Maternal Serum Soluble HLA-G Levels in Missed Abortions

Fatih Keskin[1], Ahmet Karatas[2], Mustafa Albayrak[1], Ismail Bıyık[3], Müşerref Erkan[4], Hilmi Demirin[5], Serdar Dilbaz[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Duzce University, [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Abant Izzet Baysal University, [3]Department of Obstetrics and Gynecology, Karacabey State Hospital, [4]Department of Obstetrics and Gynecology, Atatürk State Hospital, [5]Department of Biochemistry, Faculty of Medicine, Duzce University, Turkey

Key Words: *pregnancy loss; sHLA-G; early pregnancy; missed abortion.*

Summary. *Background and Objective. It is unclear how immune tolerance develops to a semi-allograft fetus in pregnancy. Human leukocyte antigen G (HLA-G) expressed by extravascular trophoblasts plays an important role in the recognition of the gestational tissues as self and the development of immune tolerance against the gestational tissues by the maternal immune system. The soluble form of the HLA-G (sHLA-G) molecule in the maternal serum is also reported to contribute to the prevention of rejection during pregnancy. The aim of the study was to compare the maternal serum sHLA-G levels of the women with missed abortions and control subjects with uncomplicated pregnancies.*

Material and Methods. The prospective cross-sectional study involving 40 with missed abortions and 40 control women, matched by age, gestational age, and body mass index, was carried out. The study group consisted of the women with singleton pregnancies, who were diagnosed with a missed abortion. Only the patients who were confirmed to have an uncomplicated term delivery during follow-up were included in the control group. The serum sHLA-G level was compared between the groups.

Results. There was no significant difference in the mean serum sHLA-G levels in terms of gravidity (P=0.761) and a history of abortion (P=0.379) in the control group. The median serum sHLA-G level in the missed abortion group was significantly lower compared with the control group (16.8 [8.5–35.8] vs. 26 [11–135] U/mL, P<0.001). All the women in the control group had uncomplicated term deliveries.

Conclusion. Our results showed that the women with missed abortions had significantly lower serum sHLA-G levels compared with the healthy pregnant controls, which may have potentially played a role in the impairment of physiological immunological tolerance during pregnancy. However, the determination of the exact role and the potential clinical utility of maternal serum sHLA-G for the detection/prediction of a missed abortion risk requires further detailed studies.

Introduction

Implantation of the embryo in the endometrium results from a complex interplay between the embryo and the uterus, and many aspects are yet to be elucidated. Although it is known that the genome of the embryo originates from the mother and the father equally and the fetus is a semiallograft for the mother, the development of immune tolerance to the gestational tissues in pregnancy still remains a mystery (1–4).

The normal development of the placenta is essential for a healthy pregnancy. The placenta functions as an interface between the mother and the gestational tissues. It is involved in a complex series of events resulting in immune tolerance and the prevention of the rejection of the fetal allograft (5). While villous trophoblasts from the chorionic villi provide oxygen and nutrient exchange, extravillous trophoblasts migrate into the decidua and invade the lumen of the spiral arteries in the myometrium (6). Extravillous trophoblasts that interact with such different maternal cells express major histocompatibility complex (MHC) class I cell surface antigens referred to as human leukocyte antigen G (HLA-G) molecules. HLA-G molecules are thought to control the trophoblastic cell invasion through the regulation of the release of various cytokines (6, 7) and to enhance immune tolerance locally in the uterus and systemically in the maternal peripheral tissues. Thus, they are thought to protect the semi-allogeneic fetus from rejection and play an important role in successful implantation (7, 8).

Among the 7 recognized isoforms of HLA-G, 4 isoforms are membrane-bound (HLA-G1, G2, G3, and G4) and 3 are soluble in the serum (HLA-G5, G6, and G7) (3, 7, 9). The soluble isoforms of HLA-G1 and HLA-G2 are called soluble HLA-G5 (sHLA-G5) and HLA-G6 (sHLA-G6), respectively.

