HLA-G is highly expressed in the serum of patients with adenomyosis

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Abstract

Objective To evaluate the expression of soluble human leukocyte antigen G(sHLA-G) in serum of patients with adenomyosis.Methods 34 patients with adenomyosis and 31 patients with uterine fibroids diagnosed histologically were selected as subjects. Serum SHLA-G expression level was detected by enzyme-linked immunosorbent assay (ELISA) in patients with adenomyosis and adenomyoma before and after treatment. while CA125 levels were determined using electrochemiluminescence immunoassay. Results The preoperative serum sHLA-g expression level of the adenomyosis group was significantly higher (18.53 ± 1.435 ng/ml) than that of the fibroids group (28.05 ± 2.466 ng/ml) ($P < 0.05$). The CA125 level was also significantly higher in patients with adenomyosis than in controls (18.59 ± 1.673 VS 134.8 ± 30.41U/ml; $P < 0.05$). Serum sHLA-G level in adenomyosis group showed a decreasing trend before and after operation(28.05+-14.38 ng/ml、18.41±8.34 ng/ml、14.45±5.77 ng/ml), serum HLA-G level decreased significantly at 2 days after surgery compared with that before surgery ($P < 0.05$). sHLA-G decreased more significantly in patients with adenomyosis who underwent total hysterectomy (n=20) than in those who underwent partial hysterectomy (n=14), the sHLA-g level of the patients who underwent total hysterectomy (16.04+-4.27ng/ml) was significantly lower than that of the patients who underwent partial hysterectomy (21.79+-11.35 ng/ml)(P<0,05).Conclusion Serum sHLA-G is highly expressed in patients with adenomyosis and showed a positive and significant response to therapy. Suggesting that sHLA-G may be closely related to the immune tolerance process of adenomyosis, and is expected to be a serological marker for prognosis assessment of adenomy.

1.Introduction

Adenomyosis (AM) is a disease caused by the growth of endometrial tissue in the myometrium. It has a high incidence in adult women and often leads to pelvic pain and even infertility. The etiology of adenomyosis remains unknown, currently, the widely accepted theory is the invagination of basalis endometrium. Some scholars have proposed theories such as metaplasia theory, Mueller's duct residue theory, and tissue remodeling theory[1, 2]. Although some scholars proposed the relationship between adenomyosis and immune system, there are relatively few researches in this field [3].Human leukocyte antigen-G (HLA-G) is a kind of classic I human leukocyte antigen class molecules, first discovered in 1987 by Geraghty[4]. It is widely expressed in malignancies, organ transplants, autoimmune diseases, inflammatory diseases, and viral infections. But there are few studies on HLA-G in AM. In our previous studies, it was found that patients with adenomyosis had high HLA-G expression in both the endometrium and the endometrium of the patients with adenomyosis [5]. Suggests HLA-G may play an important role in the process of immune tolerance of AM. Currently, there have been no studies on the expression of this molecule in the blood of patients with adenomyosis. Our study detected the expression level of serum HLA-G in patients with AM and found that HLA-G was highly expressed in the serum of AM patients. Suggesting that HLA-G may play an important role in the process of immune tolerance of AM, and has a certain correlation with disease progress and prognosis, and may be used as a serological molecular marker for the diagnosis and consequently targeted

therapy of AM.

2.Material and methods

2.1 Patients and serum specimen

Serum specimens were obtained from patients surgically operated from Feb. 2019 to Oct.2019 at the Department of Obstetrics & Gynecology, Shandong Provincial Hospital Affiliated to Shandong First Medical University. Included 34 females with a confirmed diagnostic of AM and 31 females with a confirmed diagnostic of fibroids.

Inclusion criteria for patients with AM and controls: patients with AM or fibroids had explicit surgical indication and the diagnoses were confirmed histologically. All patients with AM or uterine fibroids had menstruation before operations and complete clinical data. Both patients and controls who met any of the following exclusion criteria were not eligible to participate in this study: patients with pelvic and ovarian endometriosis, autoimmune disease, malignant tumors of reproductive system and other body parts, and those treated with GnRH-a, Danazol, or gestrinone.

