

**Pregnancy, breast-feeding and menopause: a correlation with the de-  
creased sHLA-G levels in breast cancer patients?**

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## ABSTRACT

Concerns about breast cancer (BC) etiology have increased researches on the implication of HLA-G molecules in this malignancy. HLA-G molecules act as immune-inhibitory mediators that could promote tumor growth. In this study, we investigated the levels of sHLA-G in plasma samples from women affected by BC and correlated them with previous pregnancies, breast-feeding experience as well as their menopause status.

We report, for the first time, a significant differences in sHLA-G levels between BC patients without breast-feeding experience and those who have breastfed ( $p=0.04$ ). Similarly, sHLA-G is significantly increased in BC patients without previous pregnancy experience compared to those who have been pregnant ( $p=0.02$ ). No differences in sHLA-G levels were noticed between premenopausal and postmenopausal BC patients.

Additionally, we demonstrated that patients having not been pregnant and who have not breastfed presented an advanced SBR III grade, associated with significant enhancement in tumor size compared with patients who had both experiences ( $p=0.028$ ). These later patients expressed higher levels of progesterone and estrogen receptors with an increased chance to be responsive to hormonal therapies.

Taken together, our results indicate the possible implication of breast-feeding and pregnancy in HLA-G expression during BC and in the protection against complicated stages of BC.

**Keywords:** HLA-G, Breast cancer.

## 53 1. INTRODUCTION

54 Breast cancer (BC) is considered an important cause of death among women [1-2].  
55 Several researches are involved in the determination of the mechanisms of its genesis and  
56 progression. Several molecules have been implicated in the increased risk of BC, especially  
57 classical HLA (Human leukocyte antigen) class I molecules [2-4]. Non classical HLA-I mol-  
58 ecules are considered as possibly implicated in the control of BC progression [4].

59 Among non classical HLA-I molecules, HLA-G antigens behave as an immune-  
60 tolerance mediator avoiding fetus rejection [5-6] and successful pregnancy [7]. In this con-  
61 text, HLA-G molecules are expressed as membrane-bound molecules as well as soluble ones  
62 (sHLA-G) [6]. Reciprocally, the reduced levels of HLA-G expression is involved in pre-  
63 eclampsia events [8] and other pregnancies complications [9-11].

64 HLA-G molecules are implicated in tumor evasion [12], due to their ability to control  
65 the activation of immune cells. In particular, ..... We sought to evaluate the secretion of  
66 HLA-G molecules in the plasma samples from BC patients and evaluate the implication in  
67 BC women with/without previous pregnancies, breast-feeding experiences, as well as pre-  
68 /postmenopausal BC women.

**Commento [R1]:** Add here some information on HLA-G effect on immune cells and receptors.

## 70 2 RESULTS AND DISCUSSION

71 Thirty six women with confirmed BC were enrolled in the study (Table 1). 12 patients  
72 had low-intermediate TNM stage (I and II) and 15 presented an advanced TNM stage (III and  
73 IV). sHLA-G levels were assessed in patients' plasma by Exbio kit (Exbio, Praha, Czech  
74 Republic) according to the manufacturer's instructions.

75 Negative correlation were found between sHLA-G levels and either pregnancy expe-  
76 rience (R Spearman=-0.416, p=0.012), breast-feeding experience (R Spearman=-0.341,  
77 p=0.042), and menopause (R Spearman=-0.358, p=0.044) (Table 2).

78 We observed a significant difference between patients who have had pregnancy expe-  
79 rience or not (Two tailed Mann-Whitney test:  $p=0.018$ ; Table 2). In particular, patients with  
80 previous pregnancy experience have lower sHLA-G (7.70 Units/ml) compared to patients  
81 with no pregnancy experience (40.14 Units/ml). This is in accordance with the results show-  
82 ing increased levels of sHLA-G during pregnancy [6, 13] that decrease after delivery [13].  
83 Additionally, our results are in line with results by Rizzo et al. showing that sHLA-G levels  
84 decreased after 2 years from pregnancy [14]. Interestingly, we report for the first time, that  
85 patients with no breast-feeding experience had higher sHLA-G levels (35.68 Units/ml) com-  
86 pared to women who breastfed (8.13 Units/ml) (Two tailed Mann-Whitney test:  $p=0.045$ ;  
87 Table 2). However, comparable sHLA-G levels were noticed in pre-/postmenopausal patients  
88 (Table 2). These findings indirectly support the potential implication of breast-feeding in the  
89 protection against advanced stages of cancer characterized by increased levels of sHLA-G.  
90 This hypothesis should be consolidated by further experiments with enlarged samples.

