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Low expression of soluble human leukocyte antigen G in early gestation and subsequent placenta-mediated complications of pregnancy

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Abstract

Aim: Abnormal placentation is a common pathogenic mechanism of many placenta-mediated complications of late pregnancy, including pre-eclampsia, fetal growth restriction, stillbirth, and placental abruption. During successful placentation, the trophoblast (which is a semi-allograft) is not rejected by decidual immune cells because of maternal immune tolerance, mainly induced by human leukocyte antigen G (HLA-G). Deficient HLA-G expression seems to be associated with the development of complications of pregnancy. The aim of this study was to determine whether low soluble HLA-G (sHLA-G) levels in maternal blood at the beginning of pregnancy may be associated with subsequent placenta-mediated complications.

Methods: For this retrospective case–control study, 117 cases of placenta-mediated complications of pregnancy and 234 controls with uneventful pregnancy were selected. Plasma sHLA-G levels were measured at 11–13 weeks' gestation by the enzyme-linked immunosorbent assay method in blood samples previously obtained at first-trimester prenatal screening for chromosomal fetal abnormalities.

Results: Women who subsequently developed placenta-mediated complications had significantly lower sHLA-G levels at the beginning of pregnancy (median, 43.08 IU/mL) than controls (median, 49.10 IU/mL; $P = 0.008$). An sHLA-G level lower than 43.50 IU/mL at the end of the first trimester was associated with a twofold increased risk of developing a pregnancy complication (odds ratio, 1.82; 95% confidence interval, 1.22–2.73). The strongest association, although only moderately strong, was observed with severe pre-eclampsia (odds ratio, 2.66; 95% confidence interval, 1.08–6.56).

Conclusion: Placenta-mediated complications of pregnancy may be associated with low sHLA-G levels in the first trimester, suggesting a potential role of sHLA-G in the early stages of placentation.

Key words: abruptio placentae, HLA-G, placentation, pre-eclampsia, pregnancy.

Introduction

One of the most relevant aspects of normal pregnancy is maternal immune tolerance to trophoblast invasion. During placentation, decidual immune cells do not reject the trophoblast, which is a semi-allograft for the mother.[1] Maternal immune tolerance is thought to be induced by the trophoblast itself, and the production of human leukocyte antigen G (HLA-G) by trophoblast cells seems to play a key role in this pathway. Establishment of placentation is facilitated by early interactions between trophoblast cells and maternal immune cells, mostly innate decidual natural killer (dNK) cells, along with macrophages, T lymphocytes, and dendritic cells.[2–5]

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HLA-G directly inhibits the antigen-specific cytolytic function of NK cells and blocks the maturation of CD4+ T cells and dendritic cells. HLA-G also exerts a long-term tolerogenic function by activating regulatory cells, such as antigen-presenting cells (APC), CD4+, and CD8+ T cells.[6]

Many complications of late pregnancy, including pre-eclampsia, fetal growth restriction, stillbirth, and placental abruption, share a common underlying pathogenesis due to abnormal placentation. A deficiency in the expression of HLA-G in early pregnancy may be associated with abnormal placentation and the later development of placenta-mediated complications of pregnancy.[7–13] The aims of this study were: (i) to analyze the association of low maternal plasma levels of HLA-G in early pregnancy with the development of placenta-mediated complications, such as pre-eclampsia, fetal growth restriction (FGR), stillbirth, and abruption; and (ii) to determine a threshold value of maternal plasma HLA-G suggestive of increased risk for pregnancy complications.

Methods

This retrospective case–control study was conducted on a cohort of women who delivered at the Department of Obstetrics and Gynaecology, Sant'Anna Hospital, University of Turin, Turin, Italy, between 2012 and 2014. A total of 117 cases with placenta-mediated complications of pregnancy (pre-eclampsia, $n = 65$; fetal growth restriction, $n = 37$; stillbirth, $n = 8$; and placental abruption, $n = 7$) were selected and matched 2:1 for maternal age to 234 controls (women with uneventful pregnancy). Matching was made for each type of placenta-mediated complication.

