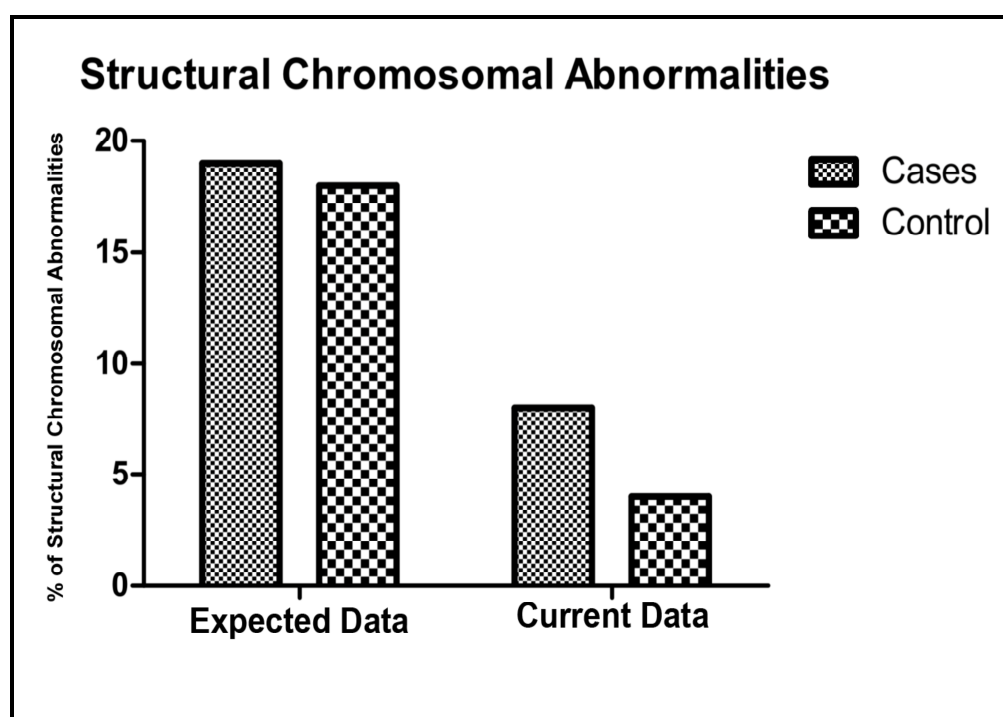


Figure 1. Represents the Structural Chromosomal Abnormalities for both studied cases (threatened aborted) and control group (CG).

Using students't test, there was a significant difference between the 2 groups in serum level of inhibin A ($t = 12.9$, $P < 0.05$).

The data suggest that threatened aborted cases (20 cases) show low levels of serum Inhibin A concentration in the first trimester. According to patient sheets —recorded under the clinical supervision— 16 cases from the total number of

threatened abortion become aborted (80%). This prove the outstanding importance for serum Inhibin A as a marker for pregnancy viability and prediction of abortion. On the other hand, there was a non-significant negative correlation between %of chromosomal anomalies and inhibin level ($r = -0.296$, $p = 0.204$).

Table 2. Concentration of Inhibin A for (group 1) and (group 2) by pg/ml.

No. Of Cases	Inhibin A in (group 1)	Inhibin A in (group 2)
1.	53.5	15.1
2.	31.5	13.3
3.	38.1	22.4
4.	38.4	25.3
5.	38.4	11.9
6.	37.5	14.6
7.	37.2	13.2
8.	39.0	14.9
9.	38.3	12.2
10.	42.0	21.6
11.	37.1	30.5
12.	33.1	17.5
13.	51.1	16.2
14.	36.3	13.9
15.	36.0	16.1
16.	33.2	13.0
17.	37.1	14.5
18.	48.1	11.4
19.	35.5	9.8
20.	34.3	19.2

Table 3. Showing the mean level of inhibin A in normal pregnant and threatened aborted women in the 1st trimester.

	Mean $\pm$ SD (pg/ml)
Subject	16.7 $\pm$ 1.15
Control	38.7 $\pm$ 1.29

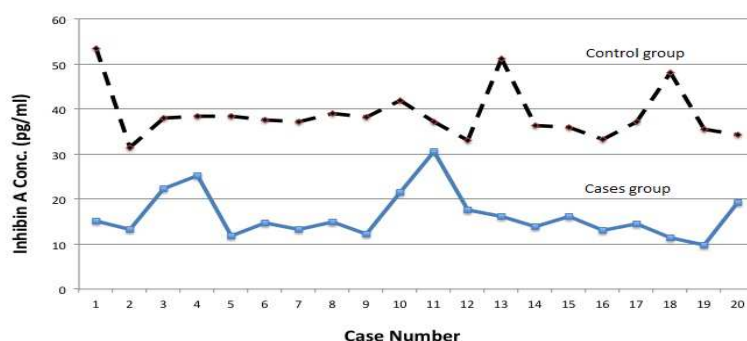


Figure 2. Mean value of serum inhibin A levels (pg/ml) of the threatened aborted cases and control group.

Discussion

Spontaneous abortion is the most common complication of early pregnancy (16). The frequency decreases with increasing gestational age. Eight to 20 percent of clinically recognized pregnancies fewer than 20 weeks of gestation will undergo Spontaneous abortion; 80 percent of these occur in the first 12 weeks of gestation, (17). The overall risk of Spontaneous abortion after 15 weeks is low (about 0.6 percent) for chromosomally and structurally normal fetuses, but varies according to maternal age and ethnicity (18).

Many women with unintended pregnancies resort to clandestine abortions that are not safe. According to the World Health Organization (WHO) (19), around 1.5 million abortions in Egypt and Middle East in 2003 were performed in unsanitary settings, by unskilled providers, or both. Complications from those abortions accounted for 11 percent of maternal deaths in the region.

Goddijn and Leschot revealed that chromosomal abnormalities are a direct cause of spontaneous abortion and therefore threatened abortion. One Meta analysis found that a chromosomal abnormality occurs in 49 percent of spontaneous abortions (5).

In our study, Cytogenetic analysis revealed that structural chromosomal abnormalities of studied cases for maternal metaphases was only (3.1%), compared to (2.6%) that were detected in the examined metaphases of control group. This proves that there is no significance between two groups. On the other hand, the measurement of inhibin A during the first trimesters of pregnancy could be useful in the diagnosis of trophoblast dysfunction, and, therefore be helpful in the management of early pregnancy problems, to predict the first trimester pregnancy outcome in patients with early pregnancy vaginal bleeding due to threatened abortion (20).

During pregnancy, serum inhibin A can be measured in maternal serum in significant concentration during pregnancy and the level increases as gestational age advances because inhibin A is produced from human placenta and fetal membranes (8, 9). There are studies which demonstrated that maternal serum inhibin A levels in women presenting with signs and symptoms of miscarriage (abdominal pain and/or vaginal bleeding) were significantly lower in these women who had a miscarriage confirmed, whether it was complete or incomplete, compared with those women who had an ongoing viable pregnancy (Illingworth et al., 1996).

Hand in hand, our study demonstrated maternal serum inhibin A levels in women presenting with signs and symptoms of threatened abortion

(abdominal pain and/or vaginal bleeding) were significantly lower with those who had an ongoing viable pregnancy.

Conclusion

Serum inhibin A can be measured during the first trimester of pregnancy. Our reference ranges might be useful for further studies, such as prediction of adverse pregnancy outcome in threatened abortion. On the other hand maternal cell culture done in the first trimester failed to be a significant predictor for threatened abortion, but this paper can let more studies for the molecular level.

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