

# Effect of cell-free fetal DNA on spontaneous preterm labor

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## ABSTRACT

Inflammatory phenomenon, including cell-free fetal DNA (cffDNA), is one of the various causes of preterm delivery. Always, there is a trend in the prediction and prevention of preterm labor. Herein, the aim of this study is to assess the value of cffDNA levels in serum of pregnant women for prediction of spontaneous preterm labor. A case-control study with nonrandomized convenience sampling was conducted. One hundred women qualifying for the study were enrolled. The participants were divided into two groups of healthy nulliparous pregnant women ( $n = 50$ ) and nulliparous pregnant women experiencing preterm labor ( $n = 50$ ). Then, venous blood was sampled from each participant and its plasma was separated and analyzed. The mean age of women in the experimental group was 22.90 years with the standard deviation of 4.04, while in the control group these two values were 23.78 and 4.37, respectively. In the experimental group, 29 patients (58%), and in the control group, 27 patients (54%) had cffDNA. The mean of the counted DNA in the experimental group was 2080/03 with the standard deviation of 909/792 while the same values for the control group were 1183/26 and 620/720, in the same order. The statistical analysis revealed that this difference was meaningful at  $P = 0.001$ . Furthermore, in the experimental group, cffDNA increased with increase in the age and the difference was meaningful. Finally, increasing pregnancy age in the experimental group led to an increase in the number of cffDNA ( $P = 0.001$ ). The results of this study indicated that the cumulative frequency of preterm labor for women with positive cffDNA was significantly higher. High levels of cells' DNA in the serum of pregnant mothers increase the risk of spontaneous preterm labor. These observations may have implications for preterm labor.

**Key words:** Cell-free fetal DNA, dilatation and effacement, early preterm labor, late preterm labor, prematurity

## INTRODUCTION

Despite considerable effort aimed at decreasing its incidence, preterm birth (PTB) remains the foremost cause of perinatal

morbidity and mortality.<sup>[1]</sup> Annually, 15 million deliveries happen prematurely and approximately one million babies die due to complications of prematurity and screening strategies are imperfect.<sup>[2,3]</sup> Currently, multiple preventive modalities are used to prevent PTB including treatment of intrauterine infection, progesterone therapy, cerclage of the cervix, nutritional supplementations, and lifestyle modifications; however, the incidence of PTB remains high.<sup>[4]</sup>

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It has been suggested that some circulating biomarkers may differ in pregnancies that experience spontaneous PTB from those delivering at term and that these differences may have predictive value.<sup>[1,5,6]</sup> In addition, it has been showed that labor is an inflammatory process, and elevated levels of inflammatory cells and pro-inflammatory agents have been found in maternal and fetal tissues during parturition. Hence, it is suggested that inflammation plays a crucial role in preterm and term labor.<sup>[7]</sup>

Cell-free fetal DNA (cffDNA) is fetal DNA which circulates freely in the maternal blood. The cffDNA is measurable in the maternal serum and increases with gestational age (GA) and its greater concentrations is found in association with some pregnancy complications.<sup>[2,8]</sup> In fact, cffDNA is a novel promising biomarker that has been used in numerous obstetrical researches, especially in prenatal diagnosis and complicated pregnancies. It is easily detectable by polymerase chain reaction tests.<sup>[9]</sup> The pro-inflammatory properties of cffDNA and its conceivable effects on pregnancy and labor are mysterious. Previous studies suggest an association between preterm labor and higher maternal serum levels of cffDNA in the second and third trimester and at onset of PTB. Along with the pro-inflammatory effects of cffDNA, its elevation with GA and during labor suggests a potential role in the pathogenesis of spontaneous PTB.<sup>[2]</sup>

Regarding the high importance of prediction and prevention of preterm labor, this study was conducted to assess the value of cffDNA levels in the serum of pregnant women for the prediction of spontaneous preterm labor.

## MATERIALS AND METHODS

### Study design

A case-control study with nonrandomized convenience sampling was conducted. One hundred women qualifying for the study were enrolled.

### Study setting and population

The selected women were then briefed about the objectives of the study and consented to participant in the study.

The participants were divided into two groups of healthy nulliparous pregnant women and nulliparous pregnant women experiencing preterm labor. There were 50 participants in each group.