Pre-eclampsia was diagnosed according to the definition of the American College of Obstetricians and Gynecologists;[14] it was severe in 33 of the 65 cases. Fetal growth restriction was defined as a fetal weight under the fifth percentile for gestational age, based on standardized tables for parity, gestational age, and fetal sex.[15] Stillbirth was defined as intrauterine death after 20 weeks of gestation. Placental abruption was defined as partial or full placenta detachment before delivery, as documented on placental histology of the tissue sample.[16] Only cases with severe placental abruption (vaginal bleeding or concealed hemorrhage and fetal distress requiring immediate delivery) were included. Subjects with potential confounders likely to have affected the outcome of pregnancy (twin pregnancy, maternal chronic diseases, and fetal chromosomal abnormalities or malformations) were excluded from the analysis.

Data on pregnancy outcome were obtained from the hospital's computerized obstetric database. The database contains information on maternal demographics, obstetric history, pregnancy complications, labor and delivery events, and short-term maternal and neonatal outcome. The hospital charts of the index pregnancies of the eligible subjects were reviewed to confirm the diagnosis. The characteristics of the subjects are shown in Table 1.

Maternal plasma HLA-G levels were measured in samples obtained at prenatal screening at the beginning of the index pregnancies and then stored at −80°C until the time of assay, after delivery. At our department, blood samples obtained at prenatal screening during the first trimester are routinely stored for 1 year, and pregnant women are asked to sign an informed consent to future use of data and samples for research. The study was approved by the local Ethics Committee (10 June 2011, protocol n. 27 352/c 28.2).

Quantitative determination of plasma HLA-G levels was performed by enzyme-linked immunosorbent assay using a murine monoclonal antibody to human sHLA-G (MEM-G 9), which recognizes both shed HLA-G1 and soluble HLA-G5 isoforms of HLA-G (Biovendor GmbH). The lower analytical limit of detection of HLA-G is 0.6 U/mL. HLA-G is characterized by a post-transcriptional alternative splicing of the mRNA from the single HLA-G gene. HLA-G1 represents the full-length isoform, whereas the other isoforms are formed by out-splicing of exons. This results in seven isoforms, four of which are membrane-bound (HLA-G1, HLA-G2, HLA-G3, and HLA-G4), and three of which are soluble (sHLA-G5, sHLA-G6, and sHLA-G7). In the presence of metalloproteinases, HLA-G1 can be shed from the surface by proteolytic cleavage, also

Table 1 Demographic and clinical characteristics of the subjects

Characteristics	Cases ($n = 117$)	Controls ($n = 234$)
Maternal age (years), mean ± SD	31.7 ± 4.8	31.7 ± 4.9
Primiparity, *n* (%)	82 (70)	162 (70)
Gestational age at delivery (weeks), mean ± SD	37.3 ± 3.6	39.6 ± 1.2
Birthweight (g), mean ± SD	2434 ± 882	3220 ± 395

SD, standard deviation.

resulting in a soluble molecule. The calibrators and samples were incubated at 2–8°C for 16–20 h in micro-wells pre-coated with monoclonal antibody anti-HLA-G. After washing, a monoclonal antibody anti-human beta2-microglobulin conjugated to horseradish peroxidase was added, and the samples were stirred at 300 r.p.m. for 60 min at room temperature. Following washing, the horseradish peroxidase conjugate was reacted with the appropriate substrate; after 25 min, an acid solution was added at room temperature to stop the reaction. Absorbance of the resulting yellow color at 450 nm was measured against a reference curve set at 630 nm. The absorbance was proportional to the total concentration of shed HLA-G1 and soluble HLA-G5 (sHLA-G). A calibration curve was plotted by comparing the observed absorbance values with the concentrations of the calibrators, and the sHLA-G concentrations of the samples were established according to the reference curve. Samples in which the sHLA-G concentration exceeded 200 IU/mL (10% in both groups) were re-analyzed after diluting the sample by a factor of 1:4.

For the statistical analysis, continuous variables are expressed as mean and standard deviation or median and interquartile range as a measure of variability. Because 10% of the subjects had sHLA-G levels outside the curve range we used a non-parametric Wilcoxon test for paired data. A P-value < 0.05 was considered as statistically significant. In order to identify a cut-off level of sHLA-G suggestive of increased risk for placenta-mediated pregnancy complications, receiver–operator curve (ROC) analysis was performed. Finally, the relative and absolute risk of developing pregnancy complications according to the sHLA-G values was estimated by logistic model.[17] All analyses were performed using R software version 2.14.[18]

Results

The maternal plasma sHLA-G concentrations at 11–13 weeks' gestation showed a high variability (range, 4–500 IU/mL) in both the cases and the controls**.** Overall, the plasma sHLA-G concentration was significantly lower in the cases than in the controls (P = 0.008). Subgroup analysis according to type of pregnancy complication showed that sHLA-G concentrations were lower in the groups of patients with pre-eclampsia (both mild and severe), stillbirth, and placental abruption, although statistical significance was reached only in the group with placental abruption (P = 0.049) (Table 2). The box-plots of individual values of sHLA-G in the different groups are presented in Figure 1.

