Comparative study between levels of maternal serum Cell Free Fetal DNA and Uric acid in pregnant women with and without Preeclampsia

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Short title: maternal serum cffDNA Vs uric acid in preeclampsia

Abstract

Objectives: In this study, we aimed to compare the value of cell-free fetal DNA (cffDNA) and uric acid in maternal serum as markers for pre-eclampsia and subsequently as possible predictors in the future.

Study Design: This cross-section study including pregnant women attending Cairo University maternity hospital, Cairo, Egypt. This study included 120 patients in two groups; study group and control group with 60 patients each.

Results: The mean level of CffDNA was 19.6 ± 5.4 and 578.9 ± 185.3 mg/dl in the control and preeclampsia patients respectively; $p<0.01$. The mean level of cell free fetal DNA was significantly higher among primigravida with severe preeclampsia; 743.2 mg/dl compared with 295.3 mg/dl in multiparous women with severe preeclampsia; $p<0.01$. Similarly, the mean level of cell free fetal DNA was significantly higher among primigravida with mild preeclampsia; 543.3900 mg/dl compared with 372.2464 mg/dl among the multiparous women with mild preeclampsia; $p<0.01$.

The mean level of uric acid in control group was 2.9 mg/dl compared with 4.7 mg/dl in the preeclampsia group; $p<0.01$. The mean level of uric acid was significantly higher among primigravida with severe preeclampsia; 749.6 mg/dl compared with 295.3 mg/dl in multiparous women with severe preeclampsia; $p<0.01$. Similarly, the mean level of uric acid was higher among primigravida with mild preeclampsia; 3.6 mg/dl compared with 3.4 mg/dl among the multiparous women with mild preeclampsia; however, there was no significant difference, $p= 0.072$.

Conclusion: We found that cffDNA is a better marker of pre-eclampsia than uric acid. Compared with uric acid, cffDNA is more sensitive as it has shown elevated levels with mild pre-eclampsia and more elevations with severe pre-eclampsia. Different age groups did not affect

the levels of both markers. However, parity seemed to affect Cell-Free Fetal DNA level as it had higher values in primigravida.

Keywords: fetal DNA, cffDNA, uric acid, Preeclampsia.

Introduction

Pre-eclampsia is a disease specific to humans, characterized by hypertension and proteinuria. The disease has a high incidence of 7 % in most populations and it is one of the leading causes of maternal and perinatal morbidity and mortality in developing countries (1,2).

Multiple theories tried to explain why pre-eclampsia happens, yet the exact pathogenesis remains unclear and that explains the difficulty to predict pre-eclampsia. In order to establish a reliable pre-eclampsia predictor from the maternal serum, one should identify first a strong marker (3,4).

In 1997, a new approach of non-invasive prenatal diagnosis appeared when Lo et al. discovered the existence of cell-free fetal DNA (cffDNA) in maternal plasma by detecting Y chromosome-specific DNA sequence in pregnant women bearing male fetuses (5).

Current data suggest that fetal DNA is detectable in virtually all pregnant women. In case of pre-eclampsia, a five-fold elevation of plasma fetal DNA has been reported (6–8). The exact mechanism for such elevation, like the etiology of the disorder itself has not been fully understood.

Another marker/predictor has been suggested for decades, that is Uric acid. The association between elevated serum uric acid and pre-eclampsia was reported for the first time early in the 20th century by Slemmons et al. in 1917 (8). Interest has been renewed over the role of uric acid in the pathogenesis of hypertension, endothelial dysfunction and renal dysfunction, which are all features of pre-eclampsia. Recent studies pointed that uric acid measurement is considered a component in the work up of pregnant women with pre-eclampsia to monitor the severity of the disease and to help management (9–11).

Hence, in this study, we aim to investigate the predictive role of cffDNA and uric acid in preeclampsia.