The following data of patients with AM or fibroids were documented: age, smoking history, BMI, menstrual cycle, reproductive history, gynecological ultrasound data, and other basic information. Patients in both groups had detected the level of serum CA125 before operation. AM group: the serum of patients was collected before operation, 2 days after operation and 1 month after operation. Fibroids group: the serum of patients was collected before operation. All specimens were clotted for 2 hours at room temperature before centrifugation for 20 min at 1000×g at 2-8, then collected the supernatant and stored in the specimen bank at -120 . All of the specimens mentioned in this study passed the ethical review and obtained the informed consent of the participants. Baseline clinical characteristics of both study groups are as follows (Table 1), there were no statistically significant differences between the patient and control groups in terms of age, BMI, marriage and childbearing history and smoking history.

Table 1 Baseline clinical characteristics of two group

	AM group (n=34)	**Fbroids group(n=31)**	**P value**
Age(y)	**Age(y)**	**Age(y)**	**Age(y)**
Mean±SD	42.68±5.56	46.10±5.39	¿0.05
range	29-53	31-52	-
BMI	23.69±3.05	23.73±3.04	¿0.05
Smoking	0%	0%	-
Reproductive history	**Reproductive history**	**Reproductive history**	**Reproductive history**
yes	34	30	-
no	0	1	-
Operation time	**Operation time**	**Operation time**	**Operation time**
proliferative phase	31	27	-
secretory phase	3	4	-
Surgical Treatment	**Surgical Treatment**	**Surgical Treatment**	**Surgical Treatment**
partial hysterectomy	20	18	-
total hysterectomy	14	13	-
Size of uterine(cm^3)	216.89±108.83	241.97±212.42	¿0.05

2.2 Surgical Treatment

All the patients received surgical treatment. In the AM group, 14 patients (41.2%) underwent the total hysterectomy, 20 patients (58.8%) underwent partial hysterectomy (the focus was excised and the cervix was

left intact). In fibroids group: 13 patients (58.1%) underwent the total hysterectomy, 13 patients (41.9%) underwent hysteromyomectomy.

2.3 ELISA

Enzyme-linked immunosorbent assay (ELISA) was used to detect the expression of sHLA-G in the retained serum of patients with adenomyosis or fibroid. The concentration of sHLA-G in serum in the patients was determined with the Human MHCG/HLA-G(Major Histocompatibility Complex Class I G) ELISA Kit(E-EL-H1663c). The absorbance values were detected at a wavelength of 450 nm, and the concentrations of sHLA-G was determined through a standard calibration curve constructed with absorbance.

2.4 Statistical Analysis

All data were analyzed via the statistical software package IBM SPSS Statistics 22.0 using a significance level of $p < 0.05$. The sHLA-G expression levels in different groups were analyzed by t test and ANOVA. As the expression levels of CA125 did not show a normal distribution, the diferences of CA125 between groups were analyzed with the Mann–Whitney U test.

3.Results

The results of different groups have been showed in table 2.

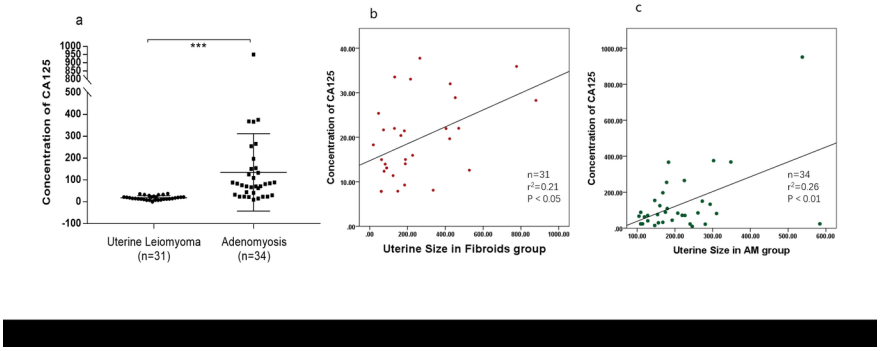
Table 2: The concentrating of CA125 and HLA-G in different groups.

