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Rapid Communication

sHLA-G1 and HLA-G5 levels are decreased in Tunisian women with multiple abortion

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ABSTRACT

Pregnancy is associated with increased levels of soluble (s) human leukocyte antigen (HLA)-G molecules, while during abortion these molecules are decreased. To date, little is known about the role of sHLA-G isoforms during abortion. In this study, we investigated the levels of total sHLA-G and its isoforms: HLA-G1 (membrane shedded isoform) and alternative spliced HLA-G5 in plasma samples obtained from 55 women who had experienced spontaneous abortion, 108 pregnant healthy women and 56 non pregnant healthy women.

We found that pregnant women exhibited higher amounts of sHLA-G compared to either non pregnant women or women with abortion. Among women who had experienced spontaneous abortion, women with recurrent abortions (RSA) had lower sHLA-G than women with only one abortion. In particular, RSA women were characterized by the absence of sHLA-G1 isoform, suggesting a possible implication in abortion event.

1. Introduction

There is accumulating and compelling evidence that non-classical human leukocyte antigen (HLA) class I molecule HLA-G is highly implicated in immune tolerance as an immune checkpoint [1]. Its role substantiates its implication in immune cell regulation. Indeed, HLA-G can inhibit NK and CD8+ T lymphocyte mediated cell lysis [2,3], dendritic cell antigen presentation [4] and T lymphocyte CD4+ alloproliferation [5]. Additionally, HLA-G molecules up-regulate T lymphocyte CD8+ apoptosis and enhance the production of regulator T cells [6]. These functions evidence the importance of HLA-G molecules in fetus tolerance by the maternal immune system during pregnancy. Indeed, HLA-G molecule expression was originally described at the feto-maternal interface in trophoblast cells' surface [7,8].

The HLA-G gene expresses seven isoforms generated through alternate mRNA splicing: four membrane-bound isoforms (HLA-G1, HLA-G2, HLA-G3 and HLA-G4) and three soluble isoforms (sHLA-G: HLA-G5, HLA-G6 and HLA-G7). Additionally, it has also been demonstrated that HLA-G1 could be cleaved by matrix metalloproteinases (MMP) generating HLA-G1 shedding molecule (sHLA-G1) [9], in particular by MMP-2 [9]. However, the most studied isoforms of sHLA-G consisted of sHLA-G1 and HLA-G5 [10].

Immune processes play a crucial role in abortion and especially in recurrent spontaneous abortion (RSA) [11,12]. Several studies support the implication of HLA-G molecule in pregnancy complications. Essentially, HLA-G isoforms, either membranous or soluble, are decreased in pregnancy complications including preeclampsia and RSA [13]. Furthermore, a recent work reported that uterine sHLA-G levels are altered in unexplained infertility [14].

Here we explored, for the first time, levels of sHLA-G in plasma samples from pregnant women in comparison to women who had experienced spontaneous abortion (one or more abortion events)

Abbreviations: sHLA-G, soluble HLA-G; sHLA-G1, shedding HLA-G1; HLA-G5, HLA-G5; RSA, recurrent spontaneous abortion.

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and non-pregnant women. We also evaluated levels of membrane-shedded HLA-G1 (sHLA-G1) and spliced HLA-G5 isoforms.

2. Materials and methods

2.1. Patients

Venous blood samples were collected from Tunisian participants, and plasmas were obtained by centrifugation avoiding sHLA-G to be trapped and/or consumed by clots [15,16]. This study implicated 55 women who have experienced spontaneous abortion recruited from the Basic Health Group of Sousse (“Groupement de santé de base de Sousse”): 44 women with one abortion and 11 women with recurrent spontaneous abortions (RSA, with at least two spontaneous abortions [17–19]). The mean age of these women equaled 34.83 ± 8.46 years (mean ± SD; age range: 20–60 years).

Two age-matched control groups were recruited for this study. The first group consisted in 56 unrelated non-pregnant fertile volunteers women with a mean age 32.23 ± 11.72 (Age range: 18–63 years). Collected blood was off menstruating. The second group is composed by 108 healthy women at different stages of uncomplicated pregnancy with a mean age 33.69 ± 8.43 (Age range: 18–68 years). None of control groups had an abortion history or a complicated pregnancy.

2.2. Soluble HLA-G dosage by enzyme-linked immunosorbent assay (ELISA)

Sandwich ELISA was performed according to the Essen Workshop [20]. sHLA-G was measured by enzyme-linked immunosorbent assay (ELISA) in plasma samples as previously reported [21–23], using the MEM-G9 Monoclonal antibody (MAb; Exbio, Praha, Czech Republic) that recognizes sHLA-G molecules associated to β2-microglobuline, or the 5A6G7 MAb (Exbio, Praha, Czech Republic) that recognizes the HLA-G5, -G6 and -G7 isoforms. Standard supernatants of HLA-G/721.221 were used for standard calibration curves generation. The intra-assay coefficient of variation (CV) was 1.4% and the inter-assay CV was 4.0%. The limit of sensitivity was 1.0 ng/ml.