Correspondence to A. Karatas, Department of Obstetrics and Gynecology, Faculty of Medicine, Abant Izzet Baysal University, 14280, Golkoy, Bolu, Turkey
E-mail: akaratas1973@hotmail.com

HLA-G1 and HLA-G2 are abundant in the maternal-fetal interface; they also pass into the maternal circulation during pregnancy. Although lower maternal serum levels of HLA-G in the soluble form (sHLA-G) similar to membrane bound forms have been reported to be a risk factor for many pregnancy-related diseases resulting in pregnancy loss including a spontaneous abortion, there are only a few contradictory studies that compared the maternal serum levels of sHLA-G between women with missed abortions and women with uncomplicated pregnancies (3, 10–12).

The aim of this study was to compare the maternal serum sHLA-G levels between the women with missed abortions and the women with uncomplicated pregnancies.

Material and Methods

This prospective cross-sectional study was carried out in the Department of Obstetrics and Gynecology, Faculty of Medicine, Duzce University, between February 2012 and July 2012. The study was approved by the Noninvasive Human Research Ethics Committee, Duzce University. Informed consent was obtained from all the women.

Study Population. The study group consisted of the women with singleton pregnancies, who were diagnosed with a missed abortion. The diagnosis of a missed abortion was made based on the presence of at least one of the following transvaginal or transabdominal ultrasound findings before the 20th week of gestation: 1) the absence of the embryo's heart beat when the crown-rump length (CRL) was $\geqslant$5 mm; 2) the absence of a yolk sac despite a gestational sac (GS) of $\geqslant$13 mm; and 3) the absence of an embryo despite a GS with a diameter of $\geqslant$25 mm on the transabdominal measurement and/or $\geqslant$18 mm on transvaginal ultrasound.

The control group consisted of the women with healthy singleton pregnancies matched by maternal and gestational age to the study group who attended our outpatient clinic for routine obstetric care with no symptoms or signs related to any kind of abortion such as vaginal bleeding and subchorionic hematoma on ultrasound. Only the patients who were confirmed to have an uncomplicated term delivery during follow-up were included in the control group.

The women with known or suspected chromosomal or structural anomalies of the fetus, abnormal results of the screening test in the first and/or second trimesters, congenital or acquired anomalies of the uterus, and the history of drug use other than antianemics or vitamins were excluded from the control group. Additionally, the women with a history of 2 abortions and more, smoking, alcohol and other substance consumption, maternal chronic systemic illnesses, abnormal pregnancy outcomes such as a preterm delivery, a preterm premature rupture of the membranes, cervical incompetence or cervical surgery, hypertension, preeclampsia, eclampsia, delivery of a baby with intrauterine growth restriction, placental detachment, diabetes, and autoimmune diseases were excluded from the study.

Gestational age was determined by the last menstrual period (LMP). In cases when there was a difference of more than 7 days between the LMP-based gestational age and the gestational age based on the crown-rump length on transvaginal ultrasonography, the latter was used.

Laboratory Analyses. The venous blood (5 mL) was collected from the study patients after the diagnosis of a missed abortion was made and before the commencement of any therapy. A similar amount of the blood was drawn from the patients who fulfilled the criteria to be included into the control group. The blood samples were centrifuged at 3500*g* for 4 minutes to separate serum and stored at –80°C until analysis. The serum sHLA-G (sHLA-G1/G5) levels were determined using the commercial ELISA kit (BioVendor-Laboratorni Medicina A.S., the Czech Republic). The researchers who performed the biochemical analysis were blinded to the pregnancy outcomes.

Statistical Analysis. When comparing the numerical parameters between the groups, the independent samples *t* test was used for the normally distributed data. The Mann-Whitney *U* test was used for the parameters that were not normally distributed, and the chi-square test was used for the comparison of the nominal data. The quantitative data that were distributed normally were given as the mean (standard deviation); and the data that were not normally distributed were given as the median (range). The nominal data were expressed as frequency and percentages (%). The receiver operating characteristic (ROC) curve analysis was used for the determination of the cutoff value of the HLA-G levels for estimating missed abortions.