	AM group (n=34)	**AM group (n=34)**	**Fibroids group(n=31)**	**P value**
CA125(U/ml)	134.8±30.41	134.8±30.41	18.59±1.67	¡0.001
sHLA-G(ng/ml)	**sHLA-G(ng/ml)**	**sHLA-G(ng/ml)**	**sHLA-G(ng/ml)**	**sHLA-G(ng/ml)**
before operation	28.05±2.46	28.05±2.46	18.53±1.43	¡0.01
two days after operation	18.41±8.34	18.41±8.34	-	-
one month after operation	14.45±5.77	14.45±5.77	-	-
	total hysterectomy(n=14)	partial hysterectomy(n=20)	-	-
before operation	26.90±11.41	28.85±16.38	-	¿0.05
two days after operation	21.79±11.35	16.04±4.28	-	¡0.05
one month after operation	14.78±7.20	14.22±4.73	-	¿0.05

3.1 The expression of CA125

Our data indicate that the level of preoperative serum CA125 was significantly higher in patients with AM than in controls (134.8±30.41 U/mL in the AM group vs 18.59±1.67 U/mL in the fibroids group, $P < 0.001$) (Fig1a). The overall analysis showed no significant correlation between serum CA125 level and uterine size in all patients ($P > 0.05$). But a certain correlation between serum CA125 level and uterine size in two groups was found when analyzed separately ($P < 0.01$ in AM group; $P < 0.05$ in fibroids group) (Fig 1b, c).

Figure 1:



3.2 The expression of HLA-G

The level of preoperative serum HLA-G was significantly higher in patients with AM than in controls (28.05±2.46 ng/mL in the AM group VS 18.53±1.43 ng/mL in the fibroids group, $P < 0.01$) (Fig 2a). However, there was no significant correlation between serum HLA-G level and uterine size in AM patients ($P > 0.05$). The serum HLA-G level before, 2 days, and 1 month after operative were 28.05±2.46 ng/ml,18.41±8.34 ng/ml,14.45±5.77 ng/ml, displayed a significant downward trend. The level of sHLA-G in patients with AM before operation was significantly higher than that at 2 days after operation ($P < 0.0001$). However, serum HLA-G level 1 month after the operation was not statistically significant compared with that 2 days after the operation (Fig 2b). The level of HLA-G showed a significant downward trend before and after treatment in both patients who underwent a total or partial hysterectomy. There was no significant difference in preoperative sHLA-G level between total hysterectomy and partial hysterectomy (28.85 ±16.38 ng/ml vs 26.89 ±11.40 ng/ml)(p¿0.05). On two days after operation, the level of sHLA-G of patients who underwent total hysterectomy was significantly lower than those who underwent partial hysterectomy. In patients who underwent total hysterectomy, the level of sHLA-G in 2 days after operation was significantly lower than that before operation (16.04 +-4.27ng/ml vs 28.85 +-16.38ng/ml) (Fig 2 c, d).

