Fetal DNA Clearance from Maternal Plasma Is Impaired in Preeclampsia

Tai-Wah Lau, Tse N. Leung, Lisa Y.S. Chan, Tze K. Lau, K.C. Allen Chan, Wing H. Tam, and Y.M. Dennis Lo

Background: Increased fetal DNA in maternal plasma/serum has been reported in pregnancies complicated by preeclampsia. We hypothesized that impaired clearance of fetal DNA might contribute, at least in part, to the above-mentioned phenomenon.

Methods: We studied 7 preeclamptic and 10 control pregnant women. All had male fetuses. Serial blood samples were obtained from before delivery to 6 h postpartum. Male fetal DNA in maternal plasma was measured by real-time quantitative PCR for the SRY gene on the Y chromosome.

Results: The median fetal DNA concentrations before delivery were significantly higher in the preeclamptic women than in the controls (521 vs 227 genome-equivalents/mL for preeclamptic and control women, respectively; Mann–Whitney rank-sum test, P = 0.017). The median fetal DNA concentrations at 6 h after delivery were also significantly different between the two groups (208 vs 0 genome-equivalents/mL for preeclamptic and control women, respectively; Mann–Whitney rank-sum test, P = 0.002). A first-order clearance model was found to best describe the kinetics of maternal plasma fetal DNA clearance. Moreover, we observed a significant difference in the median apparent clearance half-lives of fetal DNA between the preeclamptic women (114 min) and controls (28 min; Mann–Whitney rank-sum test, P = 0.007).

Conclusions: This study represents the first documentation of impaired fetal DNA clearance from maternal plasma in preeclampsia. Such an abnormality in circulating DNA clearance may also be present in other medical conditions associated with quantitative aberrations in circulating DNA concentrations.

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Preeclampsia is one of the leading causes of maternal and fetal mortality and morbidity in the developed world. Despite numerous studies, the pathogenesis remains unclear (1). Previously, our group has demonstrated that the median concentration of circulating fetal DNA in maternal serum of preeclamptic women is five times higher than that of pregnant controls matched for gestational age (2). Similar results were subsequently reported by other groups (3, 4). Theoretically, this increase in circulating fetal DNA concentration could result from either one or a combination of two processes: (a) increased liberation and (b) reduced clearance of fetal DNA from the maternal plasma.

With regard to fetal DNA clearance, our group has previously reported the rapid clearance of fetal DNA from maternal plasma, with a mean half-life of 16.3 min (range, 4–30 min), in women with no medical disease or antenatal complications (5). In this study, we used a similar approach to investigate whether there was any evidence of impaired fetal DNA clearance from the plasma of women with preeclampsia.

Materials and Methods

SAMPLE COLLECTION
Pregnant women attending the Department of Obstetrics and Gynaecology at the Prince of Wales Hospital (Shatin, Hong Kong) were recruited with informed consent. Approval was obtained from the Research Ethics Committee of The Chinese University of Hong Kong.

Two patient groups were studied: (a) a preeclamptic group and (b) a control group. Preeclampsia was diagnosed on the basis of a sustained increase in diastolic blood pressure to >90 mmHg on two or more occasions at least 4 h apart, with the presence of significant proteinuria in women with no history of hypertension. Significant proteinuria was defined as proteinuria >0.3 g/day or ≥2+ on dipstick testing in two clean-catch midstream

Departments of 1 Chemical Pathology and 2 Obstetrics and Gynaecology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR.

*Address correspondence to this author at: Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR. Fax 852-2194-6171; e-mail loym@cuhk.edu.hk.

