Sys Rev Pharm 2020; 11(6): 871 – 877
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Human Leukocyte Antigen (HLA-G) Expression and Soluble HLA-G Levels in Women with Breast Cancer from Basrah Province in Iraq

Hadeel Saleem Touma[1], Wafaa Sadoon Shani[1], Talaat Sadoon Shani[2]
[1]Department of Biology, College of Science, University of Basrah, Basrah, Iraq.
[2]Department of surgery, Al-Zubair general hospital, Basrah, Iraq
E-mail: Hadeels85@yahoo.com

Article History: *Submitted: 22.04.2020* *Revised: 20.05.2020* *Accepted: 28.06.2020*

ABSTRACT
HLA-G is one of the non-classical HLA class I molecules and it is known to be implicated in a tumor-driven immune escape mechanism in malignancy. The purpose of this study was to investigate soluble HLA-G (sHLA-G) and HLA-G expression in breast cancer. sHLA-G was determined by enzyme-linked Immunosorbent assay (ELISA) from plasma samples of 70 breast cancer patients and 35 healthy controls. HLA-G expression in breast cancer lesions was also analyzed by immunohistochemistry staining. The presence of HLA-G expression were analyzed and found to be involved in breast carcinogenesis. Levels of sHLA-G were higher in the breast cancer group compared to the control group and sHLA-G concentration could be used as a diagnostic marker for detecting breast cancer.
Keywords: HLA-G, sHLA-G, cancer, breast cancer, expression.
Correspondence:
Hadeel Saleem Touma
Department of Biology, College of Science, University of Basrah, Basrah, Iraq
E-mail: Hadeels85@yahoo.com
DOI: 10.31838/srp.2020.6.125

INTRODUCTION

Breast cancer is the leading cause of cancer mortality, resulting in more than 14% deaths annually in Iraq (Qassim, 2015). Human leukocyte antigen-G (HLA-G) is a non-classical major histocompatibility complex (MHC) class Ib molecules, In contrast with classical HLA-Ia molecules (HLA-A, B and C), these molecules display a limited polymorphism with a small number of protein encoded by few allele (Jeony *et al*., 2014). HLA-G is expressed in seven different isoforms, four of which are membrane-bound (HLA-G1, G2, G3, and G4) and three are soluble (HLA-G5, G6, and G7) (Bortolotti *et al*., 2014; Amodio and Gregori, 2017). They are defined to be selectively expressed at the maternal–fetal interface on cytotrophoblast cells, to contribute maternal–fetal tolerance, however, during the past few year, the expression of HLA-G has been detected in other healthy tissue including cornea, thymus, pancreatic islets, endothelial cell precursors and erythroblasts (Carosella *et al*., 2008;Yie *et al*., 2007; Lin and Yan, 2015).

Indeed, HLA-G plays an important role in immuno tolerance mechanisms through inhibiting cytolytic functions of natural killer cells, cytotoxic T-lymphocytes, and T-cell alloproliferative responses (Provatopoulou *et al*., 2012; Rizzo*et al*., 2017). For examples, HLA-G bind to inhibitory receptor such as immunoglobulin-like transcripts (ILT-2/CD85j, ILT-4/CD85d and killer cell immunoglobulin receptor (KIR) 2DL4/CD158d (Ristich *et al*., 2005; Liang *et al*., 2008, Carosella *et al*., 2
008).

In cancer, HLA-G may acquire or up regulate expression as protective property against immune recognition and elimination, It is now clear that HLA-G expression have been related with several types of malignancies such as hepatocellular carcinoma, esophageal cancer, lung cancer, renal cell carcinoma, and malignant melanoma (Chen *et al*., 2012; Akin *et al*., 2017). In addition, expression of the HLA-G has been associated with sHLA-G levels and with cancer outcomes in some, but not all, studies.

Aim of the study was to evaluate the potential role of HLA-G in breast carcinogenesis through investigating HLA-G expression in both tissue and plasma in the same patient population.