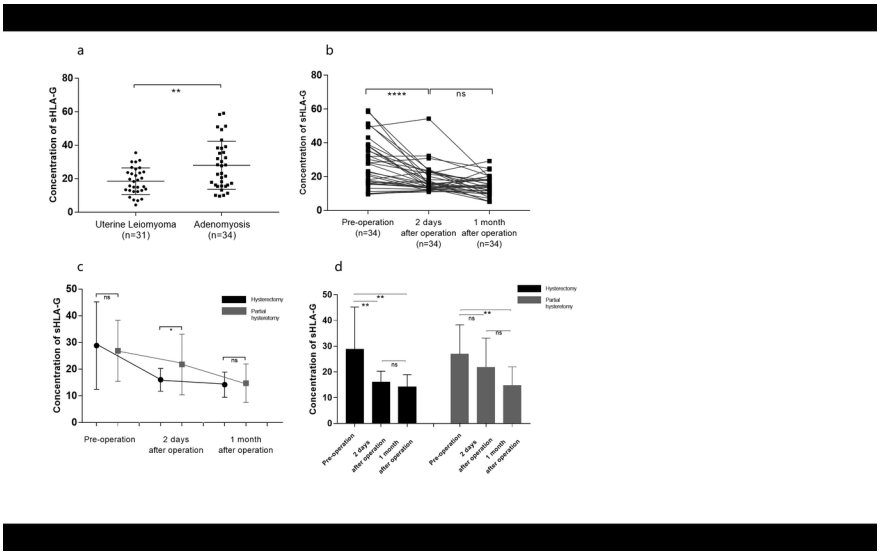


Figure 2:The level of sHLA-G. (a) The level of preoperative sHLA-G was significantly higher in AM group than in controls ($P < 0.01$). (b) The level of sHLA-G in patients with AM before operation was significantly higher than that at 2 days after operation ($P < 0.0001$). sHLA-G level 1 month after the operation was not statistically significant compared with that 2 days after the operation. (c) There was no significant difference in preoperative sHLA-G level between total hysterectomy and partial hysterectomy (p¿0.05). On two days after operation, The level of sHLA-G of patients who underwent total hysterectomy was significantly lower than those who underwent partial hysterectomy. (d)In patients who underwent total hysterectomy, the level of sHLA-G in 2 days after operation was significantly lower than that before operation (p¡0.01)

3.3 Diagnostic value analysis of single and combined detection of CA125 and sHLA-G.

In receiver operating characteristic (ROC) curve analysis, the combined detection had the largest area under the curve (0.936), followed by CA125 (0.920) and sHLA-G (0.697) (Table 3) (Fig 3a). The cut-off value of CA125 and sHLA-G was 35.0 U/ml and 26.79 ng/ml. CA125 have a good diagnostic value for AM and combined detection can improve the sensitivity of an CA125 diagnosis. Further analysis showed a positive correlation between the level of serum CA125 and serum HLA-G ($p < 0.05$) (Fig 3b).

Table 3: ROC curve analysis of single and combined detection of CA125 and sHLA-G.

Index	**AUC**	**95%CI**	**Sensitivity%**	**Specificity%**	**P-value**
sHLA-G	0.697	0.5704-0.8243	50.00	87.10	¡0.01
CA125	0.920	0.8527-0.9879	76.47	93.55	¡0.0001
Combine	0.936	0.8794-0.9934	87.54	81.35	¡0.0001