Inclusion criteria were nulliparous pregnant women with GA of <37 weeks and body mass index of between 20 and 29.9 kg/m<sup>2</sup>. Exclusion criteria were presence of any pathology in placenta including detachment, preeclampsia, or disease including diabetes mellitus, sexually transmitted diseases, or first-trimester vaginal hemorrhage (the placental health was defined according to the ultrasonography performed in the third trimester), receiving tocolytic therapy for preventing

the delivery. Further, each woman in the control group who delivered before 37<sup>th</sup> week was excluded from the study.

The study was confirmed by local research committee. All participants signed the informed consent. The data were collected by bipartite questionnaire including comprehensive information about demography, interview, and complementary ultrasonography, findings of clinical examination, offspring gender, and maternal blood sample results. The venous blood (2.5 cc) was sampled from each participant and its plasma was separated. The separated and frozen plasma was then sent to the research center of Imam Khomeini Hospital for further investigation. DNA extraction was performed using Macherey-Nagel Company kit.

### Data analysis

The data obtained from the used checklists along with data from the participants' blood samples were inputted into SPSS for Windows, Version 16.0. SPSS inc., Chicago for statistical analysis and  $P < 0.05$  was considered statistically significant. To analyze the data, descriptive statistical procedures including frequency counts and tables and inferential statistical techniques such as *t*-test and Chi-square were used.

## RESULTS

The study conducted on 100 women (including 50 cases and 50 controls). The average GA was  $23.84 \pm 4.58$  weeks in cases and  $23.06 \pm 4.04$  weeks in controls ( $P = 0.369$ ). Twenty women (40%) in cases and 19 women (38%) in controls had blood group and Rh of A+ ( $P < 0.05$ ).

Twenty-nine women (58%) in cases and 27 women (54%) in controls had cffDNA ( $P < 0.518$ ) [Table 1].

The average cffDNA in cases was  $1570.64 \pm 207.64$  copy number in GA of 28–34 in comparison with  $2555.47 \pm 198.87$  copy number in GA of 35–37 ( $P = 0.002$ ). Furthermore, the average cffDNA in controls was  $1109.50 \pm 387.46$  copy number in GA of 28–34 in comparison with  $1467.36 \pm 547.25$  copy number in GA of 35–37 ( $P = 0.057$ ). As confirmed by *t*-test, the GA was directly related with average serum cffDNA ( $P = 0.007$ ) [Diagrams 1 and 2].

The most specificity was in DNA level >3000 and the most sensitivity was in DNA level of 500. The comparison of specificity and sensitivity was performed to obtain a cffDNA level as an index for using in screening and diagnostic tests. The level of 1000 had acceptable specificity and sensitivity in this survey [Table 2].

## DISCUSSION

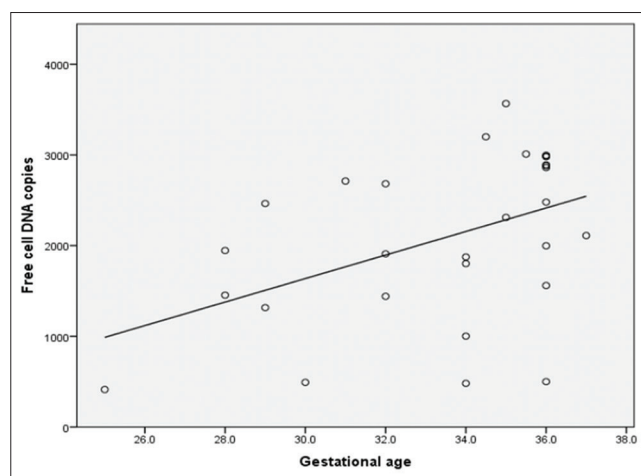
We studied pregnant women to assess the value of cffDNA levels in serum of pregnant women for prediction of spontaneous preterm labor. Currently, reducing the

**Table 1: Presence or absence of cell-free fetal DNA**

Variable	Cases		Controls		Summation
	28-34 weeks	35-37 weeks	28-34 weeks	35-37 weeks	
Presence of DNA (%)	14 (56)	15 (60)	16 (64)	11 (44)	56
Absence of DNA (%)	11 (44)	10 (40)	9 (36)	14 (56)	44
Summation	25	25	25	25	100

**Table 2: Comparison of specificity and sensitivity according to the cell-free fetal DNA level**

DNA level	Cases		Controls		Sensitivity (%)	Specificity (%)
	Positive	Negative	Positive	Negative		
500	26	24	26	24	52	48
1000	22	28	12	38	44	76
1500	18	32	8	42	36	84
2000	8	42	4	46	16	92
2500	8	42	3	47	16	94
3000	5	45	0	50	10	100
3500	3	47	0	50	6	100