The sHLA-G threshold value with the best sensitivity (54.1%) and specificity (60.7%) to discriminate subjects at higher risk of developing pregnancy complications, calculated by ROC analysis (area under curve, 58.5%), was 43.50 IU/mL. The odds ratio (OR) analysis according to this cut-off value showed that an sHLA-G concentration lower than 43.50 IU/mL at the beginning of pregnancy was associated with a significantly increased risk of developing one of the pregnancy complications included in the analysis (OR, 1.89; 95% confidence interval [CI], 1.22–2.73). The strongest association was observed with severe pre-eclampsia (OR, 2.66; 95%CI, 1.08–6.56; Table 2). Figure 2 shows the inversely proportional correlation between sHLA-G values at first trimester of pregnancy and the risk of

Table 2 HLA-G value (IU/mL) in cases and controls

Pregnancy complications	Cases' median HLA-G IU/mL (first–third quartile)	Controls' median HLA-G IU/mL (first–third quartile)	P	OR (95%CI)
Overall complications (n = 117)	43.08 (34.69–61.47)	49.10 (37.06–64.10)	0.008	1.89 (1.20–2.97)
Pre-eclampsia (n = 65)	41.30 (33.53–59.83)	46.05 (35.25–62.61)	0.13	2.12 (1.15–3.91)
Severe pre-eclampsia (n = 33)	40.70 (33.53–53.29)	45.40 (33.22–57.77)	0.234	2.66 (1.08–6.56)
Mild pre-eclampsia (n = 32)	42.99 (34.69–67.82)	47.85 (37.57–64.55)	0.376	1.77 (0.75–4.17)
Placental abruption (n = 7)	33.31 (28.03–38.61)	52.62 (33.59–73.67)	0.049	8.13 (0.72–91.61)
Fetal growth restriction (n = 37)	55.88 (37.90–65.91)	52.43 (42.70–67.00)	0.997	1.41 (0.58–3.43)
Stillbirth (n = 8)	46.08 (37.15–53.79)	56.73 (40.04–67.27)	0.338	1.03 (0.18–6.03)

OR were calculated according to a cut-off value of 43.5 IU/mL obtained by ROC analysis, and adjusted for maternal age and parity. CI, confidence interval; HLA-G, human leukocyte antigen G; OR, odds ratio.

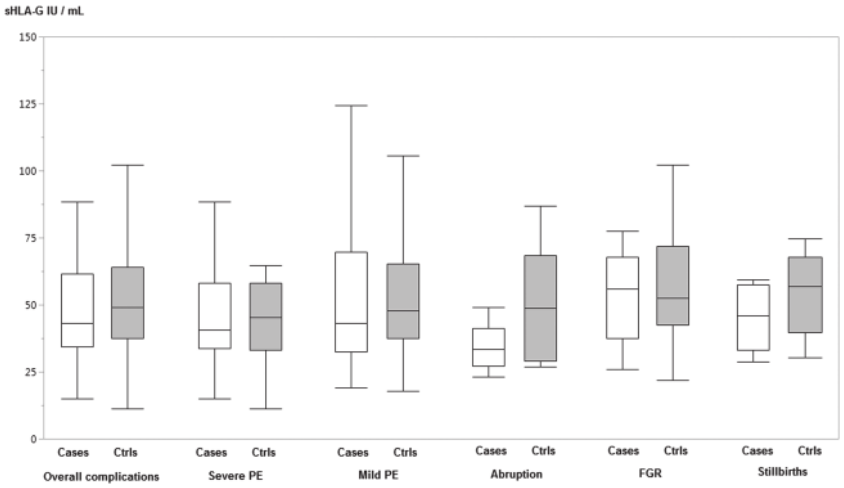

Figure 1 Box-plots of individual values of soluble human leukocyte antigen G (sHLA-G) in the different groups of cases and respective controls. Ctrls, controls; PE, pre-eclampsia; FGR, fetal growth restriction.