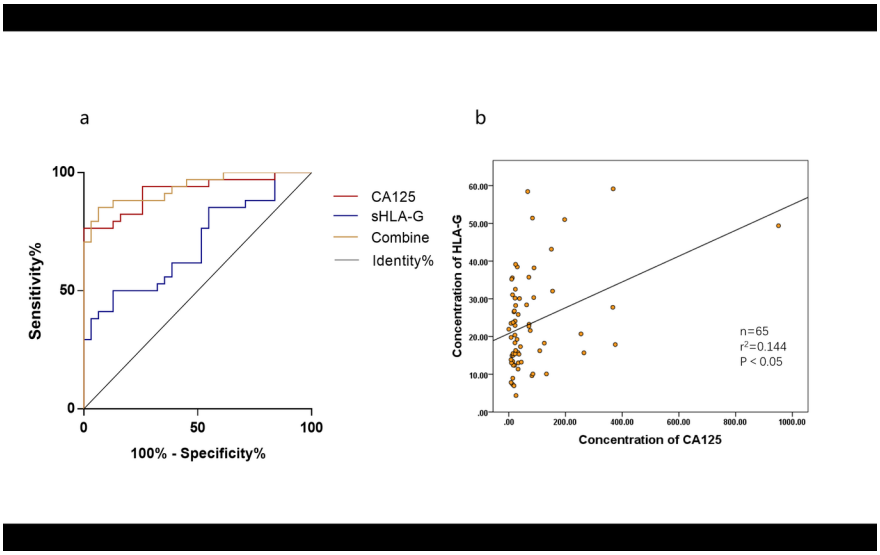


Figure 3: (a) ROC curve analysis. The area under the curve of combined detection of the two indexes is the largest, followed by CA125 and sHLA-G, showing statistically significant differences (P<0.05). (b) Linear correlation analysis showed a weak positive correlation between the level of serum CA125 and serum HLA-G ($p < 0.05$, r^2=0.144).

Discussions

AM has a high prevalence rate in women of childbearing age, and it is closely related to the symptoms of subfertility, dysmenorrhea, and menorrhagia in women of childbearing age[6]. Adenomyosis is characterized by the presence of ectopic endometrial tissue within the myometrium. The ectopic growth of endometrial tissue composed of glands and stroma affects the normal contractile function and peristalsis of uterine smooth muscle, resulting in menstrual bleeding, infertility and adverse obstetrical outcomes[7]. At present, the diagnosis of adenomyosis mainly depends on clinical symptoms and imaging examination such as ultrasonography and MRI.

CA125 and AM

Currently, there is no specific serum marker for adenomyosis, but CA125 has been reported as one of the potential serological markers for differential diagnosis of adenomyosis[8]. In this study, we found the expression of CA125 were higher in AM patients compared with fibroid patients. CA125 has been reported to rise in some severe AM with a uterine size bigger than12 weeks or volume beyond 240 cm^3[9]. Similarly, our data also showed a positive correlation between serum CA125 level and uterine size in patients with AM, and a similar correlation can also be seen in patients with fibroids though the CA125 level of fibroids patients was normal. Our study suggests that CA125 may rise with the enlargement of the uteri with AM and fibroids. In ROC curve analysis, the area under the curve (AUC) of CA125 is 0.920, when the values of CA125 were higher than the cut-off values (35.0 U/ml), the diagnostic specificity of CA125 was 76.47%. As we had ruled out patients with ovarian endometriosis and malignant tumors previously, CA125 showed a high specificity in differential diagnosis of AM and fibroids, but the diagnostic value of CA125 in AM may not be so high as its rise in many other gynecological diseases[10].

sHLA-G and AM

At present, at least 7 subtypes have been reported in HLA-G, including four membrane-bound subtypes (HLA-G1-HLA-G4) and three soluble subtypes (HLA-G5-HLA-G7) [11]. Membrane-bound HLA-G has been reported to express in a variety of malignant tumor cells, including non-melanoma skin cancer, lung cancer, prostate cancer, bladder cancer, glioma, ovarian cancer, endometrial cancer, breast cancer, gastric

cancer, colorectal cancer and renal cell carcinoma, while soluble HLA-G can also be detected in the serum of some patients with ovarian cancer and non-small cell lung cancer [12]. The expression of HLA-G in reproductive system was reported by Kovats et al in 1990, as they first found the expression of HLA-G in cytotrophoblast cells. The later studies suggested that HLA-G molecules played an important role in the process of female pregnancy. Firstly, HLA-G is considered to be related to adverse pregnancy outcomes such as recurrent spontaneous abortion. Secondly, HAL-G can promote the process of uterine spiral artery remodeling, maternal and fetal immune tolerance and affect fetal growth by mediating the interaction of immune cells [13]. The pathogenesis of adenomyosis has long been considered to be related to abnormal immune function and can cause changes in immune function[3]. Some scholars found the relationship between human leukocyte antigen DR(HLA-DR) molecule and endometriosis in the early stage[14]. There are many studies on the expression of HLA-G in endometriosis, but there is still a lack of research in adenomyosis. Although the location of AM is different from that of endometriosis, there may be similar changes in immune function. In previous studies, it was found that HLA-G was highly expressed in ectopic endometrium [15]. Rached et al detected that the concentration of sHLA-G in serum of patients with endometriosis was different from that of patients without endometriosis [16].As one of the important molecules mediating immune tolerance, the detection of soluble HLA-G level in body fluid may reflect the changes of immune function, but there were no studies on the expression of serum HLA-G in adenomyosis.

