HVEM/BTLA Immune Checkpoint Expression in Development of Gastric Cancer

Maryam Azarafza
Mazandaran University of Medical Sciences
Mohsen Tehrani
MazUMS: Mazandaran University of Medical Sciences
Reza Valadan
Mazandaran University of Medical Sciences
Iradj Maleki
Mazandaran University of Medical Sciences
Seyed Mohammad Mehdi Ghaffari Hamedani
Mazandaran University of Medical Sciences
Alireza Ghanadan
Tehran University of Medical Sciences
Reza Alizadeh-Navaei
Mazandaran University of Medical Sciences
Abolghasem Ajami (ajami36@gmail.com)
Mazandaran University of Medical Sciences https://orcid.org/0000-0002-0062-8792

Research Article

Keywords: HVEM, BTLA, Gastric cancer, soluble HVEM, Immune checkpoint

Posted Date: May 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-522573/v1

Abstract

Purpose: Regarding the role of B- and T-lymphocyte attenuator/Herpesvirus entry mediator (BTLA/HVEM) in tumorigenesis, this research was conducted to determine the HVEM/BTLA expression in development of gastric cancer.

Methods: The statistical population of this study consisted of 114 patients, who were divided into 3 groups of non-ulcer dyspepsia (NUD; n=32), intestinal metaplasia (IM; n=19), and gastric cancer (GC; n=63). BTLA and HVEM in gastric biopsies were evaluated using SYBR Green-based on real-time PCR and immunohistochemistry. In this research, soluble HVEM (sHVEM) concentration and anti-*Helicobacter pylori* IgG antibody were assessed in sera of all study subjects.

Results: Our result showed that in contrast to mRNA, HVEM protein expression was significantly higher in the GC group, compared to that in the NUD and IM groups ($P<0.0001$ and $P=0.0002$, respectively). It was found that BTLA mRNA expression was significantly higher in the GC group than in the IM and NUD groups ($P=0.004$ and $P=0.0003$, respectively). It was also significantly elevated in the advanced stages of GC ($P=0.039$). IHC results showed significant expression of BTLA in GC and IM groups, compared to the NUD group ($P=0.0002$, and $P=0.008$, respectively), and advanced stages than early stages of gastric cancer ($p = 0.005$). The sHVEM concentration was also higher in the GC group than in the NUD groups ($P=0.001$).

Conclusions: High expression of BTLA/HVEM and sHVEM suggested that this inhibitory pathway is involved in immune regulation and progression of IM and GC. Therefore, these molecules can be used for the diagnosis and prognosis of GC.

Introduction

Gastric cancer (GC) is one of the most common malignancies with high morbidity and mortality throughout the world. Despite recent advances in diagnosis and treatment, GC is still the third leading cause of cancer-related deaths worldwide, with an estimated 783,000 deaths in 2018 [1]. The pathogenesis of GC is suggested by the Correa pathway as the disease progresses from gastritis to atrophic gastritis, intestinal metaplasia (IM), dysplasia, and eventually to cancer [2]. In this process, inflammatory and immune responses against *Helicobacter pylori* are known as critical risk factors for IM and GC [3]. Since most GC patients are diagnosed in advanced stages, current treatments are not sufficient to increase the survival of patients [4]. Therefore, understanding the molecular mechanisms of disease can play an essential role in improving prognosis and treatment.

Tumors evade the immune system through various mechanisms, one of which is using immune checkpoint receptors that inhibit the function of tumor-specific T-cells. The role and therapeutic aspects of immune checkpoint receptors (e.g., B7/cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1(PD-1)/programmed death-ligand 1 (PD-L1) have been implicated in different cancers [5, 6]. However, some tumors do not appropriately respond to these treatments [7]. The

results of recent studies suggest that other inhibitory receptors can also play a critical role in antitumor immune responses [8], among which, the potential role of herpesvirus entry mediator (HVEM) has been highlighted [9-11].

