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RESEARCH ARTICLE

IMMUNOHISTOCHEMICAL TARGETING OF HLA-G ANTIGEN IN MISCARRIAGE PLACENTAL TISSUES OF IRAQI WOMEN INFECTED WITH HUMAN HERPES VIRUSES TYPES 1, 2, 4 AND 5

DR. BASIM S. AHMED[1], DR. SAAD H. MOHAMMED ALI [2], DR. ZAINAB A. HAMID*[3], DR.ALI H. BAYATI[4]

[1]Department of Pathology, College of Medicine, AL-Mustansyria University, Baghdad, Iraq.[2] Communicable Diseases Research Unit, Baghdad Medical College, Baghdad, Iraq.[3] Department of Microbiology, Baghdad Medical College, Baghdad, Iraq.[4] community health department, Sulaimani Polytechnic University.Email: zainabhamid96@gmail.com

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ABSTRACT

Background: Microbial infections represent a major cause of miscarriage, of which viruses appear to be the most frequently involved pathogens. Among many viral causes of miscarriage, maternal infections caused by Herpes Virus type 1and 2, Cytomegalovirus (CMV) and Epstein-Barr virus infections are important causes. Among many possible causes, HLA-G was implicated in immune mechanisms, and might be involved, in pregnancy loss. **Methods:** Immunohistochemistry technique was used to detect placental infection with Herpes Virus type 1 and 2, Cytomegalovirus, and Epstein-Barr virus as well as HLAG in 40 women with spontaneous miscarriage and in 40 healthy pregnant women in Baghdad/Iraq. **Results:** The HSV-1 protein was detected in (10 %) of placental tissues from miscarriage women, HSV-2 in (37.5%) , EBV- VCA in (22.5%) , and CMV in (37.5%). The positive HLA-G markers were detected in (55.0%) of placental tissues from women with live-born children and (22.5%) of miscarriage placental tissues group. The association between HLAG with HSV-1 among the placental tissues from miscarriage patients was constituting (5%), (10%) with HSV-2, (7.5%) with EBV and (2.5%) with CMV. **Conclusion:** In conclusion, this research work could point for an important role and participation of this marker in protection the fetuses during succeeded full-term pregnancies as well as the highest expressions of HLAG with HSV-2, lower rates of each viral agents especially in the placental tissues of healthy pregnant women than other pointing for its important protecting role.

Key Words: Herpes Virus type 1 and 2, Cytomegalovirus; Epstein-Barr virus; Immunohistochemistry; Miscarriage; Placenta; HLAG.

INTRODUCTION

The causes of abortions in many cases are still unknown [1]. However microbial infections represent a major cause of abortion, of which viruses appear to be the most frequently involved pathogens [2].

Among many viruses, Human Herpes viruses infections of the placenta may be harmful in pregnancy leading to disorders in fetal growth, premature delivery, miscarriage, or major congenital abnormalities [3], and some of them can produce chronic or recurrent maternal infection. In particular, CMV during pregnancy can reach the placenta by viremia, following both primary and recurrent infection, or by ascending route from the cervix, mostly the following reactivation. Herpes simplex virus type 2, and less frequently type 1, cause recurrent infections of the genital tract and can lead to abortion [4]. Epstein-Barr virus, among herpesviruses, has been least associated with occasional abortions [5].

Among many possible causes of abortions, immunological factors might be attributed to abortion, yet these factors have not been clearly elucidated. The HLA-G implicated in immune mechanisms and involved in pregnancy loss.

The aim of this study was to have an insight in the immunological profile in close relationship with the state of abortion through the determining the possible correlation between the expressions of the HLA-G antigens and placental infection with each of viruses under study: HSV-type 1, HSV-type 2, CMV, and EBV, and comparing results with virally- uninfected placentae using immunohistochemistry technique.