In this study, we first found that the expression level of serum HLA-G in patients with AM was significantly higher than in patients with fibroids. We also found that the level of HLA-G showed a significant downward trend before and after treatment in AM patients. The level of sHLA-G in AM patients decreased significantly at 2 days after operation. Currently, surgical treatment for patients with adenomyosis mainly includes total hysterectomy or partial hysterectomy[17]. Different surgical procedures may relate to the speed and extent of this decline, the level of HLA-G in AM patients who underwent total hysterectomy may decrease faster than patients who underwent partial hysterectomy. But at 1 month after operation, the level of sHLA-G in AM groups who underwent total hysterectomy or partial hysterectomy all decreased to the level of the control group. sHLA-G also showed a certain diagnostic value in the differential diagnosis of adenomyosis and fibroids. The AUC of the combined diagnosis of the two indexes is larger than that of the two alone, however, the value of combined diagnosis of adenomyosis is not as good as expected. Since the sHLA-G level does not increase with the enlargement of uteri, combine detection may improve the specificity of diagnosis in some conditions.

HLA-G have been found to be up-regulated in diseases such as cancer, viral infection, pregnancy, organ transplants, autoimmune disease, and inflammations in previous studies. It has been found that autoimmune regulatory protein (Aire) can up-regulate HLA-G in thymocytes by increasing cell aneuploid transcription [18]. Cytokines also play an important role in regulating the expression of HLA-G. IFN- γ can be involved in maintaining the expression of HLA-G, while IL-10 may be involved in regulating different subtypes of HLA-G expression [19]. Some hormones are also involved in the regulation of HLA-G expression, such as growth hormone has been shown to up-regulate the expression of HLA-G in liver cells and serum of patients with growth hormone deficiency [20]. Many transcription factors are also involved in the regulation of HLA-G expression. Long-term chromatin cyclization mediated by transcription factors in trophoblasts can control the expression of tissue-specific HLA-G at the maternal-fetal interface [21], and then mediate maternal-fetal immune tolerance. Although the above studies have found many factors regulating the expression of HLA-G, the regulation mechanism of HLA-G expression in tumor cells, grafts and ectopic tissues needs to be further studied. Patients with adenomyosis may mediate the high expression of HLA-G through the above pathway. HLA-G plays an important role in the process of immune tolerance. The role of HLA-G in body fluid mainly depends on binding to specific receptors on the surface of immune cells such as monocytes, T cells, B cells and NK cells, thus inhibiting the function of immune cells and mediating immune tolerance [22]. However, the mechanism of the effect of serum soluble HLA-G on the immune system is still not clear. This study found the high expression of serum HLA-G in patients with AM, suggesting that there may be an immunologic dysfunction of the whole immune system in patients with AM.

Conclusions

In conclusion, through the detection of serum HLA-G in patients with adenomyosis, the high expression of HLA-G in serum of patients with adenomyosis was found for the first time, and through the detection of serum HLA-G level before and after operation, it was found that there was a certain correlation between the decline rate of serum HLA-G and different surgical procedures of AM patients. At the same time, we found that serum HLA-G has a certain value in the diagnosis of adenomyosis, and its combined detection with CA125 can improve the diagnostic efficiency to a certain extent. The main limit of this research is the small number of cases enrolled which has limited the statistical analysis of patient's subgroups.

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