HVEM is a member of the tumor necrosis factor receptor superfamily (TNFRSF) that is expressed on a wide range of hematopoietic and non-hematopoietic cells [12]. This mediator has been proposed as a molecular switch in the immune system due to the diversity of ligands and their signaling pathways [13]. It transduces co-stimulatory signals and stimulates the immune responses via binding to its ligand, homologous to lymphotoxin, exhibits inducible expression and competes with herpes simplex virus glycoprotein D for binding to herpes virus entry mediator, a receptor expressed on T lymphocytes (LIGHT) and lymphotoxin-α (LTα). In contrast, it transduces co-inhibitory signals via binding to BTLA and CD160 [9, 14].

The BTLA/HVEM inhibitory pathway seems to be more dominant in the HVEM network, as indicated by increased T-cell activation in HVEM-deficient mice [15]. BTLA belongs to the immunoglobulin superfamily, which is expressed on different subsets of T-cells, as well as on antigen-presenting cells and natural killer (NK)cells [16]. Herpesvirus entry mediator interacts with BTLA in cis- or trans-configurations, where cis interaction helps T-cells to stay in their naive state, while, trans interaction induces an inhibitory signal via immunoreceptor tyrosine-based inhibition motifs (ITIM) and survival signals [17].

Recently, HVEM molecular network has been investigated in hematopoietic cancers and solid tumors, which showed that the BTLA/HVEM pathway not only leads to decreased proliferation and functions of tumor-specific cytotoxic (CD8+) T-cell but also plays a prognostic role [18-21]. The BTLA/HVEM interaction could be a promising candidate for cancer immunotherapy as blockade of this interaction led to the restoration of tumor-specific (CD8+) T-cell functions [20]. Furthermore, immune responses could be modulated by soluble HVEM (sHVEM). In autoimmune and inflammatory diseases, the elevated level of sHVEM in serum has been correlated to disease severity and can be used as a prognostic factor [22]; however, little is known about its role in tumor immunity [23, 24].

There is scarce knowledge about the role of the BTLA/HVEM pathway in Gastric cancer [25-27]. Moreover, the expression of BTLA/HVEM and sHVEM in a trend of gastritis to intestinal metaplasia and GC have not been investigated. This study aimed to determine the expression of BTLA/HVEM at the levels of messenger ribonucleic acid (mRNA), protein, and soluble form in the Correa pathway in gastritis, metaplasia, and different stages of Gastric cancer in the context of *H. pylori* infection.

Materials And Methods

Study population

The statistical population of this research (n=114) consisted of 80 male and 34 female patients, undergoing esophagogastroduodenoscopy in Imam Hospital or Tooba Outpatient Clinic, affiliated with Mazandaran University of Medical Sciences, Sari, Iran, within 2017-2019. Initially, the cases were

subjected to endoscopic and histopathological examinations and were then divided into three groups of non-ulcer dyspepsia (NUD, n=32) with normal histology serving as a control group, intestinal metaplasia (IM, n=19), and gastric cancer (GC, n=63) (Table 1). None of the study subjects had a history of chronic inflammatory or autoimmune disorders or *H. pylori* eradication therapy or received non-steroidal anti-inflammatory drugs during the past 2 weeks. Informed consent was obtained from all the cases before endoscopy/biopsy and blood sampling. The patients' demographic information was gathered from their medical records. As a protocol, two biopsies were obtained from antrum and corpus separately from NUD and IM cases and the tumor tissue of gastric cancer objects. At the time of endoscopy, 2.5 mL of the blood sample was collected from the patients. According to the medical records, tumors were classified according to the tumor, nodes, and metastases staging system. Histological sections from formalin-fixed and paraffin-embedded gastric biopsies were prepared and stained using modified Giemsa and hematoxylin-eosin. The presence of *H. pylori* infection was determined by histopathological examination, including Giemsa staining, and a positive result for a rapid urease test performed on at least one additional biopsy sample. This study was approved by the Ethics Committee of the Mazandaran University of Medical Sciences.