2.3. Statistical analysis

Statistical analysis was performed with SPSS (16.0) and by Graphpad prism 5. Comparison between ages and sHLA-G levels was evaluated respectively by unpaired *t*-test or Mann–Whitney test. Fisher exact test was performed to compare HLA-G positive subjects’ percentage. Two-tailed *P*-value under 0.05 was considered as statistically significant.

3. Results

3.1. sHLA-G levels are decreased in women with abortion

The three studied cohorts, women with abortion, pregnant and non pregnant women, presented similar ages (Unpaired *t*-test: P (abortion vs. non pregnancy) = 0.186; P (abortion vs. pregnancy) = 0.419).

The comparison of the three groups for sHLA-G plasmatic levels evidenced that women with abortion had lower sHLA-G concentrations than pregnant women (mean ± SEM: 0.50 ± 0.30 ng/ml and 2.8 ± 0.54 ng/ml, respectively $P < 0.0001$, Mann–Whitney test) (Table 1). Similarly, we observed lower levels of both sHLA-G1 and HLA-G5 in women with abortion than in the pregnant ones ($P = 0.0003$ and $P = 0.0001$, respectively, Mann–Whitney test) (Table 1). On the contrary, no significant difference in sHLA-G level was observed between non pregnant women and women with abortion (Table 1).

3.2. RSA women showed lower levels of sHLA-G

Women with abortion were stratified according the number of abortions (one or more than one abortion (RSA)). Both subgroups showed lower levels of sHLA-G compared to the pregnant women (pregnant vs. one abortion: $P < 0.0001$; pregnant vs. RSA $P = 0.0046$, Mann–Whitney). Interestingly, although without significance, the subgroup of RSA women showed lower sHLA-G levels in comparison to the subgroup of women with only one abortion ($P = 0.53$; percentage of positive samples = 9%) (Fig. 1). No differences were observed between women with abortion group and non pregnant women cohort.

The analysis of sHLA-G1 and HLA-G5 isoforms evidenced higher levels in pregnant women compared to both abortion subgroups (Fig. 1).

3.3. sHLA-G1 absence characterizes women with RSA

Considering the number of positive samples for sHLA-G and its isoforms in each group, we reported a high positivity percentage in pregnant women (Fig. 1) (sHLA-G: 53.7%, sHLA-G1: 31.4%; HLA-G5: 37%) in comparison to both women with abortion subgroups ($P < 0.0001$, Fisher exact test).

Interestingly, no positive samples for sHLA-G1 were found in the subgroup of RSA women (women with RSA vs. non pregnant women: $P < 0.0001$ and women with RSA vs. women with one abortion: $P = 0.01$; Fisher exact test) (Fig. 1).

4. Discussion

Pregnancy is an immunological paradox [24] in which the fetus, considered as “semi-allogenic”, is maintained in the mother body through different immune-tolerance mechanisms particularly brought by HLA-G molecules [21–25]. Indeed, a decrease in HLA-G expression is associated with complications in pregnancy [23].

In accordance to previous studies showing that sHLA-G is increased in pregnant women plasma [21,23], we have reported here the increase of this molecule in plasma samples from healthy pregnant women. We also observed a decrease in total sHLA-G in women with abortion as previously described [21,26]. Further investigations should shed light on the real mechanism implicating sHLA-G and would explain whether low sHLA-G is the cause or only a by-product of the spontaneous abortion. sHLA-G decrease could be attributed to the changes in pro-inflammatory and anti-inflammatory cytokines balance associated to abortion [27]. In particular, previous studies reported a decrease in IL (Interleukin)-10 expression, a positive regulator of HLA-G production in normal pregnancy [28], in RSA women [29]. This defect in IL-10 secretion could in part explain the observed decrease of sHLA-G expression in our cohort of women with abortion. Further studies need to address this aberrant expression of cytokines to clearly substantiate their association to HLA-G production.

To the best of our knowledge, this is the first time that a decrease in HLA-G5 and sHLA-G1 isoforms is reported in women with abortion. Similarly to preeclamptic women, where HLA-G5 is the only isoform observed, even if with lower levels in comparison with healthy pregnant women [24], we found only the HLA-G5 isoform in RSA women (Fig. 1). On the contrary, women with only one abortion were characterized by the presence of sHLA-G1 isoform and also HLA-G5. These data suggest a peculiar