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urine specimens collected at least 4 h apart. Severe disease was diagnosed when the systolic blood pressure was >160 mmHg or the diastolic blood pressure was >110 mmHg on two occasions at least 4 h apart, or when there was proteinuria >5 g/day or 3+ on dipstick testing, or the women presented with oliguria, pulmonary edema, impaired liver function, hepatic rupture, thrombocytopenia, HELLP (hemolysis, increased liver enzymes, low platelets) syndrome, or cerebral signs, including headache, blurred vision, or altered consciousness. The control group included pregnant women with no preexisting medical diseases or antenatal complications. Both groups were delivered by cesarean section. The indications for elective cesarean section in the control group were either elective repeat or breech presentation. Because women with uncomplicated pregnancies did not undergo cesarean section during preterm gestation, there was a difference in the median gestational age of the preeclamptic and control groups, namely, 32 and 38 weeks, respectively. However, as will be explained in the Discussion, this should not affect the interpretation of our results.

**Sample Preparation**
Maternal antecubital venous blood (5–10 mL) was collected into tubes containing EDTA at the following time points: before delivery and at 5, 15, 30, 45, 60, 120, and 360 min after delivery of the babies by cesarean section. Blood samples were processed as described previously (6), with the plasma stored separately at −20 °C.

**DNA Extraction**
DNA was extracted from the plasma by use of the QIAamp DNA blood mini reagent set (Qiagen) according to the “blood and body fluid protocol” as recommended by the manufacturer (7). We used 200–800 μL of plasma per column for DNA extraction. The exact amount used was documented to enable calculation of the target DNA concentration.

**Real-time Quantitative PCR**
Real-time quantitative PCR analysis was performed with a PE Applied Biosystems 7700 Sequence Detector, with strict precautions against PCR contamination as described in detail elsewhere (7). Amplification primers and fluorescent probes, designed to detect the SRY gene on chromosome Y, were used for detecting circulating fetal DNA (7). As a control for the amplifiability of plasma DNA, all samples were subjected to a TaqMan assay for the β-globin gene on chromosome 11.

**Calculation of Half-lives of DNA Clearance**
In most of the patients, the decay of fetal DNA followed a transient increase after delivery. The half-lives were calculated using data from the peak fetal DNA concentrations to the first non-zero trough concentration. The slope (-k) of the resulting plot of the natural logarithms against time was calculated by linear regression using SigmaStat 2.0 software (SPSS). The half-life was then computed using the following equation:

$$\text{Half-life} = \frac{0.693}{k}$$

**Results**
Of the seven preeclamptic women recruited, five had severe and two had mild preeclampsia. The median circulating fetal DNA concentration was increased in the preeclamptic group (521 genome-equivalents/mL; interquartile range, 311–914 genome-equivalents/mL; range, 274–3089 genome-equivalents/mL) relative to the control group (227 genome-equivalents/mL; interquartile range, 148–338 genome-equivalents/mL; range, 38–468 genome-equivalents/mL; Mann–Whitney rank-sum test, P = 0.017). The data are shown in Fig. 1. Because of the relatively small number of cases, no further subgroup analysis was performed.

Significant differences in fetal DNA concentrations were observed at 6 h postpartum between the preeclamptic and control groups (Fig. 1). The median fetal DNA concentrations at 6 h postpartum in the preeclamptic and control groups were 208 genome-equivalents/mL (interquartile range, 74–405 genome-equivalents/mL; range, 60–779 genome-equivalents/mL) and 0 genome-equivalents/mL (interquartile range, 0–12 genome-equivalents/mL; range, 0–111 genome-equivalents/mL), respectively (Mann–Whitney rank-sum test, P = 0.002). In 7 of 10 controls, fetal DNA reached 0 genome-equivalents/mL at
6 h postpartum; fetal DNA concentrations in three of these controls reached 0 genome-equivalents/mL at 2 h postpartum. In contrast, in the preeclamptic group, none had fetal DNA concentrations of 0 genome-equivalents/mL at 6 h postpartum.