Results

A total of 40 women with a missed abortion and 40 controls were subsequently included in the study. There were no significant differences in maternal age, weight, height, body mass index (BMI), and gestational age between the groups (Table). However, 11 women (27.5%) in the missed abortion group and 8 women (20%) women in the control group had a history of abortion (P=0.43). The median serum sHLA-G level in the missed abortion group was lower than that in the control group (16.8 [8.5–35.8] U/mL vs. 26 [11–135] U/mL) (P<0.001).

For a specificity of 50% and a sensitivity of 90%, the serum sHLA-G cutoff value was determined as 25.9 U/mL in the ROC curve analysis for the women

Table. Age, Body Mass Index, Gestational Age, and sHLA-G Levels in Women With Missed Abortion and Controls

Characteristic	Controls (n=40)	Missed Abortion (n=40)	*P*
Age, years	26.7 (4.5)	27.7 (5.0)	0.36
BMI, kg/m^2	24.2 (3.7)	25.3 (3.6)	0.17
Gestational age based on LMP, weeks	9 (2.5)	9.3 (2.6)	0.63
Gestational age based on USG, weeks	8.6 (2.7)	7.9 (2.6)	0.12
sHLA-G, median (range), U/mL	25.9 (11–135)	16.8 (8.4–35.8)	<0.001

Values are mean (standard deviation) unless otherwise stated. BMI, body mass index; LMP, last menstrual period; USG, ultrasound; sHLA-G, soluble human leukocyte antigen-G.

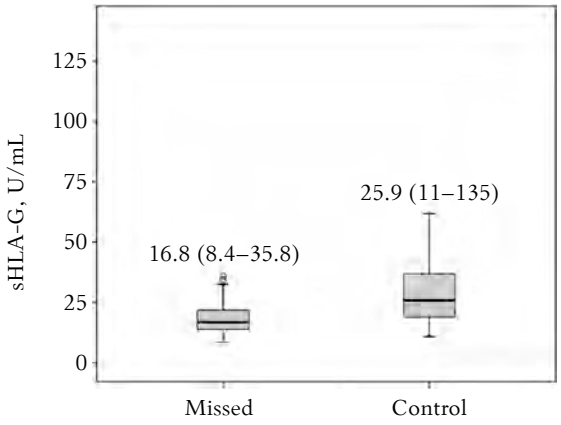

Fig. 1. The maternal serum HLA-G levels in the women with missed abortions and the control women

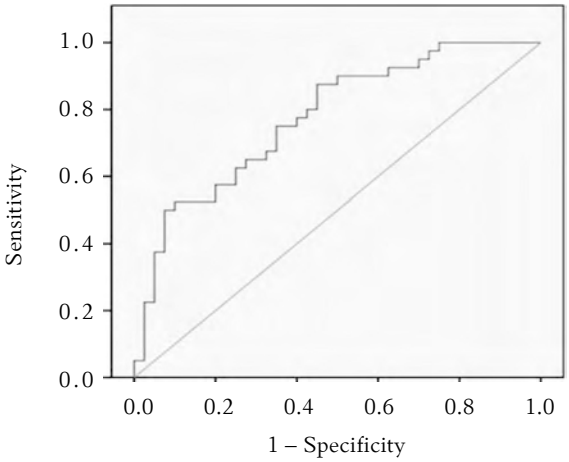

Fig. 2. The receiver operating characteristic curve of soluble HLA-G level

with a missed abortion (area under the curve, 0.7; $P<0.001$) (Figs. 1 and 2).

Moreover, 25 women (62.5%) in the control group and 28 women (70%) in the missed abortion group were multiparous ($P=0.47$). There was no significant difference in the mean serum sHLA-G level between 15 primigravid and 25 multiparous women in the control group (26 [17–127] U/mL vs. 26 [11–135] U/mL, $P=0.761$). All the women in the control group had uncomplicated term deliveries.

Additionally, the sHLA-G level in the women with a history of abortion in the control group (n=8; 33.3 [10.8–135] U/mL) was the same with that in the women in the control group who did not have a history of abortion (n=32; 26 [11–135] U/mL) ($P=0.379$). However, the sHLA-G level of these 8 women was still higher than that in the missed abortion group ($P=0.031$).