METHOD

This study was designed as a retrospective research work that enrolled paraffin embedded placental tissues which were collected from histopathological archives of Teaching Laboratories at AL-Yarmouk Teaching Hospital /Iraq and belonging to (40) female patients with miscarriage as patients' group ,where their ages were ranged between 19 to 43 years, while the (40) placental tissues of the control group were collected from obstetrics and gynecology wards of the same hospital . Expose Mouse and Rabbit Specific HRP \DAB Detection IHC Kit (ab80436 /2013/ Abcam), was used for detection of HSV1- HSV2- CMV - EBV – and HLAG after using their specific primary antibodies. Statistical analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences- version 22). The significance of difference percentages (qualitative data) was tested using Pearson Chi-square test (χ^2-test) with the application of Yate's correction or Fisher Exact test whenever applicable.

RESULTS

The IHC- expressions of the proteins of any of the tested markers were detected as a brownish discoloration or signal at nuclear or cytoplasmic localizations. The HSV-1 protein was detected in 4 out of 40 (10 %) placental tissues from miscarriage women, Herpes simplex virus type-2 antigen was noticed in 15 out of 40 (37.5%) of IHC reactions in the miscarriage placental tissues group. Expression of Epstein-Barr - viral capsid antigen as an IHC signal was detected as a brownish discoloration at nuclear localization. at the placental tissue samples of aborted women showed 22.5% (9 out of 40). The overall expression of CMV protein at nuclear localization was detected in 37.5%. Regarding immunohistochemical staining of HLA-G protein in the placental tissue sections from miscarriage and successfully delivered women, the positive HLA-G markers were detected as a brownish discoloration at cell surface localization. The HLA-G was detected by(IHC) in 55.0% (22 out of 40) of placental tissues from women with live-born children and 22.5% (9 out of 40) of miscarriage placental tissues group.The association between HSV-1 IHC with HLAG proteins among study groups showed that the positive results for both HSV-1 and HLAG were constituting 2.5% (1 out of 40) cases in placental tissues of the miscarriage group.

In control placental tissue group, the positive association results for both HSV-1 and HLAG were 5% (2 out of 40). No significant associations of HSV-1 with HLAG (P>0.005) among the study groups (Table 1).

The positive results for both HSV-2 and HLAG constituted 10% (4 out of 40) cases in placental tissues of the miscarriage group. In control placental tissue group, no positive association results for

both HSV-2 and HLAG was found. No significant associations of HSV-2 with HLAG (P>0.005) among the study groups (Table 2).

The positive results for both EBV-IHC and HLAG-IHC were constituting 7.5% (3 out of 40) of placental tissues among miscarriage group. In control placental tissues group, no positive association results for both EBV and HLAG were found. No significant associations of EBV with HLAG (P>0.005) were observed among the study groups (Table 3). The positive results for both CMV-IHC and HLAG-IHC constituting 2.5% (1 out of 40) of placental tissues among miscarriage group. In control placental tissues group, the positive association results for both CMV and HLAG constituted 10 %(4 out of 40). No significant associations of CMV with HLAG (P>0.005) were observed among the study groups (Table 4).

Table 1: Association between HSV-1 and HLAG in the studied placental tissues.

HLAG IHC Score	Miscarriage Group			Control Group		
	HSV-1 IHC Score			HSV-1 IHC Score		
	Negative	Positive	Total	Negative	Positive	Total
Negative	28 (70%)	3 (7.5%)	31 (77.5%)	18 (45 %)	-	18 (45%)
Positive	8 (20%)	1 (2.5%)	9 (22.5%)	20 (50%)	2 (5%)	22 (55%)
Total	36 (90%)	4 (10%)	40 (100%)	38 (95%)	2 (5%)	40 (100%)
P value		0.900			-	

Table 2: Association between HSV-2- IHC and HLAG in placental tissue among the miscarriage groups.

