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Soluble HLA-E and Gastroesophageal Reflux Disease: A Novel Association

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ABSTRACT

Background: Gastroesophageal reflux disease (GERD) is a prevalent clinical condition, that affects millions of individuals worldwide.

Objective: To assess the level of soluble HLA-E (sHLA-E) as a biomarker in the diagnosis and immunopathogenesis of GERD patients.

Methods: The case-control prospective study included 40 GERD patients who were consulted at the Gastroenterology Unit of Al-Kindy Teaching Hospital, as along with 40 healthy control subjects. The study period extended from January 2023 to May 2024. Blood was drawn from both groups and serum was separated to assesssHLA-E using a sandwich enzyme-linked immunosorbent assay (ELISA) kit.

Results: There was a statistically significant difference in sHLA-E levels between GERD patients and healthy controls (P=0.021). The median sHLA-E level was significantly higher in GERD patients (0.370 ng/mL) compared to controls (0.148 ng/mL). A receiver operating characteristic (ROC) curve was generated to evaluate the diagnostic performance of soluble HLA-E (sHLA-E) in predicting GERD. The area under the ROC curve (AUC) was calculated to assess the discriminatory ability of sHLA-E with a vlue of 0.649 (95% CI: 0.534-0.752, P=0.021). The optimal cutoff value for sHLA-E was determined to be ≤0.65 ng/mL, with a sensitivity of 85.1%, specificityof 27.3%, positive predictive value of 65.9%, negative predictive value of 69.4%, and accuracy of 35.0%.

Conclusion: The study provides evidence of an association between elevated sHLA-E levels and GERD. It also suggests that sHLA-E has a moderate discriminatory ability as a biomarker in predicting GERD.

Keywords: GERD; sHLA-E; Dyspepsia

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INTRODUCTION

Gastroesophageal reflux disease (GERD) is a common illness that is often treated in gastroesophageal units in many hospitals (1). It typycally diminishes patient's quality of life due to persistent symptoms such as heartburn, epigastric pain, and other extra esophageal symptoms like chronic cough, and pharyngitis (2). There are many risk factors associated with the development of GERD including age, race, gender, *H pylori* infection, and presence of a hiatus hernia (3). Lifestyle factors such as smoking, alcohol consumption, obesity and the use ofdrugs like non-steroidal anti-inflammatory drugs can also contribute to the development of GERD (4). The severity of esophageal mucosa damage is usually directly related to the intensity of clinical symptoms (5). Early diagnosis is crucial in order to prevent complications such as Barrett's esophagus, and adenocarcinoma of the esophagus (6). Diagnosis of GERD is typically based on the clinical presentation of patients, upper endoscopy, esophageal pH monitoring, and capsule monitoring (7). However, data suggests that genetic factors, the presence of local inflammation in the esophageal mucosa, and signals from the nervous system can determine the clinical manifestations of GERD (8). There is a familial genetic predisposition to GERD that is common in both monozygotic and dizygotic twins (9). Additionally, the Human Leukocyte Antigen (HLA) system, which is polymorphic and a marker for disease predisposition has shown an association between HLA-DRB1 *15:01 and GERD in patients who are also *H. pylori* positive (10). Soluble HLA-E (sHLA-E) is an important non-classical HLA molecule that modulates the activation of natural killer cells and cytotoxic T lymphocytes (11). Therefore, this study aims to shed light on the importance of assessing the level of sHLA-E as a biomarker in the diagnosis and immunopathogenesis of GERD.

MATERIALS AND METHODS

This prospective case-control study involved 40 patients with GERD who were consulted at the Gastroenterology Unit of Al-Kindy Teaching Hospital, as well as 40 healthy control subjects. The study period spanned from January 2023 to May 2024. The Scientific and Ethical Committee of Al-Kindy Medical College-University of Baghdad approved the research and consent was obtained from the patients. The inclusion criteria for this study were male or female patients who complained of heartburn, dyspepsia, and epigastric pain. GERD diagnosis was confirmed via upper gastroesophageal endoscopy using a gastroscope: GIFH260; Olympus, Tokyo, Japan and a display screen; Olympus OEV-261H liquid crystal display monitor; Olympus, Tokyo, Japan. Only patients with Grade B were selected and enrolled in this study. The classification was done using, the 2013 ?.

Grade A shows mucosal discontinuity less than five mm in length which may involve one or more areas but does not extend to the top of the mucosal folds Grade B demonstrates mucosal lesion longer than five mm that also do not reach the tops of the mucosal folds.

Grade C involves mucosal lesions that are continious between the mucosal folds and affect less than 75% of the esophagus.