MATERIALS AND METHODS

The study involved 105 women, who were distributed into two groups of patients and one group of control. The patients were the women who had a breast tumor, and according to the therapy, they were distributed as before and after therapy groups, each with 35 patients. Before therapy group included patients whose age was range between 21 and 62 years, while such range in after therapy group was 21-76 years. The patients before therapy were referred to the center for early detection of breast tumor at AL-Basra hospital for gynecology and obstetrics, the diagnosis was made by the consultant medical staff, which was based on a triple assessment technique (i.e. physical breast examination, ultrasonography, with or without mammography and fine needle aspiration cytology). While the patients after therapy were referred to the specialist center for oncology and blood disease at AL-Sadar teaching hospital in Basra city, during the period march 2016 to 2017. The data were collected from all studied groups using a short structured questionnaire, that included information on age, address, lymph node metastases, histological type and grade, PR, ER and HER-2 status, these questioned through face to face interview. With respect to controls, which were 35.

Assay for plasmas HLA-G

Peripheral blood samples were collected in potassium ethylene diamine tetra acetic acid (EDTA) and were left for 30 min at room temperature. Subsequently, the samples were centrifuged at 4400xg for 10 min at 8^0C, and the plasma was aliquots and stored at -20^0C until assay. Plasma sHLA-G levels were quantified using a commercially available ELISA kit, according to the manufacturer's instructions.

Immunohistochemistry for HLA-G antigens in tissue

A total of 35 formalin-fixed and paraffin-embedded biopsies of the breast were collected from 35 patients and stratified according to lesion grade, stage,…etc. 4H84 Abs were used to recognized denatured HLA-G molecules (de Kruijf, 2010). Tissue sections of 4 um thick were cut from the paraffin-embbed breast tissue blocks. Tissue sections were deparaffinized and rehydrated. For antigen retrieval was treated with 0.01M Trizma EDTA buffer (pH 9) for 10 min in microwave oven. Endogenous peroxidase was blocked for 20 min in 0.3% hydrogen peroxide solution. Anti HLA-G mAbs (1/50) was added and incubated for 30 min at 20^0C. After washing with 0.01M phosphate buffer solution (PBS), the sections were incubated with secondary Ab undiluted goat anti-mouse HRP, tissue sections were counterstained with haematoxylin, and then dehydrated and mounted. All of the slides were stained synchronously to avoid inter assay variation. For staining, breast tissue of normal controls served as positive control. HLA-G immunoreactivity was assessed in a binary manner, considering any specific staining of tumor cells as positive expression and no staining as no expression.

Statistical Analysis

Statistical analyses were performed using SPSS statistics program version 22. The Chi square test was used to analyze the difference in HLA-G expression and evaluate the associations between various clinicopathological parameters and HLA-G expression. P values of (≤ 0.05) were used as the level of significance.

RESULTS

Soluble HLA-G concentration

Highest median concentration of sHLA-G was noted in post therapy patient's 1.55 mg/ml which was higher significantly from the median concentration in pre-therapy (0.89 mg/ml) and control (0.77 mg/ml) (table1). The median concentration of sHLA-G in pre-therapy was also increased as compared with control but showed no significant difference between their means. The sHLA-G levels in the study population points out that patient who post or during treatment (post) exhibited higher levels of plasma sHLA-G compared to patients who did not receive therapy (pre) and control.

Table 1: Levels of sHLA-G among the study population

Groups	Number	sHLA-G Level (µg/ml)*
Control	35	0.77±0.35
Pre-therapy	35	0.89±0.56

Correlations between sHLA-G and various indices

Specific five correlations between sHLA-G concentration and various histological variables can be seen in table (2). These correlations were calculated for all subjects and a significant correlation was found between sHLA-G and each of lymph node, metastasis, ER and PR, (table 2).

The relation between tumor staging and mean levels of sHLA-G showed highest level was in patients at stage IIII that the sHLA-G level reached to 2.18 µg/ml, while the patients from stage II and III the HLA-G level was 1.24 and 1.30 respectively, while reached to 1.70 in patients from stage I, statistically no significant difference between them when sHLA-G level compared at ($P \leq 0.05$).

Also the study determined the relationship between tumor grade and sHLA-G levels, Plasma sHLA-G concentrations were detected to range from 0.5 to2.5 µg/ mL with a mean of 1.62 µg/mL in grade I cancer patients, and from 0.4 to 3 µg/mL with a mean of 1.33 and 1.62 µg/mL in grade II and III, respectively. There was no significant difference in sHLA-G level between grade I, II and III patients, and sHLA-G was also not significantly associated with HER-2, at ($P \leq 0.05$).