HLAG IHC Score	Miscarriage Group			Control Group		
	HSV-2 CISH Score			HSV-2 CISH Score		
	Negative	Positive	Total	Negative	Positive	Total
Negative	**20 (50%)**	**11 (27.5%)**	**31 (77.5%)**	**15 (37.5%)**	**3 (7.5)**	**18 (45%)**
Positive	**5 (12.5%)**	**4 (10%)**	**15 (37.5%)**	**22 (55%)**	**-**	**22 (55%)**
Total	**25 (62.5%)**	**15 (37.5%)**	**40 (100%)**	**37 (92.5%)**	**3 (7.5)**	**40 (100)**
P value		**0.625**			**-**	

Table 3: Association between EBV and HLAG in the studied placental tissue groups.

HLAG IHC Score	Miscarriage Group			Control Group		
	EBV CISH Score			EBV CISH Score		
	Negative	Positive	Total	Negative	Positive	Total
Negative	25 (62.5%)	6 (15%)	25 (62.5%)	18 (45%)	-	18 (45%)
Positive	6 (15%)	3 (7.5%)	15 (37.5%)	22 (55%)	-	22 (55%)
Total	31 (77.5%)	9 (22.5%)	40 (100%)	40 (100%)	-	40 (100)
P value		**0.643**			**-**	

Table 4: Association of CMV with HLAG in the studied placental tissues groups.

HLAG IHC Score	Miscarriage Group			Control Group		
	CMV IHC Score			CMV IHC Score		
	Negative	Positive	Total	Negative	Positive	Total
Negative	20 (50%)	11 (27.5%)	31 (77.5%)	16 (40%)	2 (5%)	18 (45%)
Positive	8 (20%)	1 (2.5%)	9 (22.5%)	18 (45%)	4 (10%)	22 (55%)
Total	28 (70%)	12 (30%)	40 (100%)	34 (85%)	6 (15%)	40 (100)
P value		**0.160**				**0.533**

***Significant difference between proportions using Pearson Chi-square test at 0.05 levels**

DISCUSSION

The most frequently involved pathogens during pregnancy appear to be viruses. Herpes simplex virus type 2, and less frequently herpes simplex virus type 1, and occasionally EBV, can cause recurrent infections of the genital tract, so as involve the fetoplacental tissue units [6]. The maternal-fetal interface is known to protect the fetus from destruction by the immune system of its mother [7]. The Human leukocyte antigen (HLA - G) has been considered to play a crucial role in ensuring a successful pregnancy by its participant on the fetal-maternal tolerance [8]. Based on these results, the observed immunological profile of placental tissue of miscarriage group which is nearly in duplicate bulk difference when compared to that of successful pregnant women and in turn these results could point for that the pregnancy losses are aggravated by a 'malfunction' of that arm of immunity system, which seems to fail to protect these fetuses.

No significant association has been revealed in this study between HSV1 infection and HLA-G expression in the placental tissues of miscarriage groups.The current study revealed that the percentage of positive correlation results for both HSV-1 and HLAG was 2.5 (1/40) of miscarriage patients, we hypothesized that infection of extravillous trophoblast cells by HSV-1 may block the expression of cell surface HLA-G molecules that prevent immune rejection of the semi allograft fetal [9].