Immunohistochemistry

Formalin-fixed and paraffin-embedded tissues from gastric biopsies were collected and cut into 2-3 μm sections, which were mounted on poly L-lysine coated slides, and deparaffinized in xylene, and rehydrated. Then the slides were autoclaved for 15 min in citrate buffer (pH 6.0) for antigen retrieval and, subsequently, incubated with 3% H_2O_2 to block endogenous peroxidase activity for 15 min. Each slide was blocked with normal goat serum (Cyto Matin Gene, Iran) for 15 min at room temperature in a humid chamber. Then each section was incubated with Rabbit polyclonal anti-BTLA antibody (1:200 dilution, PA5-22248, Invitrogen, USA) at room temperature for 2 h or Rabbit polyclonal anti-HVEM antibody (1:50, PA5-26103, Invitrogen, USA) at 4°C overnight.

The slides were washed in TBS buffer and incubated in secondary antibody (goat anti-rabbit secondary antibody, Thermofisher, USA) at room temperature for 2h. Afterward, each slide was detected with diaminobenzidine reagent sets (Biopharma, China) for 5-7 min. For negative control, rabbit IgG was used in the immunostaining procedure. Finally, the slides were counterstained with haematoxylin, dehydrated by gradient alcohol, and mounted. Immunohistochemistry (IHC) results were evaluated by a pathologist in a blinded manner; therefore, a semi-quantitative H-score approach was used to assess the intensity and percentage of positively stained cells. The H-score was calculated by the following equation:

The percentage of positively stained cells was classified into 5 groups, namely less than 5% staining of cells recorded as negative (0), 5-25%; 1, 26%-50%; 2, 51%-75% ; 3, and more than 75%; 4. The staining intensity was graded as 0=negative staining, 1=weak, 2=moderate, and 3=strong. The final immunostaining score was calculated using multiplying the intensity and the percentage of positively stained cells.

Quantitative real-time polymerase chain reaction

Total RNA was extracted from fresh gastric biopsies in RNAlater solution using the Qiagen RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The quality and quantity of RNA were evaluated by a nano-spectrophotometer (Thermo Fisher Scientific Inc, Massachusetts, USA) and agarose gel electrophoresis, respectively. A volume of 1 µg of RNA was reversely transcribed by complementary a DNA synthesis kit (Yekta Tajhiz, Iran), according to the manufacturer's protocol.

Polymerase chain reaction (PCR) was performed using Takara SYBR Green MasterMix with ROX kit (Takara, Japan) on a 96x Bio-Rad real-time PCR system (Bio-Rad, USA) with the following primers: BTLA forward: CCATATCTGGACATCTGGAACATC, reverse: CACAGTATTTCACAGGGCATTCTA; HVEM forward: CTGACTCTCGGTGCCTCC, reverse: CCACTTCTGCATCGTCCA. Hypoxanthine guanine phosphoribosyltransferase (HPRT) forward: CTGGCGTCGTGATTAGTGATGATG, reverse: CAGAGGGCTACAATGTGATGGC was used as an internal control. All reactions were performed in duplicate.

Polymerase chain reaction thermal cycle parameters were: initial denaturation at 95°C for 30 seconds, followed by 40 cycles at 95°C for 15 seconds, 60°C for 45 seconds, and 72°C for 30 seconds. A melting curve analysis was displayed to ensure the specificity of the PCR products.

Enzyme-linked immunosorbent assay

Soluble HVEM concentration was measured (in pg/ml) in patients' sera using human HVEM/TNFRSF14 DuoSet enzyme-linked immunosorbent assay (ELISA) Kit (R&D Systems, USA) according to the manufacturer's instructions. Moreover, anti-H. *pylori* IgG antibody was assessed in sera of all patients using the AccuBindTM ELISA kit (Monobind, USA) according to the manufacturer's instructions. The concentrations higher than 20 U/mL were considered positive.