Discussion

In this study, we found that the median maternal serum sHLA-G levels in the women with missed abortions were lower compared with the healthy controls matched by maternal age, BMI, and gestational age. To our knowledge, this is the first study directly investigating the maternal serum soluble sHLA-G levels in the women with missed abortions in comparison with the healthy pregnant controls.

Immune system modulation during pregnancy is essential for the development of immune tolerance to the fetal semiallograft. It is believed that sHLA-G molecules are involved in modulating the immune response, enhancing immune tolerance, protecting the semiallogeneic fetus from rejection, and providing a systemic attachment of the allograft (3, 9, 13). It is known that sHLA-G levels gradually increase in early pregnancy, reach the highest levels in the first trimester, and remain constant thereafter with no statistically significant differences among the 3 trimesters (11). sHLA-G is thought to exert these salutary functions on the immune system through the regulation of the release of various cytokines involved in allograft rejection and tolerance (4, 14). It reduces the production of interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), which are cytokines of Th1 involved in allograft rejection produced by monocytes in the decidua and monocytes in the peripheral blood. Additionally, they induce an increase in the production of interleukin-4, a Th2 cytokine involved in preventing the rejection of the fetus (4).

Although controversial, an increased immune response against the gestational tissues resulting from lower maternal serum sHLA-G concentrations has been implicated in the pathogenesis of various pregnancy-related diseases such as preeclampsia, intrauterine growth restriction, and recurrent abortions resulting in pregnancy loss (11–16). Additionally, a lower sHLA-G release in the embryo culture has also been proposed to be associated with implantation failure in in vitro fertilization (IVF), and HLA-G levels have also been reported to be positively correlated with successful pregnancy rates in IVF (4, 17, 18).

Although many researchers commonly report lower maternal serum sHLA-G levels as a cause of recurrent abortion (14, 16, 19), to our knowledge, there is no study in literature that directly investigated maternal serum levels in women with missed abortions in comparison with women with a normal pregnancy. Remaining few studies have not focused on the relationship between maternal serum sHLA-G and abortion, and they were somewhat contradictory (11). Alegre et al. (3) have reported lower maternal serum sHLA-G levels in 8 women who experienced a spontaneous abortion before the 12th week of pregnancy compared with those with a successful pregnancy. Additionally, Pfeiffer et al. have concluded that the circulating maternal sHLA-G serum levels are lower in pregnancies that undergo abortion during the first 9 weeks of gestation compared with normal pregnancies in IVF (10). Contrary, Steinborn et al. have reported no significant differences in maternal serum sHLA-G levels between women with a normal pregnancy outcome and those of 11 women aborted before the 25th week of gestation (11). The discrepancy between these studies including ours may have resulted from the previously reported profound variability and overlapping nature of serum sHLA-G levels among individuals with normal and pathological pregnancies (11) besides the low number of the subjects in these studies. In addition, the genetic polymorphism and the assay methods of sHLA-G across the population may have accounted for the differences across the studies. Nevertheless, although a high sHLA-G level may not be mandatory for a healthy pregnancy (11), our results imply that a lower maternal sHLA-G level seems to be involved in a missed abortion.

Conclusions

Our results showed that the women with missed abortions had significantly lower serum sHLA-G levels compared with the healthy pregnant controls, which may have potentially played a role in the impairment of physiological immunological tolerance during pregnancy. However, the determination of the exact role and the potential clinical utility of maternal serum sHLA-G for the detection and prediction of a missed abortion risk requires further detailed studies.

Acknowledgments

This study was financially endorsed by the Scientific Study Research Commission, Faculty of Medicine, Duzce University (registration No. 2012-486).

Statement of Conflict of Interest

The authors state no conflict of interest.