Materials and methods

This is a case control study including pregnant women attending Cairo University maternity hospital, Cairo, Egypt. This study included 120 patients in two groups; study group and control group with 60 patients each.

A) Study group:

Inclusion criteria:

Singleton, viable pregnancy with no obstetrical or medical complications of pregnancy apart from severe pre-eclampsia (with the presence of any of the following criteria):

1- B/P more than or Equal to 160/110 in 2 separate occasions without history of hypertension prior to pregnancy and not responding to treatment. 2- Urine analysis showed persistent proteinuria of 3+ or 4+. 3- IUGR or oligohydramnios. 4- Placental separation. 5- HELLP Syndrome. 6- Abnormal kidney or liver functions. 7- Eclampsia. 8- Gestational age ≥ 28 weeks.

Exclusion criteria:

1- Any maternal complication found other than Preeclampsia. E.g: diabetes mellitus. 2- Past history of Hypertension. 3- Pregnancy with detected fetal congenital anomalies.

B) Control group:

Inclusion criteria:

1- Singleton, viable pregnancy, 2- No obstetrical or medical complications of pregnancy, 3- Gestational age ≥ 28 weeks, 4- Pregnancy with fetal congenital anomalies will be excluded.

All patients were subjected to history taking, general examination including blood pressure management and fetal ultrasound scanning. Blood and urine samples were collected afterwards for the detection of Albumin in urine, serum Uric acid and Cell Free Fetal DNA. Uricase method was used to determine the level of uric acid. Real time 7500 fast SDS software v. 2.05 (Applied biosystems, Foster City, USA) was used to monitor the cffDNA. It should be noticed that routine investigations were also done.

Statistical Analysis:

All of the statistical indices were done using statistical package: SPSS version 18 and Microsoft Excel 2007. Mean, Median, Mode, Standard Error, Standard Deviation, and Interquartile Range. Maternal peripheral blood samples (3ml of blood) were collected and serum is separated in a plain tube. For uric acid level in serum, uricase method is used.

Results

For better evaluation of the two markers, the study group was subdivided into mild (22 patients, 36.7%) and severe (38 patients, 63.3%) groups. In order to understand the relation of the Cell Free Fetal DNA and Uric acid to the parity of the women of the study, we divided the cases into Primigravida (35 control and 41 pre-eclamptic including 30 severe cases), and Multiparas (25 control and 19 pre-eclamptic including 8 severe cases), **figures 1 and 2.**

CffDNA characteristics: (Table 1)

The mean level of CffDNA was 19.6 ± 5.4 and 578.9 ± 185.3 mg/dl in the control and preeclampsia patients respectively. There was a statistically significant difference between preeclampsia patients compared with the control group; p<0.01. The mean level of cell free fetal DNA was significantly higher among primigravida with severe preeclampsia; 743.2 mg/dl compared with 295.3 mg/dl in multiparous women with severe preeclampsia; p<0.01. Similarly, the mean level of cell free fetal DNA was significantly higher among primigravida with mild preeclampsia; 543.3900 mg/dl compared with 372.2464 mg/dl among the multiparous women with mild preeclampsia; p<0.01.

	CffDNA Characteristics among different groups		
	Parameter	**Mean ± SD**	**p-value**
	Control (n=60)	19.6 ± 5.4	<0.01
Pre-eclampsia severity	**Pre-eclampsia patients (n=60)**	578.9 ± 185.3	
	Mild preeclampsia subgroup (n=22)	457.8 ± 101.2	
	Severe preeclampsia subgroup (n=38)	648.9 ± 187.5	
Gravidity and preeclampsia levels	**Primigravida with Severe preeclampsia (n=30)**	743.2 ± 34.3	<0.01
	Multi para with Severe preeclampsia (n=8)	295.3 ± 3.3	
	Primigravida with mild preeclampsia (n=11)	543.4 ± 68.8	<0.01
	Multi para with mild preeclampsia (n=11)	372.2 ± 25.4	

Table 1: Shows characteristics of CffDNA among different groups.