Statistical analysis

The collected data were analyzed in SPSS software (version 21) using one-sample Kolmogorov-Smirnov test (for normal distribution of data), as well as t-test, Mann-Whitney U test, ANOVA, and Kruskal-Wallis tests. The p-values of less than 0.05 were considered significant.

Results

BTLA and HVEM expression in GC, IM, and NUD patients

BTLA and HVEM mRNA expression levels in gastric biopsies from GC, IM, and NUD groups were evaluated by qRT-PCR and normalized to HPRT. There was a positive increasing trend of BTLA mRNA levels in gastric tissues from patients with GC, compared with those in the IM and NUD groups (P=0.004 and P=0.0003, respectively). However, no significant increase was observed in BTLA expression between patients with metaplasia and those in the NUD group (P=0.716) (Figure 1A). According to Figure 1B, the

HVEM mRNA expression showed a slight increase in IM patients, compared to that in the NUD group; however, this increase was not statistically significant (P=0.685). Furthermore, HVEM mRNA expression in patients with GC was not significantly increased, compared to that in the NUD and IM groups (P=0.493 and P=0.984, respectively).

BTLA and HVEM mRNA expression in advanced clinical stages of GC

The expression of HVEM and BTLA mRNA in different GC stages was also investigated to clarify the role of HVEM and BTLA in the progression of GC. Patients were divided into two groups of early (I and II) and advanced stages (III and IV). The results showed that the expression of BTLA mRNA was significantly higher in advanced stages (III and IV) than in early stages (P=0.039) (Figure 2A), while HVEM mRNA expression showed no significant difference in advanced stages of the disease, compared to the early stages (P=0.975) (Figure 2B).

HVEM and BTLA protein expression in GC, IM, and NUD groups

Herpesvirus entry mediator HVEMand BTLA protein expressions of gastric biopsy samples were analyzed using IHC based on a semi-quantitative H-score system to evaluate the relation between mRNA and protein expression. and BTLA are often observed in the cytoplasm of cancer and immune cells, although nucleus staining has also been observed in some cancer cells. In the semi-quantitative H-score system, GC tissues showed higher expression of HVEM, compared to NUD and IM groups (P<0.0001 and P=0.0002, respectively). However, there was no significant difference in HVEM protein expression between the IM and NUD groups (P=0.242) (figures 3A,B,C,G). BTLA protein was also more expressed in patients with GC and IM than those in the NUD group (P=0.0002 and P=0.008, respectively); nevertheless, no significant difference was revealed between the GC and IM groups in this regard (P=0.813) (figures 3D,E,F,H).

BTLA and HVEM protein expression in GC patients at advanced clinical stages

Although the expression of HVEM protein was higher in advanced stages of GC, the semi-quantitative H-score system did not show a significant increase in advanced stages, compared to early stages (P=0.616) (figure 4 (A,B,C). Evaluation of BTLA in different stages of GC displayed a growing trend with disease progression; consequently, BTLA expression in advanced stages (III and IV) was significantly up-regulated, compared to early stages (P=0.005) (figures 4(D,E,F).

sHVEM concentration in GC, IM, and NUD groups

To further illustrate the HVEM/BTLA role in the development of GC, serum concentrations of HVEM were measured in all study groups. Based on Figure 5A, sHVEM concentration was found significantly higher in the sera of GC patients than in those of NUD patients (P=0.001). In contrast, sHVEM concentration in the IM group was not significantly different, compared to that in the NUD and GC groups (P=0.095 and P=0.721, respectively). Furthermore, increased sHVEM level in advanced disease stages showed no

statistical significance, compared to early stages (P=0.09) (Figure 5B). These data suggested that sHVEM level was correlated with the development and progression of GC.