To obtain a more detailed picture of the kinetics of fetal DNA clearance in preeclamptic and control women, multiple samples between before delivery and 6 h after delivery were analyzed. Representative cases illustrating the dynamics of fetal DNA clearance in the preeclamptic and control groups are shown in Fig. 2. We compared the closeness of fit of these data into either a zeroth- or first-order model. The rationale of using these two models will be discussed in the Discussion. For this analysis we focused on the clearance pattern from the peak plasma fetal DNA concentration. For zeroth-order clearance, one would expect a linear relationship when maternal plasma fetal DNA concentration was plotted against time. The closeness of fit was analyzed by linear regression analysis, and the \( R^2 \) values [denoted \( R^2 \) (linear)] were documented. For first-order clearance, one would expect a linear relationship when the natural logarithm of the fetal DNA concentration was plotted against time. The closeness of fit was analyzed by linear regression analysis (using the natural logarithm of fetal DNA concentration) and the \( R^2 \) values [denoted \( R^2 \) (log)] were documented. One control pregnancy case (case 348M) was excluded from this analysis because the fetal DNA concentration fell to 0 genome-equivalents/mL by 30 min.

In 15 of the 16 cases analyzed, the \( R^2 \) (log) values were closer to 1 than the corresponding \( R^2 \) (linear) values. The median \( R^2 \) (log) value was 0.86 (interquartile range, 0.73–0.96; range, 0.23–0.99), whereas the median \( R^2 \) (linear) value was 0.74 (interquartile range, 0.50–0.87; range, 0.37–0.98). A comparison of the paired \( R^2 \) (log) and \( R^2 \) (linear) values indicated that this difference was statistically significant (Wilcoxon signed-rank test, \( P = 0.001 \)). This analysis thus indicated that a first-order clearance model was a better description of fetal DNA clearance than a zeroth-order model, and the former was used in subsequent analysis.

Having demonstrated that a first-order clearance model is a better description of fetal DNA clearance, we went on to determine the apparent half-life of fetal DNA clearance for each case. The clearance rate of fetal DNA was significantly different between the two groups, with median apparent clearance half-lives of 114 min in the preeclamptic group (interquartile range, 81–168 min; range, 46–210 min) and 28 min in the control group (interquartile range, 16–47 min; range, 7–114 min; Mann–Whitney rank-sum test, \( P = 0.007 \)).

**Discussion**

Previous data from our group and others have shown that preeclampsia is associated with an increase in fetal DNA concentrations in maternal plasma/serum (2–4). In this report, we present evidence that suggests that impairment in fetal DNA clearance from maternal plasma represents one of the mechanisms that produces this phenomenon.

We analyzed the kinetics of fetal DNA clearance and showed that a first-order clearance model provides a reasonable description of the present data set. This first-order kinetic model also fits the data previously generated in an animal model of fetal DNA clearance (8). However, the median of \( R^2 \) (log) was only 0.86. There are at least two explanations for this observation. One explanation is that this may imply that even the first-order kinetics model provides only a partial, albeit reasonably satisfactory, kinetic description of the clearance of fetal DNA from maternal plasma. In this regard, it is important to realize that the process of DNA clearance is likely to be complex and to involve more than one mechanism. The second explanation is that the imprecision of the current generation of DNA extraction and PCR amplification technologies may lead to degradation in the quality of the data set, which will adversely affect the subsequent mathematical analysis.

Clearance of fetal DNA from maternal plasma in healthy pregnant women has been shown to be very rapid, with a mean apparent half-life of 16.3 min (5). In the present study, the majority of the healthy controls (7 of 10), who had no antenatal complications, had undetectable circulating fetal DNA by 6 h postpartum. Indeed, three of these healthy women had undetectable fetal DNA at 2 h postpartum. The median apparent half-life for fetal DNA clearance was 28 min in these healthy women.

In contrast, the preeclamptic women had a much slower clearance rate, as evidenced by a median apparent fetal DNA clearance half-life that was four times longer than the value for the healthy women (114 min in the preeclamptic group vs 28 min in the control group). In addition, none of the preeclamptic women had completely cleared their plasma of fetal DNA by 6 h postpartum. On the basis of a first-order clearance model, a fourfold increase in clearance half-life would increase the steady-state fetal DNA concentration fourfold, assuming that there is no change in the liberation rate of fetal DNA into maternal plasma. It is important to note that our study, in providing one mechanism leading to an increase in circulating fetal DNA concentration in maternal plasma, does not necessarily exclude other possible mechanisms. An additional mechanism is the possible increased liberation of fetal DNA into the maternal circulation, which should be explored in future studies.