91 Decreased sHLA-G in BC patients with previous pregnancy and/or breast-feeding  
92 experience may be protected directly or indirectly by sHLA-G molecules. Further studies still  
93 needed to elucidate the mechanism under this regulation. Elsewhere, the observed increased  
94 levels of sHLA-G in patients with no history of pregnancy or breast-feeding may be associat-  
95 ed to worse outcome since HLA-G is found in advanced stages of BC disease [15-18]. In-  
96 deed, in this study, we found that in the group of patients with no pregnancy nor breast-  
97 feeding experiences presented SBR grade II and III, whereas patients with pregnancy and  
98 breast-feeding experience presented SBR grade II, III and also grade I (Table 3). SBR I/II are  
99 increased in patients who have had both experiences of pregnancy and breast-feeding  
100 (82.35%) compared to those without these experiences (75%). Inversely, SBR III are in-  
101 creased in patients who have no pregnancy experience nor breast-feeding experience (25%)  
102 compared to those with these experiences (17.65%; Table 3).

103 Interestingly, patients without pregnancy and breast-feeding showed an enhanced  
104 tumor size (Mean=43.82 cm) compared to patients with BC with a previous history of preg-  
105 nancy and breastfing (Mean=34.8 cm). This difference is statistically significant (Mann-  
106 Whitney:  $p=0.028$ ).

107 Elsewhere, we observed that patients with both pregnancy and breast-feeding experi-  
108 ences had more progesterone and estrogen receptors compared to those who did not have  
109 these experiences (Table 3). This observation is important because it shows that patients with  
110 both pregnancy and breast-feeding experiences have more chance to respond correctly to  
111 hormonal therapies than those who had neither pregnancy nor breast-feeding experience.  
112 However, kinetics studies should be made to confirm this hypothesis.

113 Stratifications of patients according with pregnancy and breast-feeding experience  
114 and menopause are reported in Table 4. We found that pre-/postmenopausal patients with  
115 previous pregnancy experience had low sHLA-G. These levels are lower in post-menopausal  
116 women (sHLA-G: Premenopausal patients/ 11.82 Units/ml versus Postmenopausal pa-  
117 tients/2.05 Units/ml). Similarly, the increase of sHLA-G in women with no pregnancy expe-  
118 rience is more pronounced in postmenopausal patients compared to premenopausal ones  
119 (sHLA-G: Premenopausal patients/ 36.52 Units/ml versus Postmenopausal patients/56.27  
120 Units/ml). In the same way, patients who breastfed presented lower levels of sHLA-G com-  
121 pared to women without breast-feeding experience, and this difference is more pronounced in  
122 post-menopausal patients ( $p=0.06$ ; Table 4).

123 Basta et al. reported increased sHLA-G in postmenopausal patients with leiomyoma  
124 compared to controls [19]. Similarly, the sHLA-G increase observed in our post-menopausal  
125 patients is confirmed for patients without pregnancy and/or breast-feeding experience. Recip-  
126 roccally, patients with pregnancy and breastfeeding experience may express protective mole-

cules controlling the secretion of HLA-G. Further, molecular experiments may shed light on the mechanism of sHLA-G regulation.

As HLA-G expression is positively regulated by progesterone hormone [20-21], we speculated that decreased sHLA-G in postmenopausal women could be due to the decrease of progesterone and estrogen hormones. Nevertheless, this hypothesis was not validated in patients without breast-feeding and pregnancy experience. This result may emphasize the probable and exclusive role of hormones in those patients with both pregnancy and breast-feeding experience. However, patients without these experiences may have other positive regulators of sHLA-G molecules.

Overall, the data obtained in the present study suggest that sHLA-G is influenced by the pregnancy and, for the first time, by the breast-feeding experience. Patients with BC who had breastfed in their pregnancy had lower sHLA-G compared to patients without these experiences. They have also a lower tumor stage. Large number of patients would confirm these new findings.

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