Table2: Correlation between plasma sHLA-G concentrations and clinicopathological parameters.

Variable		sHLA-G (µg/ml)
Stage	T1	1.70±1.13
	T2	1.24±0.89
	T3	1.30±0.68
	T4	2.18±1.62
Grade	G1	1.62±0.95
	G2	1.33±1.04
	G3	1.62±0.89
Lymph node	Positive	1.61±1.001*
	Negative	0.87±0.53
ER	Positive	1.61±1.003*
	Negative	0.96±0.55
PR	Positive	1.61±1.003*
	Negative	0.96±0.55

HER-2	Positive	1.62±1.21
	Negative	1.28±0.80
Metastasis	Positive	1.87±1.17*
	Negative	1.11±0.64

Expression of HLA-G

Overall, 54.29% (19/35) of breast cancer lesions were classified as HLA-G positive of which 31.58% (6/19) were weakly expressed, 21.05% (4/19) were regarded as strongly and 47.37% (9/19) moderately expressed (table3, figure1,2,3). Heterogeneous HLA-G staining was observed in breast cancer lesions. The intensity of staining varied from tumor to tumor and/or from one area to another within the same tumor. The HLA-G expression was detected in the cell membrane and cytoplasm of breast cancer cells. HLA-G positive expressions were also identified in the benign tumor.

HLA-G expression and association with clinical

Overall, most of tumors examined in the study showed both ER and PgR expression (ER+/PgR+) and patients with strong/moderate HLAG expression had strong/moderate ER and PgR expression, majority of stage I patients were HLA-G-positive and HLA-G positivity decreased as the clinical stage advanced (table4). HLA-G positive patients had less metastasis (p<0.004) and more nodal involvement. Intensity of HLA-G expression tended to correlate with tumor grade, however, this correlation was not statistically significant.

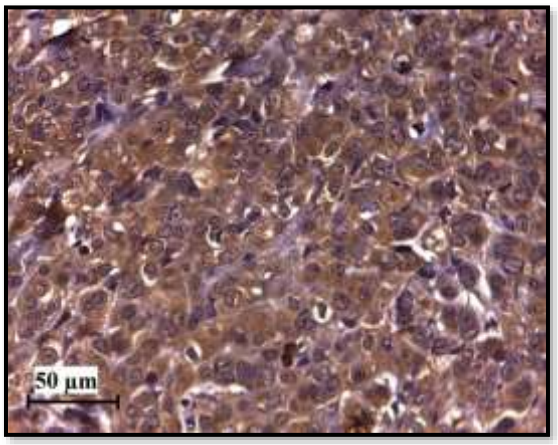

Figure1: Breast cancer section showed immunohistochemical stain for HLA-G with strong positive (40X)

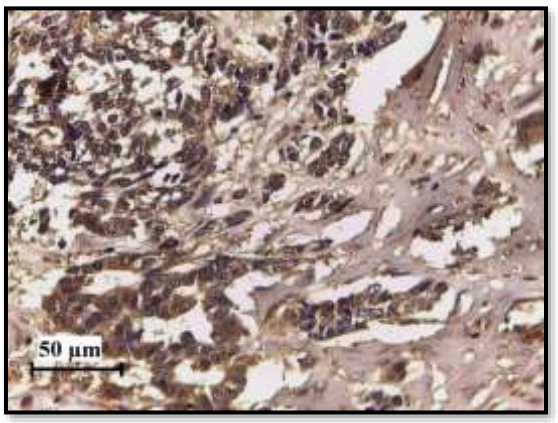

Figure 2: Breast cancer section showed immunohistochemical stain for HLA-G with moderate positive (40X).