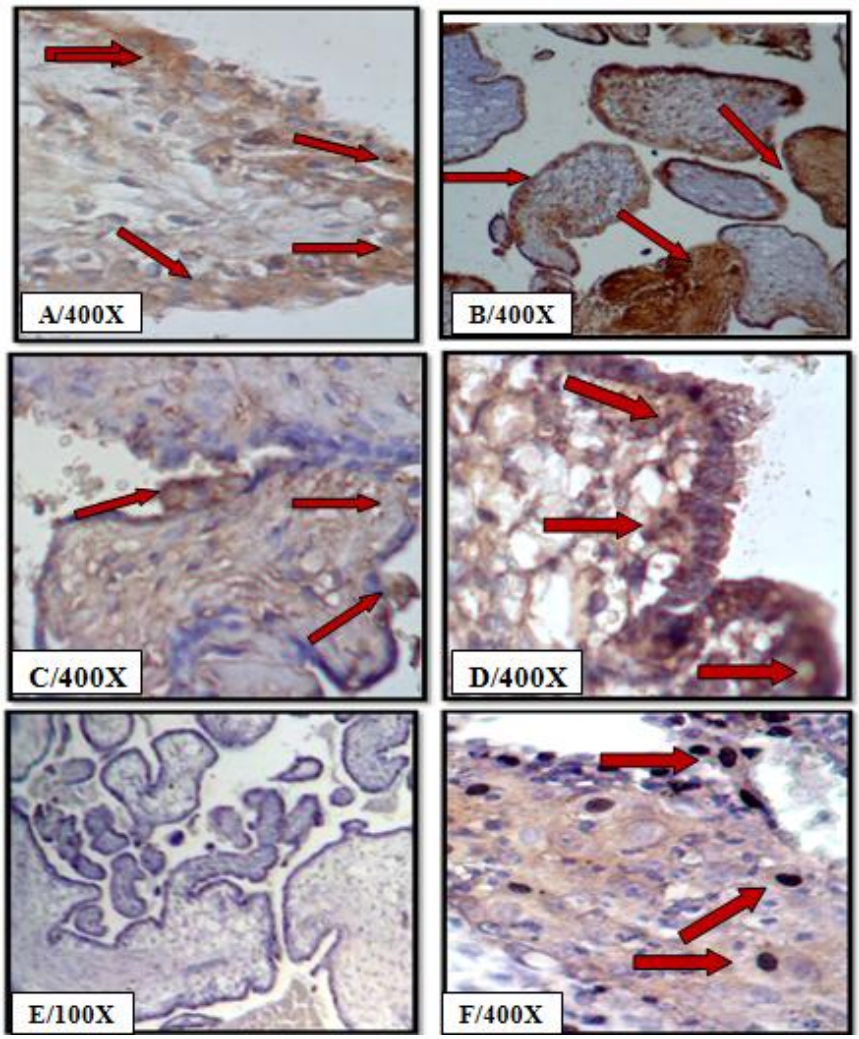

Figure 1: Microphotograph of IHC staining of trophoblastic placental tissues from miscarriage patients (red arrow): A- HSV1 in cell cytoplasm .B- HLAG in cell membrane staining C- HSV2 in the cell cytoplasm. D- EBV in cell nucleus intensity .E- Negative IHC staining for EBV. F- IHC staining for CMV in the cell nucleus.

In the current study, low expression of HLAG was noticed in placental tissues among miscarriage women which involved extravillous trophoblast cells by HSV-1 , This results match with Hideki and colleague ,who showed a reduce or an absent of HLAG expression in placental tissues in aborted patients compared with normal delivered women [10] . The reasons for these discrepancies are not entirely clear. One of the explanation of such decrease in the HLAG expression in placental tissues could be related to the blocks the expression of cell surface HLA-G molecule by HSV1 that prevent immune function of this molecule as a result rejection of the fetal semi allograft. In addition , Hideki and associate have denoted that , extravillous trophoblast cells are uniquely susceptible to HSV-1 infection. These findings support by Norskov-Lauritsen [11] , who had observed that HSV-induced cytopathic effects in mononuclear trophoblast cells , and these cells are susceptible to this virus , moreover Monique [12] have recorded that HSV-1 has induced mostly the HLA- G expression which is up-regulated in these virally infected cells as well as those uninfected neighboring cells.

The association between HSV-2 with HLAG showed that although there were no significant associations in the pattern expression which revealed between the expression of HLAG protein in association with HSV-2 infection among all study groups , the current results revealed that 10% (4 out of 40) showed positive result for both HSV-2 infection and HLA-G molecule expression, while no association was found between HSV-2 infection and HLAG expression in the healthy control group . The present finding could support the suggestion that the expression of HLA – G molecules could abrogate in HSV-infected cells, mediated by the virally encoded inhibitor of the transporters associated with Ag presentation.These observations might provide a link between HSV-2 infection and spontaneous fetal loss and as supported by the results of a previous study [9].