Uric acid characteristics: (Table 2)

The mean level of uric acid in control group was 2.9 mg/dl compared with 4.7 mg/dl in the preeclampsia group. There was a statistically significant difference between preeclampsia patients compared with the control group; p<0.01. The mean level of uric acid was significantly higher among primigravida with severe preeclampsia; 749.6 mg/dl compared with 295.3 mg/dl in multiparous women with severe preeclampsia; p<0.01. Similarly, the mean level of uric acid was higher among primigravida with mild preeclampsia; 3.6 mg/dl compared with 3.4 mg/dl among the multiparous women with mild preeclampsia; however, there was no significant difference, p= 0.072.

	Uric acid Characteristics among different groups		
	Parameter	**Mean ± SD**	**p-value**
	Control group (n= 54)	2.9 ± 0.39	<0.01
Pre-eclampsia severity	**Pre-eclampsia patients (n=60)**	4.7 ± 1.2	
	Mild preeclampsia subgroup (n=22)	3.5 ± 0.5	
	Severe preeclampsia subgroup (n=38)	5.5 ± 1	
Gravidity and preeclampsia levels	**Primigravida with Severe preeclampsia (n=30)**	5.7± 0.92	<0.01
	Multi para with Severe preeclampsia (n=8)	4.7 ± 0.83	
	Primigravida with mild preeclampsia (n=11)	3.6 ± 0.5	0.072
	Multi para with mild preeclampsia (n=11)	3.4 ± 0.5	

Table 2: Shows characteristics of uric acid among different groups.

Discussion

Studying the control group showed a level of Cell Free Fetal DNA with a mean of 22.28 Genome equivalent/mL which is consisting with most of other studies as shown by Lo et al. (5) and Smid et al (12) with their mean levels of 76 and 24 Genome equivalent/mL respectively.

Same studies have shown mean levels of Cell Free Fetal DNA of 381 and 256 Genome equivalent/mL respectively denoting 6- and 12-folds increase, compared to our study having a mean of mild cases of 412 and 500 Genome equivalent/mL in severe cases which is showing an increase of 17 and 20-fold approximately. Our study has shown higher levels than those 2 studies yet lower than other studies as Swinkels at al (13) with a mean of 781 Genome equivalent/mL and 1599 Genome equivalent/mL by Zhong et al (8). But it should be clear that these two studies showed an increase of 6 to 7 folds of the mean level of Cell Free Fetal DNA in cases of pre-eclampsia comparing it with its equivalent in the control group, taking into consideration that some cases in these studies showed an increased level of Cell Free Fetal DNA by more than 25 folds compared to the level of Cell Free Fetal DNA of the same patient before and after the onset of pre-eclampsia.

Due to this great variation in the results using the Genome equivalent/mL as a unit for our research, we decided to focus on the

proportional increase in the level of the Cell Free Fetal DNA instead of its absolute value. It is important to denote that all of the studies showed an increase of the mean of the Cell Free Fetal DNA between the control and the study group of 5 to 20 folds (6–8).

The normal Uric acid level with pregnancy is still controversial. This is because it differs throughout pregnancy, being very low in its beginning (2.8 mg/Dl) and reaching 3.6 mg/dL in late pregnancy (14). Our study has shown results consistent with these numbers, having a level of uric acid of 2.9 mg/dL in the control cases, rising to 3.5 and 5.1 mg/dL in mild and severe cases of pre-eclampsia respectively.

There are no studies determining a specific value above which we can precise that Uric acid is abnormally elevated. That is why Uric acid level has prognostic value not a diagnostic one. Although mean serum uric acid values are elevated in women with preeclampsia, its clinical utility of identifying pre-eclampsia seems to be limited, however, a serum uric acid level of ≥5.5 mg/dL could identify women with preeclampsia (15).

