On the basis of clinical information, samples were classified according to progression/recurrence, no change, or regression/response. The sensitivity and specificity of Elecsys CA 15-3 for detection of recurrence were evaluated in the subgroup of patients treated for UICC stage II or III breast cancer with no evidence of disease at the beginning of follow-up (Table 1). Only the first recurrence was rated per patient. Increases occurring only before or at the time of recurrence were evaluated as true positive. Patients with an increase of values at the end of follow-up were excluded when later clinical information was not available. Analogously, progression was evaluated in patients with metastatic disease at the beginning of follow-up, and response to treatment was tested in patients with metastatic breast cancer from the beginning of or during follow-up (Table 1). The findings confirm the previously described suitability of CA 15-3 for detection of recurrences (10, 11) and for analyzing the course of disease and treatment response in patients with metastases (12).

The Elecsys CA 15-3 test has an imprecision (CV) <7.5%, is linear at 30–300 kilounits/L, and has resistance to various types of interferents. Diagnostic sensitivity is comparable to that of the AxSYM method as is detection of recurrence/response to therapy. Despite the clinical comparability of the Elecsys CA 15-3 and AxSYM CA 15-3 methods in the evaluation of marker changes in individuals during follow-up, to switch from one method to another requires parallel determination by both methods because results for individual patients may show method-dependent differences.

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References


Cell-free Fetal DNA Is Increased in Plasma of Women with Hyperemesis Gravidarum, Akihiko Sekizawa,* Yumi Sugito, Mariko Iwasaki, Akira Watanabe, Masatoshi Jimbo, Shinichi Hoshi, Hiroshi Saito, and Takashi Okai (Department of Obstetrics and Gynecology, Showa University School of Medicine, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan; * author for correspondence: fax 81-33784-8355, e-mail sekizawa@d8.dion.ne.jp)

Nausea and vomiting of moderate intensity are especially common complaints in early gestation. Hyperemesis gravidarum (HG), which is the most severe form of this disorder, occurs in 0.5–2% of pregnancies and is associated with weight loss, ketonemia, ketonuria, electrolyte imbalance, dehydration, and possible hepatic and renal damage.

Recently, the presence of cell-free fetal DNA in maternal plasma or serum has been demonstrated (1). It has been reported that the concentration of fetal DNA in maternal plasma is increased in pregnancies involving preterm delivery (2), preeclampsia (3, 4), and trisomy 21 (5, 6). In the present study, we evaluated the concentration of fetal DNA in maternal plasma in HG patients.

Pregnant women diagnosed with HG and admitted to Showa University Hospital were recruited. In this study, HG was defined according to the following criteria: (a) persistent nausea and vomiting; (b) weight loss (>2 kg); and (c) ketonuria (>2+, urine dipstick). Maternal blood samples were obtained at the time of admission from 35 patients with HG carrying a single fetus between 7 and 16 weeks of gestation. Sixteen of 35 blood samples collected from the HG patients carrying a male fetus were used in the present study (median period of gestation, 10.0 weeks; range, 7–16 weeks). Twenty-three pregnant women who were carrying a male fetus and did not have any complaints of nausea or vomiting were also enrolled, as were a control group (median period of gestation, 11.0 weeks; range, 8–15 weeks), and their maternal age and gestational age were matched with the HG group. Written informed consent was received from all participants, and approval for this study was obtained from the ethics committee of the University.

Maternal blood samples (10 mL) collected into tubes containing EDTA were separated by centrifugation at 3000g within 3 h of collection. After an additional centrifugation, the supernatant was collected into fresh tubes and stored at −20 °C until further processing.

DNA was extracted from 1.5 mL of the plasma samples using the QIAamp Blood Mini Kit (Qiagen) and was eluted from columns with 50 µL of water. Subsequent PCR quantification of the Y-chromosome-
specific DYS14 sequence in maternal plasma was performed with a LightCycler (Roche Diagnostics) according to protocols described previously (7). The concentration of male DNA present in the plasma sample was determined by a typical dilution curve of male genomic DNA. In the same maternal plasma, we also measured the human chorionic gonadotropin-β (hCGβ) concentration using the Ball ELSA Free Beta HCG reagent set (CIS Bio International). Statistical analysis was performed by StatView software (Ver. 5.0). Significance was established at $P < 0.05$.

The fetal DNA concentrations of all cases are shown in Fig. 1. The median concentrations of fetal DNA in plasma of HG patients and controls were 48.8 genome-equivalents/mL (range, 21.6–311.2 genome-equivalents/mL) and 31.4 genome-equivalents/mL (range, 6.6–59.6 genome-equivalents/mL), respectively. The median concentration of fetal DNA in HG patients was significantly higher than in controls (Mann–Whitney U-test, $P < 0.05$). Four HG patients who showed a high fetal DNA concentration (>100 genome-equivalents/mL) had severe symptoms of HG. The median concentration of hCGβ in maternal plasma in HG patients was 110 μg/L (range, 35–371 μg/L), which was higher than that in the control group (median concentration, 72 μg/L; range: 13–269 μg/L), but this difference was not statistically significant. The concentrations of the DYS14 gene and hCGβ in maternal plasma were correlated significantly (Pearson’s correlation coefficient, $r = 0.788$; $P < 0.001$).

Although the origin of fetal DNA in maternal plasma is not clear, it is thought that the majority of fetal DNA is derived from destroyed trophoblasts at the feto-maternal interface. The pathogenesis of HG is likely related to a mechanism that increases fetal DNA in maternal plasma. The pathogenesis of HG may be related to immunologic interactions of mother and fetus. The mother does not reject the fetus because trophoblasts express HLA-G antigen (8). However, if the maternal immune system completely tolerates the fetus, growing trophoblasts might invade the uterine muscle. Activated natural killer and cytotoxic T cells in the uterine decidua have also been reported to protect the mother from the invasion of trophoblasts (9). One study reported that functional activation of natural killer and cytotoxic T cells is more prominent in the uterine decidua in patients with HG than in women with normal pregnancies (10). The over-expression, therefore, may be responsible for the onset of HG, although adequate activation of the maternal immune system is essential for maintenance of pregnancy. Therefore, the increased fetal DNA in maternal plasma might be associated with overactivation of maternal immune cells. Thus, the pathogenesis of HG could be related to an inadequate immune response in the process of establishing maternal immune tolerance of the maternal–fetal semiallograft.

In this study, the fetal DNA concentration in maternal plasma was closely correlated with the plasma hCGβ concentration. HG is reportedly related to the amount of hCG produced by trophoblasts (11), and examination of the relationship between hCGβ and fetal DNA in maternal plasma would help to clarify this issue.

In conclusion, we reported here the first description that fetal DNA in maternal plasma is increased in patients with HG and that the concentration of fetal DNA is significantly correlated with the concentration of plasma hCGβ. It is suggested that the pathogenesis of HG could be related to an abnormality of the immune interaction between the mother and fetus.

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References

Fig. 1. Fetal DNA concentrations in maternal plasma of patients with HG (HG) and of normal pregnant women (Normal).