The current finding supported by the observations of Nathalie and associated researchers [13], who denoted that inhibition of intracellular HLA-G transport in human placental cells which were infected with herpes simplex virus type 2.

The association between EBV with HLAG revealed no significant difference as shown in this study between HLAG expression and EBV infections in placental tissues in the studied groups. The association between EBV infection and HLAG expression was revealed 7.5% (3 out of 40) while no association has been noted between EBV infection and HLAG expression in healthy control groups. In fact, the expression levels of HLAG might be associated with the survival of embryos. HLA-G protein expression has been associated with pregnancy success, so this fact suggesting the important role for HLA-G in pregnancy[14; 15]. In the current study, viruses might up-regulate the HLA-G to

escape the host immune attack and as stated also by [14]. The present results are in agreement with Aldrich [16] who noticed that high levels of expression of HLAG appeared to protect the examined allogeneic tissues in the studied cases of pregnancies. On the other hand, in previously discussed studies denoted that HLA-G is suggested to be an important factor in complicated cases of pregnancy such as spontaneous abortion[17]. HLA-G might be a key protein in the shift of the pro-inflammatory response to Th2, which is essential for a successful pregnancy [18]. Odile showed that (EBV)- induced gene that expressed at a high level by B cell lines transformed *in vitro* by EBV. And this gene expressed at a very high level in term placenta, that induce a peptides presented by trophoblast HLA-G molecules [19]. The recognition of MHC class I complex by KIR molecules and the subsequent inhibition of NK cells have been shown to be peptide-dependent. Therefore, this peptide may be involved in HLAG/NK cell receptor interaction in the decidua and may contribute to maternal tolerance. So this may reflect the association between EBV and HLAG in the current study, on the other hand, this correlation may reflect the immune mechanism of HLAG toward this virus. Although HLA-G displays restricted polymorphism but may act like classical MHC class I molecules, is capable of binding peptides and is expressed at the cell surface [19].

The association between CMV with HLAG marker showed no significant difference have revealed between the expression of HLAG and CMV infections in placental tissues among all study groups. Herein, the association between CMV infection and HLAG expression revealed in 2.5% (1 out of 40) of placental tissue of miscarriage women while 4 times increased levels 10% (4 out of 40) have been revealed to have of placental control tissue association between this viral infection and this marker. The current results are pointing to the role of such HLAG immunological markers in affecting the pregnancy outcome. In this respect and supporting our results Hviid, has denoted that cytokines and the epigenetic status of the HLA-G gene are the most important factors which could regulate the HLA-G expression in placental tissues. In addition, in the early pregnancy and at maternal-fetal interface, intrauterine HCMV infection was found to be closely related to the HLA-G expression [20]. Most previous studies on this HLA-G biomarker have suggested that a decreased the expression of HLAG is a companied an alteration and modification of cytokine expression which are commonly observed during HCMV infection [21]. It is known that during CMV infection, the most important cytokines in relation to such viral infection are IL-10 and IFN-γ which has been reported to induce HLA-G expression[22; 23]. Furthermore, the majority of evidence has suggested that a breakdown in immunological maternal-fetal interactions may lead to recurrent or occasional fetal loss [24]. Another possible observation that downregulation of HLAG that the cytoplasmic tail of HLAG appears to confer resistance to human cytomegalovirus mediated downregulation, in addition, the HCMV derived cytokine mimetic IL-10 gene As a result up-regulates HLAG [25].In conclusion the highly significant rate positive - HLAG protein which was detected in placental tissue of women with live-born- children could point for an important role and participation of this marker in protection the fetuses during succeeded full-term pregnancies as well as the highest expressions of HLAG with HSV-2, lower rates of each viral agents especially in the placental tissues of healthy pregnant women than other pointing for its important protection role .

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