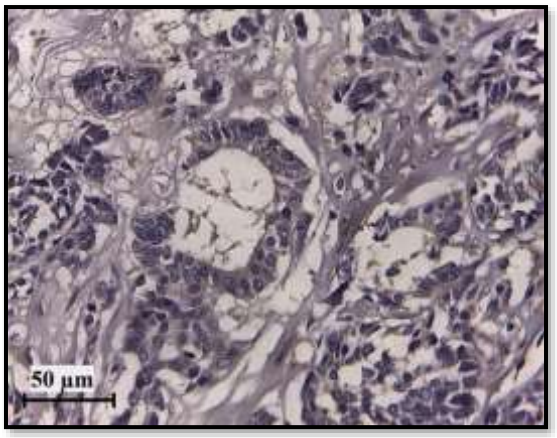

Figure 3: Breast cancer section showed weak immunohistochemical stain for HLA-G with (40X).

Table 3: Distribution ofBreast cancer patients with HLA-G expression.

Studied marker	Tumor reaction	Total		Reaction score					
				Weak		Intermediate		Strong	
		No	%	No	%	No	%	No	%
HLA-G	Positive	19	54.29	6	31.59	9	47.37	4	21.05
	Negative	16	45.17						
P value	0.612								

Table 4: Association between clinical factors and human leukocyte antigen HLA-G expression in breast cancer patients.

Variable		HLA-g expression		P value
		Positive (n=19)	Negative (n=16)	
Stage	T1	7	1	0.079
	T2	6	6	
	T3	3	5	
	T4	1	4	
Grade	G1	3	0	0.229
	G2	3	4	
	G3	1	12	
Lymph node	Positive	12	2	0.24
	Negative	7	14	
Metastasis	Positive	3	10	0.004*
	Negative	16	6	
ER	Positive	15	4	0.001*
	Negative	4	12	
PR	Positive	11	4	0.288
	Negative	8	12	
HER-2	Positive	2	4	0.258
	Negative	17	12	

Table 5: Association between clinical factors and human leukocyte antigen HLA-G and foxp3 expression in breast cancer patients.

Variable		HLA-g expression		P value
		Positive (n=19)	Negative (n=16)	
Stage	T1	7	1	0.079
	T2	6	6	
	T3	3	5	
	T4	1	4	
Grade	G1	3	0	0.229
	G2	3	4	
	G3	1	12	
Lymph node	Positive	12	2	0.24
	Negative	7	14	
Metastasis	Positive	3	10	0.004*
	Negative	16	6	
ER	Positive	15	4	0.001*
	Negative	4	12	
PR	Positive	11	4	0.288
	Negative	8	12	
HER-2	Positive	2	4	0.258
	Negative	17	12	

DISCUSSION

Many factors may regulate immune response during cancer such as immunosuppressive cytokines or immunogenetic factors (most important one HLA-G). According to the fact indicated that there was multiple mechanisms must be existing to modulate and moderate the immune system. HLA-G expression represents one of such mechanism for protecting the tumor from cytotoxic immune response.

Recorded data indicates that the median concentration of sHLA-G was higher significantly among women suffering from breast cancer than control, and showed that plasma level of sHLA-G was high in post therapy and pre therapy while much lower values were observed in healthy women, this may be attributed to an important role for sHLA-G1/ sHLA-G5 circulating protein in plasma as immune regulatory molecules in the maintenance of cancer and increased level of one or both can be acted as a risk factor for cancer prognosis. Soluble HLA-G was circulating in the blood in many cancers and at all phases of tumor with a higher level than in health, soluble HLA-G molecules are able to suppress the innate and the adaptive immune system.

He *et al*. (2010) showed that plasma sHLA-G levels were significantly higher in breast cancer patients than in healthy controls ($p< 0.001$) and also found that HLA-G may have potential clinical implications in diagnosis, prognosis and immunotherapy of patients with breast cancer.