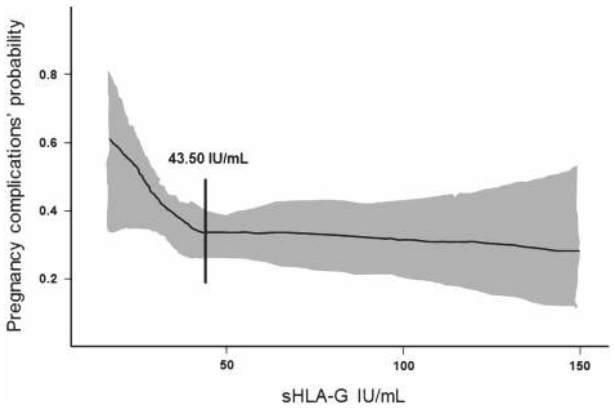

Figure 2 Probability to develop one pregnancy complication depending on soluble human leukocyte antigen G (sHLA-G) values calculated using a logistic regression model.

developing one of the pregnancy complications included in the analysis. The correlation was lost when sHLA-G levels were above 43.50 IU/mL.

Discussion

Non-classical major histocompatibility complex class I HLA-G, specifically expressed at the maternal–fetal interface, has been characterized as an immunosuppressor that may play an important role in the induction and maintenance of immunological tolerance of the fetus by the mother.[19] Low HLA-G expression is associated with pregnancy complications, particularly pre-eclampsia and recurrent miscarriages.[20] The expression of HLA-G by the embryo, as well as the presence of high levels of soluble HLA-G (sHLA-G) in maternal blood circulation, is associated with better pregnancy rates after infertility treatments.[21,22] Another possible role of HLA-G has been proposed in the context of remodeling of uterine spiral arteries during normal placentation.[23] Many complications of late pregnancy, including pre-eclampsia, fetal growth restriction, stillbirth, and placental abruption, are thought to share abnormal placentation as a common underlying pathogenic mechanism.[24] To date, few studies have assessed the correlation between maternal plasma sHLA-G levels and placenta-mediated complications. In most studies, HLA-G levels were assessed during overt complications. In pre-eclamptic patients, HLA-G levels were found to be significantly lower than in controls or even undetectable.[8–10] Moreover, Yie *et al.*[9] observed that both serum and placental HLA-G levels were markedly decreased during pre-eclampsia compared with normal pregnancy, and that there was a significantly direct correlation between serum and placental levels of HLA-G. This finding strengthens the idea that the trophoblast is the main source of sHLA-G in maternal plasma. Steinborn *et al.*[12] found that sHLA-G levels in women with placental abruption were more than three times lower than in normal pregnancy at the same gestational age.

What our study adds to the current knowledge is that sHLA-G levels at the beginning of pregnancy are lower in women who subsequently develop placenta-mediated complications, particularly severe pre-eclampsia and abruption, than in women with uneventful pregnancies. Our results are in agreement with those of Yie *et al.*[11] We observed that sHLA-G levels lower than 43.5 IU/mL at the end of the first trimester were associated with a twofold increase in

the risk of developing placenta-mediated complications during the later course of pregnancy, particularly severe pre-eclampsia, which is known to be linked to abnormal placentation, and placental abruption. The strongest association was for severe pre-eclampsia (OR, 2.66); however, it was only a moderately strong association. The association was much less robust for the other complications considered. As previously observed by Yie *et al.*,[11] there was considerable individual variation in sHLA-G levels among normal controls and patients with later placenta-mediated complications, which probably affected statistical significance in subgroups analysis, together with the small sample size of subgroups. Although sHLA-G levels were particularly high in 10% of the subjects, clustering around 500 IU/mL, exclusion from the analysis of the out-of-range values did not change the statistical results. A possible explanation for the high levels of sHLA-G in some subjects may be that activated maternal antigen-presenting cells express and release HLA-G5,[25] inducing in some subjects a strong activation of the immune system at the beginning of pregnancy. The high mean value of sHLA-G levels in women with later fetal growth restriction was a surprising and unexplained finding. No studies have previously addressed this item, which deserves further investigation.

In conclusion, our results suggest that low sHLA-G levels in early pregnancy may be associated with subsequent placenta-mediated complications, and underline the potential role of sHLA-G in the early stages of placentation. These findings, if confirmed in larger studies, indicate that HLA-G levels in early pregnancy might be a marker of abnormal placentation. At present, however, the mechanisms responsible for immune tolerance in pregnancy remain unclear, with the role of HLA-G still resistant to easy analysis.

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Disclosure

The authors declare that there is no conflict of interest regarding the publication of this article.

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