Because the current data set was generated based on pregnant women who had undergone cesarean section, it still must be formally shown that the clearance pattern after vaginal delivery is the same. However, it is important to note that both the preeclamptic and control women in the present study had undergone cesarean section and that this variable had therefore been controlled for in the current study.

Tsumita and Iwanaga (8) reported that >90% of in-
jected calf thymus DNA was removed from the circulation of mice within 30 min and that the major organ of uptake was the kidney. The liver has been suggested by Emlen and Mannik (9) to be the main organ for the removal of circulating DNA. In this regard, it is relevant to note that preeclampsia is a multisystem disorder that is...

Fig. 2. Dynamics of fetal DNA clearance in preeclamptic and control women. (A), preeclamptic women (cases 338M, 343M, and 355M); (B), controls (cases 348M, 349M, and 352M). The x axis represents the time from delivery, with 0 as the time of delivery. The y axis represents the fetal DNA concentrations in genome-equivalents/mL.
associated with damage and dysfunction of many organ systems, including the liver and kidney (1). It is thus possible that such organ damage may produce the observed abnormalities in fetal DNA clearance.

In the current study, the 2.3-fold difference in maternal plasma fetal DNA concentrations between the preeclamptic and control groups before delivery was less than the 5-fold previously reported by our group (2). One explanation may be the difference in gestational age between the two groups. The median gestational age of the preeclamptic women was 32 weeks (interquartile range, 30–37 weeks) and that of the controls was 38 weeks (interquartile range, 37.7–38.3 weeks). This difference was attributable to the fact that healthy pregnant women with uncomplicated pregnancies would not be delivered by elective cesarean section before term. It was previously reported that the concentration of fetal DNA is significantly higher in the late gestational period compared with early gestation (7). Therefore, the higher gestational age of the control group, with its bias toward higher circulating fetal DNA concentrations, may contribute to a smaller difference in fetal DNA concentration between the two groups compared with our earlier study with gestational age matching between the preeclamptic and control groups (2).

Recently, Invernizzi et al. (10) presented data indicating that fetal DNA may persist in maternal plasma for decades after pregnancy. The results presented by Invernizzi et al. are particularly puzzling because the concentrations of plasma fetal DNA in many of these nonpregnant women (140 ± 140 genome-equivalents/mL) (10) were higher than those in many pregnant women (7). Apart from the apparent contradiction to the data presented here and previously by our group (5), the data presented by Invernizzi et al. (10) are also in contradiction to the large number of reports by various groups on the very high accuracy of prenatal genetic analysis based on maternal plasma analysis (11–14). In other words, such accuracy would not have been possible if fetal DNA were to persist in a significant proportion of women after delivery. Indeed, Costa et al. (14) specifically studied 27 women who were carrying a female fetus in a current pregnancy but who had had at least one previous pregnancy involving a male fetus and found no false-positive Y-chromosomal DNA signal in the maternal plasma in the current pregnancy. Similarly, Faas et al. (12) also observed no evidence of false-positive prenatal fetal RhD status determination in women who were carrying a RhD-negative fetus in the current pregnancy but who had previously had a RhD-positive child. One possible explanation for the data presented by Invernizzi et al. (10) might be the incomplete removal of nucleated cells from the “plasma” fraction of blood (6, 15). Another possible explanation for the results reported by Invernizzi et al. (10) is the inadvertent introduction of contamination. On the basis of the above discussion, we believe that maternal plasma fetal DNA is cleared very rapidly from the maternal circulation, and the data presented here further emphasize this rapidity.

In conclusion, our study is the first documentation of the abnormality of fetal DNA clearance in maternal circulation in preeclampsia. These findings may stimulate further studies on the underlying pathology of the abnormal clearance of plasma DNA. Furthermore, analogous abnormality in circulating DNA clearance may also be present in other medical conditions associated with quantitative aberration in circulating DNA concentrations (16).

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