Identified data during cancer in this study was similar to Singer *et al.* (2003) who found increasing sHLA-G concentrations in ascites specimens obtained from breast cancer patients than those from patients with benign lesions; these data suggest that HLA-G levels might be used as a diagnostic tool to distinguish between malignant and benign tumors. König*et al*. (2016) reported that sHLA-G in MV positively correlated with worse prognosis; free sHLA-G positively correlated with better outcome. However, further investigations showed that plasma levels of sHLA-G were higher in patients than in healthy controls, and sHLA-G levels inversely correlated to numbers of peripheral activated T cells, thus suggesting that sHLA-G promotes tumor immune escape through the inactivation T cell responses (Xu*et al*., 2015). This result was conflict with Davidson *et al*. (2005) and Berta (2015) who found reduced expression of HLA-G in effusions obtained after the start of chemotherapy, these data suggest that HLA-G as a possible marker for tumor sensitivity to chemotherapy. The cause of elevation of HLA-G after therapy it's possible that HLA-G in the circulation goes up after therapy as response to global cell death or inflammation, and it has nothing to do with the tumor. Lots of cells make HLA-G so it could be released from dying cells or as part of some general response to this sort of cellular trauma.

Also our study described the immunohistochemical patterns of HLA-G in breast cancer. Overall, 54.29% of breast cancer lesions were classified as HLA-G positive, this agree with de Kruij*et al*. (2010) who reported that the average positivity was shown to be 55% for HLA-G and 60% for HLA-E expression by immunohistochemical analysis, and also agree with that He *et al*. (2010) who showed that HLA-G protein immuno reactivity was observed in 66% (155/235) of malignant cases. The frequency of HLA-G expression varies between patients, theses agree with several studies demonstrated that the expression of HLA-G varies between different types of cancer and even between different studies

in the same type of tumor probably because of the criteria of patient's selection and the methodology used. Previous studies have found elevated expression levels of the HLA-G in tumor tissues (Yie*et al*., 2007). Normally, HLA-G is not expressed on nonmalignant cells. Corresponding to this fact, we found in our study that HLA-G Ab did stain in a considerable number of tumor tissues but in a negligible number of normal breast tissues. In additional, numerous studies observed that 41-66% of breast cancer lesions expressed HLA-G (Yan, 2011; He *et al*., 2010; Jeony*et al*., 2014)

The expression of HLA-G in cancer represents a strategy employed by tumors to avoid immune destruction; *In vitro* data has suggested that HLA-G expression in tumor cells could protect them from CTL and NK cells. Immunohistochemistry's observations of HLA-G expression found on tumor cell as well as infiltrated lymphocyte. These findings are consistent with the observation of many previous studies showed that The expression and potential clinical relevance of HLA-G expression in solid malignancies; was preferentially detected in the tumor lesions and rarely in the adjacent non-tumor tissue, suggesting that it might play a role in tumor development and stimulates the interests in evaluating the potential of HLA-G expression in malignant diagnosis, prognosis and immune therapy target for various cancers (Yan, 2011). The HLA-G positive infiltrating cells bearable consist of macrophage and dendritic cells, thus assented with several studies officiated on tumors found HLA-G expression in macrophages and dendritic cells infiltrating the tumor tissue (Pangault*et al*., 1999). Expression of HLA-G was significantly associated with tumor metastasis and ER; the positive cases of malignant breast tumor were distributed as weak, intermediate and strong, these results, similar to the data of He *et al*. (2010).

In conclusion, our findings revealed that both lesion HLA-G expression and plasma sHLA-G levels were up regulated in breast cancer patients, Furthermore, sHLA-G levels in breast cancer patients might be a useful preoperative biomarker for diagnosis.