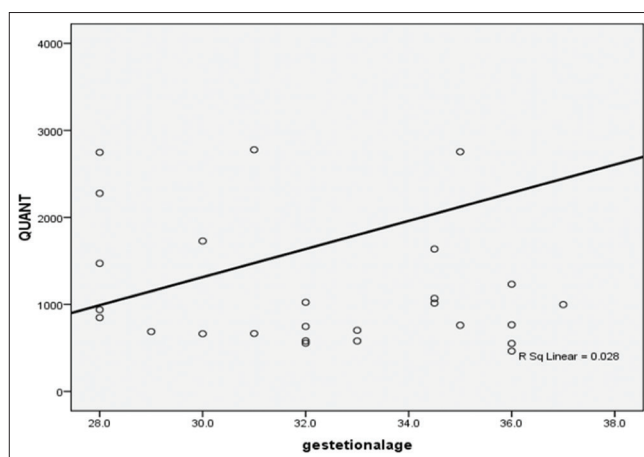
**Diagram 1:** The relation with gestational age and serum cell-free fetal DNA in cases

incidence of spontaneous PTB is one of the challenges in obstetrics and gynecology wards.<sup>[4,10]</sup>

Despite the great researches and supreme efforts made for decreasing preterm labor, it remains as the leading cause of perinatal mortality and morbidity. We studied nulliparous pregnancies because women with previous prenatal loss are at higher risk of PTB.<sup>[11,12]</sup>

The difference between GA in cases and controls was not statistically significant. Further, according to the results of ANOVA test, the difference between Blood Group (BG) of Participants and Premature birth in cases and controls was not statistically significant. However, we could not find any published study comparing the BG in such patients.

Twenty-nine women (58%) in cases and 27 women (54%) in controls had cffDNA, and resemble to previous studies,<sup>[13]</sup> the difference was not significant. However, the average

**Diagram 2:** The relation with gestational age and serum cell-free fetal DNA in controls

count of cffDNA was statistically significant between the studied groups ( $P = 0.001$ ).

In a study by Leung *et al.*, serum levels of cffDNA were significantly higher in cases than controls.<sup>[14]</sup> In addition, Farina *et al.* suggested that high maternal serum cffDNA is associated with an increased risk of spontaneous preterm delivery. This observation may have implications for the understanding of the mechanisms of disease that is associated with preterm labor.<sup>[15]</sup> These findings are compatible with our results.

However, Quezada *et al.* investigated the alteration of cffDNA in pregnant women and concluded that in the spontaneous preterm delivery groups (<34 weeks' gestation, 34–37 weeks, <37 weeks), compared to the term delivery group, there was no significant difference in cffDNA and its measurement is not predictive of spontaneous preterm delivery.<sup>[13]</sup>

We found statistically significant and direct correlation between GA and cffDNA, both in cases and controls. As gestation advances, serum cffDNA levels increase.<sup>[16]</sup> Leung *et al.*<sup>[14]</sup> and Dugoff *et al.*<sup>[17]</sup> suggested that serum levels of cffDNA were significantly higher in cases than controls and Goldfarb *et al.* concluded that elevated levels of cffDNA in maternal serum may initiate the labor.<sup>[18]</sup>

Women in labor have a greater serum cffDNA concentration.<sup>[16]</sup> Compatible with our findings, Farina *et al.* showed that the cumulative rate of early preterm delivery (GA <30 weeks) was significantly higher for women with cffDNA concentrations, and high levels of fetal cffDNA were associated with a high rate of preterm delivery.<sup>[15]</sup> Quezada *et al.*<sup>[13]</sup> and Illanes *et al.*<sup>[19]</sup> could not find any significant correlation between cffDNA and GA at delivery. They suggested that cffDNA does not reliable for the prediction of preterm labor.<sup>[13,19]</sup>

The diversity in the achieved results may be due to differences in GA or preterm labor. We assessed the correlation of maternal serum cffDNA and spontaneous preterm labor.

### Limitations

This is a small study that includes only the results of one institution, and therefore, it is not necessarily representative of the nation as a whole.

### CONCLUSIONS

As showed by our results, cffDNA biomarker can be used as a simple and noninvasive test for prediction of preterm labor with specificity of 46%, sensitivity of 58%, positive predictive value of 51%, and negative predictive value of 52%. However, the need for further research is highlighted.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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