Grade D includes mucosal lesions that affect more than 75% of the esophagus (12).

Exclusion criteria included patients with esophageal or gastrointestinal tumors, esophageal varicose veins, positive *Helicobacter pylori* infection, or current use of drugs such as proton pump inhibitors, antacids, glucocorticosteroids, nonsteroid anti-inflammatory drugs, H2-histamine receptor blockers, calcium channel antagonists, and nitrates.

Demographic data were obtained from both groups (patients and controls), including age, gender, weight, height, body mass index (BMI), smoking status, and address.

Blood was aspirated from both groups and serum was separated for assessment

of sHLA-E using a sandwich enzyme-linked immunosorbent assay (ELISA) kit for quantitative assessment of total HLA-E in serum according to the manufacturer's instructions (Cat. No YLA1602HU, Biont, Bioassay Technology Laboratory, China).

Statistical Analysis

The results of this study were collected using Excel software and analyzed using MedCalc and SPSS software version 25.0. Continuous variables are expressed as mean±Standard Error Mean (SEM), and were analyzed using Student's t-test to assess the level of significance. Other categorical variables are expressed as numbers and percentages and were analyzed using the Chi^2 test with a 95% confidence interval (CI). Receiver operating characteristic (ROC) curve analysis was used to assess the area under the curve (AUC), 95% CI, cut-off value, sensitivity and specificity of the test. Pearson and Spearman correlation analysis were applied to determine the correlation coefficient between different variables. The statistical significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 presents the demographic characteristics of GERD patients and healthy controls. There was a higher proportion of females in the GERD group compared to the control group (72.5% vs. 42.6%) (P=0.006). GERD patients were significantly older than healthy controls (median age 42 vs. 28), and had a significantly higher mean BMI compared to healthy controls (24.8 kg/m² vs. 22.3 kg/m²). As for risk factors a higher proportion of GERD patients were smokers compared to controls (62.5% vs. 35%). These results were in agreement with another study that reported nonerosive GERD disease was more common in females than in males with an increase in age, and sex and hiatal hernias were potential risk factors of GERD (13). The main cause may be due to hormonal factors or differences

Table 1. Demographic Characteristics and sHLA-E levels of GERD Patients and Controls.

Characteristics	GERD Patients No.=40		Healthy Control No.=40		95% confidence interval (CI)	P value
	No.	%	No.	%		
Gender (Male)	11	27.50	23	57.40	0.3151-p0.5406	0.006*
Gender (Female	29	72.50	17	42.60	0.4594- 0.6849	
Age (years)X±SEM	43.93±2.87 (15-85)		34.45±3.13 (11-76)		1.36-17.59	0.023**
Age (Median)	42		28		0.020***	
Age - Q1	30		18			
Age -Q3	56		54			
Age- IQR	26.5		36.25			
Smoking positive	25 (62.5%)		14 (35%)		0.013*	
BMI kg/m² X±SEM	24.799 ±0.36 (17-31)		22.27 ±0.671 (14-29)		0.001**	
sHLA-E (Median)	0.370		0.148		-0.229to-0.015	0.021***
Upper limit	1.530		1.939			
Lower limit	0.038		0.001			
sHLA-E -Q1	0.181		0.082			
sHLA-E - Q3	0.500		0.666			
sHLA-E - IQR	0.319		0.583			

*Significant (Chi^2 test); **Significant (student's t-test); ***Significant (Mann-Whitney test)

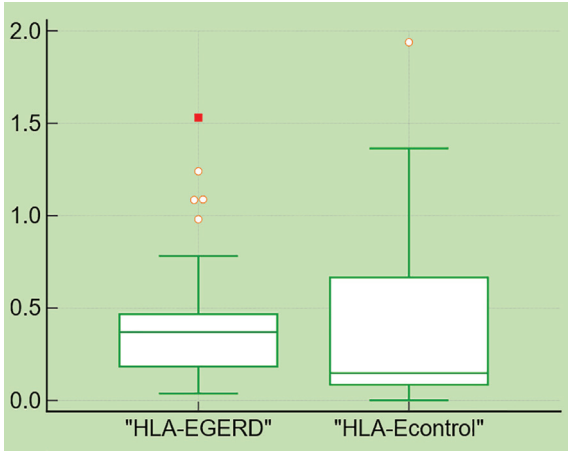

Fig. 1. Median levels of sHLA-E in GERD patients and controls.