REFERENCES

1. Akin, M., Aral, L. A., Yavuz, A., Karabacak, H., Dikmen, K. and Bostanci, H. (2017). Plasma human leukocyte antigen-G (HLA-G) in patients with thyroid cancer. Turk J Med Sci 47: 1263-1266.
2. Amodio, G. and Gregori, S. (2017). The discovery of HLA-G bearing extracellular vesicles: new perspectives in HLA-G biology.Annals of Translational Medicine, Vol 5, No, 6.
3. Bortolotti, D., Gentili,V., Rotola, A., Cassai, E., Rizzo, R. and Dario Di Luca, D. (2014). Impact of HLA-G analysis in prevention, diagnosis and treatment of pathological conditions.*World J MethMetho.* 26; 4(1): 11-25 ISSN 2222-0682
4. Carosella, E.D., Moreau, P., Lemaoult, J. and Rouas-Freiss, N. (2008). HLA-G: from biology to clinical benefits. Trends Immunol. 29, 125-132.
5. Chen, Y., Gao, X. J., Deng, Y. C. and Zhang, H. X. (2012). Relationship between HLA-G gene polymorphism and the susceptibility of esophageal cancer in Kazakh and Han nationality in Xinjiang. Biomarkers 17: 9–15.
6. Davidson, B., M. B. Elstrand, M.B., McMaster, M.T. and *et al*. (2005). HLAG expression in effusions is a possible marker of tumor susceptibility to chemotherapy in ovarian carcinoma. Gynecologic Oncology, 96(1), 42-47.
7. De Kruijf, E. M., Sajet, A., van Nes, J. G. and *et al*. (2010). HLA-E and HLA-G expression in classical HLA class I-negative tumors is of prognostic value for clinical outcome of early breast cancer patients. J *Immunol*; 185: 7452–9.
8. He, X., Dong, D. D., Yie, S. M., Yang, H., Cao, M. and et al. (2010). HLA-G expression in human breast cancer: implications for diagnosis and prognosis, and effect on Allocytotoxic lymphocyte response after hormone treatment in vitro. Ann SurgOncol 17: 1459-1469.
9. Jeony, S., Park, S., Park, B, Park, Y., Kwon, O., Kim, H. (2014). Human leukocyte-G (HLA-G) polymorphism and expression in breast cancer patients. PLOS ONE. Volume 9, issue 5, e98284.
10. König, L., Kasimir-Bauer, S., Hoffmann, O. and *et al*. (2016). "The prognostic impact of soluble and vesicular HLA-G and its relationship to circulating tumor cells in neoadjuvant treated breast cancer patients,"*Human Immunology*, vol. 77, no. 9, pp. 791–799.
11. Liang, S., Ristich, V., Arase, H., Dausset, J., Carosella, E. D. and Horuzsko, A. (2008). Modulation of dendritic cell differentiation by HLA-G and ILT4 requires the IL-6--STAT3signaling pathway. ProcNatlAcadSci U S A, 105:8357–62.
12. Lin, A. and Yan, W. (2015). Human Leukocyte Antigen-G (HLA-G) Expression in Cancers: Roles in Immune Evasion, Metastasis and Target for Therapy. M O L M E D 2 1 : 7 8 2 - 7 9 1.
13. Pangault, C., Amiot, L., Caulet-Maugendre, S., Brasseur, F., Burtin, F., Guilloux, V., Drenou, B., Fauchet, R. and Onno, M. (1999). HLA-G protein expression is not induced during malignant transformation. Tissue Antigens 53:335.
14. Provatopoulou, X., Kalogera, E., Sagkriotis, A., Zagouri, F., Nonni, A., Zografos, G. and Antonia Gounaris, A. (2012).Soluble Human Leukocyte Antigen-G Expression in Patients with Ductal and Lobular Breast Malignancy.ANTICANCER RESEARCH *32*: 1021-1026.
15. Ristich, V., Liang, S., Zhang, W., Wu, J. and Horuzsko, A. (2005). Tolerization of dendritic cells by HLA-G. Eur J Immunol. 35:1133–42.
16. Rizzo, R., Fainardi, E., Rouas-Freiss, N. and Morandi, F. (2017). The Role of HLA-Class Ib Molecules in Immune-Related Diseases, Tumors, and Infections 2016. Journal of Immunology Research. Article ID 2309574, 2 pages.
17. Singer G, Rebmann V, Chen YC, Liu HT, Ali SZ, Reinsberg J, *et al*. (2003). HLA-G is a potential tumor

marker in malignant ascites. Clin Cancer Res. 9:4460–4.

18. Xu, Y. F., Lu, Y., Cheng, H. and *et al*. (2015). High expression of human leukocyte antigen-G is associated with a poor prognosis in patients with PDAC," *Current Molecular Medicine*, vol. 15, no. 4, pp. 360–367.
19. Yan, W. H. (2011). Human leukocyte antigen-G in cancer: are they clinically relevant? Cancer Leu 311: 123-130.
20. Yie, S. M., Yang, H., Ye, S. R., Li, K., Dong, D. D. and Lin, X. M. (2007). Expression of human leukocyte antigen G (HLA-G) is associated with prognosis in non-small cell lung cancer. Lung Cancer 58: 267-274.