For better understanding of our results, we decided to clarify if the levels of Cell Free Fetal DNA and Uric acid are influenced by other factors as parity.

An interesting finding was found in the interpretation of the levels of Cell Free Fetal DNA. We noted that the mean of mild and severe cases of primigravidas with pre-eclampsia was higher that its correspondent in multiparous women, though the mean level of Cell Free Fetal DNA in control multiparous is exceeding that of primigravidas in the control group (25 and 15 Genome equivalent/mL respectively). This should be further evaluated as most studies denoted that the fetal DNA is cleared very rapidly from maternal plasma, with a half-life in minutes (5). These results suggest that maternal plasma DNA analysis would not be complicated by fetal DNA persistence from a prior gestation, making false-positive results unlikely. Apart from the diagnostic importance of this observation, these data also raised questions with regard to the possible organ system(s) that is (are) responsible for the rapid clearance of circulating fetal DNA. Recent intriguing results suggest that some circulating fetal DNA may pass through the glomerulus and then be detectable in maternal urine (16). The incomplete understanding of this clearance gives rise to the importance of the re-evaluation of this topic.

No marked differences were noted between levels of Uric acid in primigravidas versus multiparous women in any of our 3 groups of patients.

It is clear that Uric acid level is not influenced by the factors of maternal age and parity, as most studies noted, it is surely influenced by the duration of pregnancy (14,17) which is not included in our studies as all of our cases were in their third trimester of pregnancy. On the other hand, it is not clear whether Cell Free Fetal DNA could be influenced by maternal age and parity as specific patterns were proven statistically in our study with no backup medical explanation raising the importance of more studies in this field.

Conclusion

To sum up our results, we concluded that Cell Free Fetal DNA is a better marker of pre-eclampsia than Uric acid. It is more sensitive as it rises with mild preeclampsia having even higher levels with severe preeclampsia. The uric acid shows no rise with mild preeclampsia. Different age groups did not affect the levels of both markers. Parity seems to have an effect on Cell Free Fetal DNA level as it has higher values in primigravida which needs further future evaluation.

Study Approval: this study protocol was reviewed and approved by the Fayoum university ethical committee, Fayoum, Egypt.

Consent to participate: Informed consent was obtained from all patients according to the ethical committee of Fayoum University.

Conflict of interest Statement: All authors declare that there are no conflicts of interest

Acknowledgements: Not Applicable

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data Availability:

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from B. H.M badranhm@yahoo.co.uk upon reasonable request.

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Tables Legends:

Table 1: Shows characteristics of CffDNA among different groups.

Table 2: Shows characteristics of uric acid among different groups.