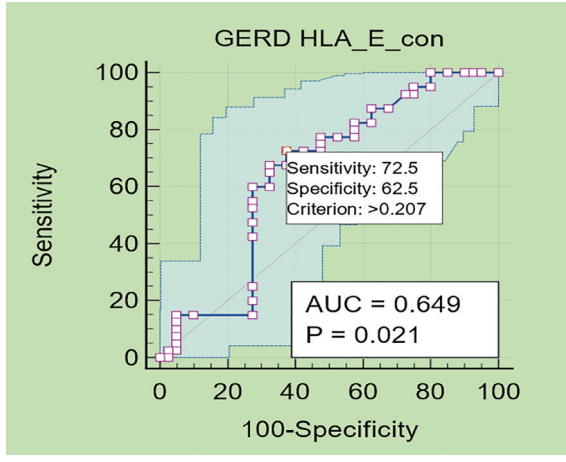

Fig. 2. Receiver Operating Characteristic (ROC) curve plot of sHLA-E for predicting GERD (Area Under the Curve of 0.649 (95% CI=0.534 to 0.752, P=0.021), SEM=0.064, positive predictive value=65.9, negative predictive value=69.4, and accuracy=0.350.

in lifestyle behaviors (14). GERD disease may be more common with aging due to changes in the tone of the lower esophageal sphincter and esophageal motility. One of the main risk factors is increased intra-abdominal pressure which is associated with obesity and a high body mass index (15). Another factor was smoking which is a well-established risk factor for GERD, and its higher frequency in the GERD group aligns with previous studies (16).

A statistically significant difference was observed in sHLA-E levels between GERD patients and healthy controls (P=0.021). The median sHLA-E level was significantly higher in patients with GERD (0.370 ng/mL) compared to controls (0.148 ng/mL) (Fig. 1). This difference may indicate an altered immune response. While there is limited research on sHLA-E and GERD, previous studies have shown associations between sHLA-E andother diseases such as viral and bacterial infections, cancer, and autoimmune disorders (17).

Pearson and Spearman correlation analyses were conducted to assess the relationship between sHLA-E levels and demographic variables (age, BMI, gender, and smoking) in GERD patients (Table 2). No significant correlations were observed between sHLA-E levels and age, BMI, or gender. A weak positive correlation was found between sHLA-E levels and smoking status (r=0.209, P>0.05) but, this correlation was not statistically significant. The lack of significant correlations between sHLA-E levels and age, BMI, or gender suggests that these factors might not be major determinants of sHLA-E expression in GERD patients. The weak non-significant positive correlation with smoking status is intriguing. It is possible that smoking might influence the immune response and contribute to altered sHLA-E expression. However, studies have explored the role of HLA-E in other inflammatory diseases such as oral squamous cell carcinoma, and autoimmunity due to the immunosuppressive effect of HLA-E on NK cells. Women showed significantly higher sHLA-E levels than men and there were a gender-specific differences (18). HLA-E present peptides to the NKG2A receptor on NK (natural killer) cells, which helps regulate immune tolerance and modulate NK cell activity. The interaction between sHLA-E and the NKG2A receptor inhibits NK cell activation and can also interact with T cells to regulate their function.

Additionally, Fig. 2 showed a receiver operating characteristic (ROC) curve that was generated to assess the diagnostic performance of soluble HLA-E (sHLA-E) in predicting GERD. The area under the ROC curve (AUC) was calculated to evaluate the discriminatory ability of sHLA-E. AUC=0.649

Table 2. Pearson and Sperman's correlation analysis between sHLA-E and other variables in GERD patients.

Variables	sHLA-E Patients pg/ml No.=40	Age (years)	BMI	Gender	Smoking
sHLA-E Patients pg/ml No.=40	1	-0.085	-0.092	-0.202	0.209

(95% CI: 0.534-0.752), P=0.021. The optimal cutoff value for sHLA-E was determined to be ≤0.65 ng/mL, with a sensitivity of 85.1%, specificity of 27.3%, positive predictive valueof 65.9%, negative predictive value of69.4%, and accuracy of 35.0%. The ROC curve analysis indicates that sHLA-E has moderate discriminatory ability in predicting GERD, with an AUC of 0.649. However, the sensitivity and specificity of sHLA-E at the optimal cut-off value are relatively low, suggesting that it might not be a precise diagnostic biomarker. The positive and negative predictive values are moderate in validity, indicating its limited clinical utility in predicting GERD. Limited research is found on this subject, however, other studies have suggested Takayasu arteritis and schizophrenia as useful biomarkers of disease activity (19, 20).

CONCLUSION

The study provides evidenceof an association between elevated sHLA-E levels and GERD, showing moderate discriminatory ability as a biomarker in predicting GERD.

LIMITATION OF THE STUDY

The small sample sizesuggests that further research is needed to explore sHLA-E as a diagnostic or therapeutic target for GERD.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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