References

1. Le Bouteiller P, Mallet V. HLA-G and pregnancy. Rev Reprod 1997;2:7-13.
2. Hunt JS, Langat DK, McIntire RH, Morales PJ. The role of HLA-G in human pregnancy. Reprod Biol Endocrinol 2006;4 Suppl 1:S10.
3. Alegre E, Díaz-Lagares A, Lemaoult J, López-Moratalla N, Carosella ED, González A. Maternal antigen presenting cells are a source of plasmatic HLA-G during pregnancy: longitudinal study during pregnancy. Hum Immunol 2007; 68:661-7.
4. Noci I, Fuzzi B, Rizzo R, Melchiorri L, Criscuoli L, Dabizzi S, et al. Embryonic soluble HLA-G as a marker of developmental potential in embryos. Hum Reprod 2005;20:138-46.
5. Guzeloglu-Kayisli O, Kayisli UA, Taylor HS. The role of growth factors and cytokines during implantation: endocrine and paracrine interactions. Semin Reprod Med 2009; 27:62-79.
6. Abrahams VM. Immunology of the maternal-fetal interface. Available from: URL: http://www.uptodate.com/contents/immunology-of-the-maternal-fetal-interface?
7. Roussev RG, Coulam CB. HLA-G and its role in implantation (review). J Assist Reprod Genet 2007;24:288-95.
8. Ober C, Aldrich CL, Chervoneva I, Billstrand C, Rahimov F, Gray HL, et al. Variation in the HLA-G promoter region influences miscarriage rates. Am J Hum Genet 2003;72: 1425-35.
9. Hviid TV. HLA-G in human reproduction: aspects of genetics, function and pregnancy complications. Hum Reprod Update 2006;12:209-32.
10. Pfeiffer KA, Fimmers R, Engels G, van der Ven H, van der Ven K. The HLA-G genotype is potentially associated with idiopathic recurrent spontaneous abortion. Mol Hum Reprod 2001;7:373-8.
11. Steinborn A, Varkonyi T, Scharf A, Bahlmann F, Klee A, Sohn C. Early detection of decreased soluble HLA-G levels in the maternal circulation predicts the occurrence of preeclampsia and intrauterine growth retardation during further course of pregnancy. Am J Reprod Immunol 2007; 57:277-86.
12. Rizzo R, Andersen AS, Lassen MR, Sørensen HC, Bergholt T, Larsen MH, et al. Soluble human leukocyte antigen-G isoforms in maternal plasma in early and late pregnancy. Am J Reprod Immunol 2009;62:320-38.
13. Hunt JS, Jadhav L, Chu W, Geraghty DE, Ober C. Soluble HLA-G circulates in maternal blood during pregnancy. Am J Obstet Gynecol 2000;183:682-8.
14. van der Meer A, Lukassen HG, van Cranenbroek B, Weiss EH, Braat DD, van Lierop MJ, et al. Soluble HLA-G promotes Th1-type cytokine production by cytokine-activated uterine and peripheral natural killer cells. Mol Hum Reprod 2007;13:123-33.
15. Yie SM, Li LH, Li YM, Librach C. HLA-G protein concentrations in maternal serum and placental tissue are decreased in preeclampsia. Am J Obstet Gynecol 2004;191:525-9.
16. Hackmon R, Koifman A, Hyodo H, Glickman H, Sheiner E, Geraghty DE. Reduced third-trimester levels of soluble human leukocyte antigen G protein in severe preeclampsia. Am J Obstet Gynecol 2007;197:255.e1-5.
17. Fuzzi B, Rizzo R, Criscuoli L, Noci I, Melchiorri L, Scarselli B, et al. HLA-G expression in early embryos is a fundamental prerequisite for the obtainment of pregnancy. Eur J Immunol 2002;32:311-5.
18. Fanchin R, Gallot V, Rouas-Freiss N, Frydman R, Carosella ED. Implication of HLA-G in human embryo implantation. Hum Immunol 2007;68:259-63.
19. Cecati M, Giannubilo SR, Emanuelli M, Tranquilli AL, Saccucci F. HLA- G and pregnancy adverse outcomes. Med Hypotheses 2011;76:782-4.

Received 12 February 2013, accepted 30 October 2013