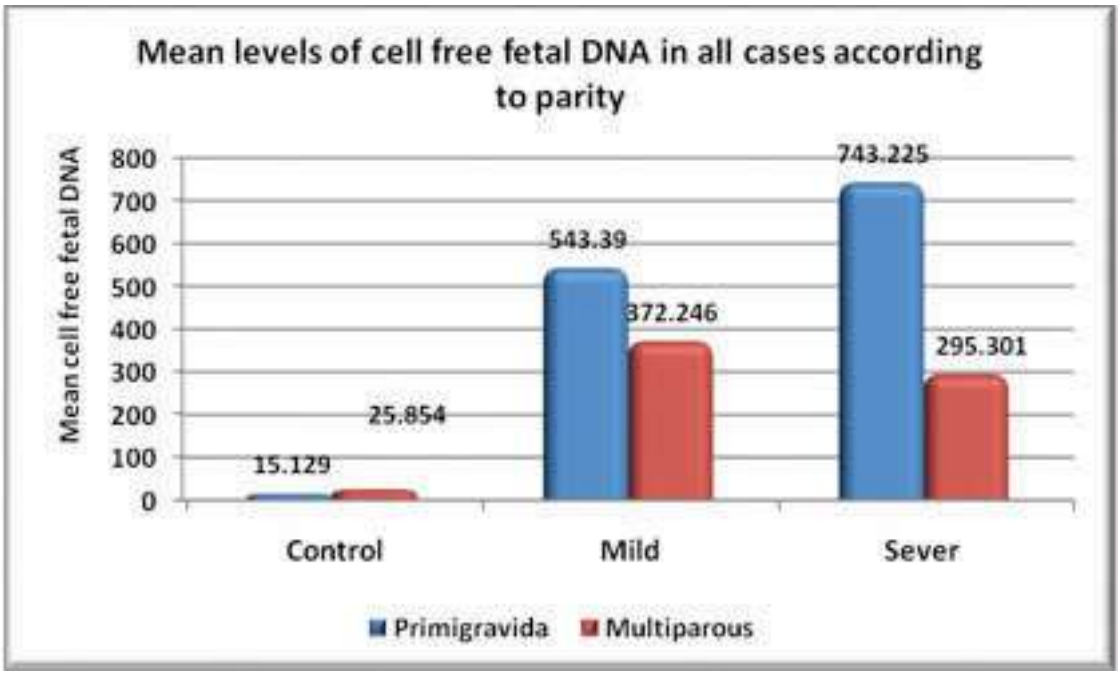

Figure 1 shows mean levels of cell free fetal DNA in all cases according to parity

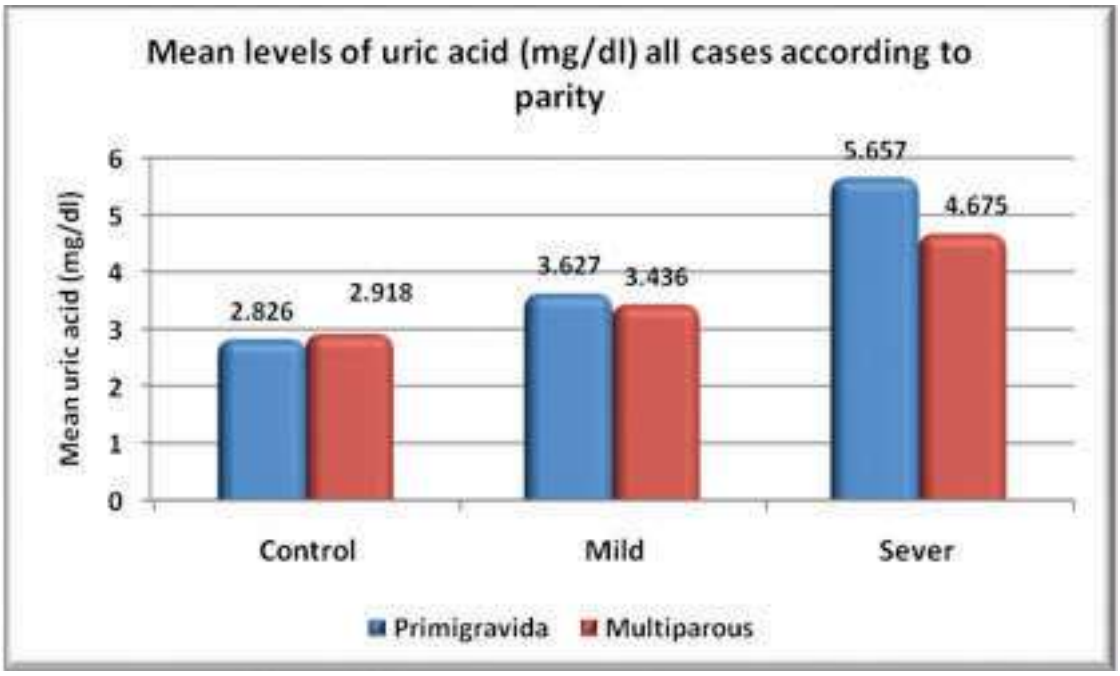

Figure 2 shows mean levels of uric acid (mg/dL) in all cases according to parity