Association of HVEM and BTLA protein expression with *Helicobacter pylori* infection

The serum concentration of anti-*H. pylori* IgG was measured using the ELISA kite, according to which, concentrations higher than 20 U/mL were considered positive. As a result, 63.3%, 70.6%, and 52.9% of the patients with NUD, IM, and GC were diagnosed positive, respectively. The findings of recent studies confirmed that the expression of immune-checkpoint receptors could be associated with *H. pylori* infection [28]. Therefore, in this study, the BTLA/HVEM expression was assessed in *H. pylori*-positive and -negative samples. Regarding, *H. pylori*-positive samples had a lower H-score of HVEM than *H. pylori*-negative ones (P=0.01). However, BTLA protein expression was not significantly different between *H. pylori*-positive and -negative samples (P=0.274).

Discussion

Based on the result of the current study, there was a significant difference in BTLA expression among NUD, IM, GC groups, as well as between early and advanced stages of GC. Therefore, BTLA may be involved in disease progression from gastritis to gastric cancer. Several studies have focused on investigating the functions of BTLA signaling inhematopoietic and non-hematopoietic malignancies [29-31]. In melanoma, HVEM expression on melanoma cells inhibited the proliferation and interferon-gamma production by BTLA+ tumor-specific CD8+ T-cells, which subsequently led to disease progression. Additionally, CpG vaccination of melanoma patients led to the downregulation of BTLA, along with the functional restoration of tumor-specific CD8+ T-cells [20]. In a mouse model of mammary carcinoma, downregulation of BTLA on NKT cells promotes tumor immune control [32]. On the other hand, the up-regulation of BTLA on tumor-specific immune cells in advanced stages of gall bladder carcinoma [33], hepatocellular carcinoma [34], and non-small cell lung carcinoma [35] has been reported to be associated with tumor progression.

BTLA shares structural similarities [36] and redundancy function with PD-1 [37]. In this regard, co-blockade of PD-1/PD-L1 and BTLA/HVEM could be considered a useful tool in GC immunotherapy [38, 39].

The results of our study showed an increase in HVEM protein among patients in the IM and GC groups; nonetheless, no increase was observed in mRNA expression. Different patterns of HVEM protein and mRNA expression have been reported in human malignancies. The elevated levels of HVEM expression on cancer cells in esophageal [40], colorectal [10], ovarian [41], and renal cell cancers [42] were associated with the lower number of tumor-infiltrating lymphocytes and lower survival rate. This finding was inconsistent with those reported in other studies [43, 44]. HVEM, as an immune checkpoint receptor, has a dual function. In other words, it transduces stimulatory signals to T-cells via ligation to LIGHT or LTα, while it transduces inhibitory signals to T- and B-cells via binding to BTLA [9, 14]. Since the obtained data

in this research showed higher expression of BTLA in IM and GC, it can be concluded that BTLA/HVEM up-regulation could lead to reduced immune responses against tumor cells.

The role of HVEM is not restricted to the membrane form; in this regard, a soluble form of HVEM has also been described [27, 45]. Therefore, in this study, the sHVEM concentration was examined in the sera of all subjects. Our findings showed that sHVEM concentrations were significantly higher in GC than in NUD and IM groups. It was also higher in advanced stages of GC, although the latter data was not statistically significant. These results were in line with those of a previous study [27]. The increased concentrations of sHVEM in allergic and autoimmune diseases, such as allergic asthma, rheumatoid arthritis, and atopic dermatitis, have been associated with disease progression [22, 46]; however, little is known about the role of sHVEM in tumor immunology. In cutaneous T-cell lymphoma, high levels of sHVEM were reported to be associated with disease severity and activation of T-helper (Th) 2 responses [23]. Moreover, increased sHVEM concentrations in sera of hepatocellular carcinoma patients were accompanied by decreased membrane-bound (m)HVEM at peripheral blood lymphocytes and advanced disease stages [47].

The results of studies have shown that sHVEM has a higher affinity for binding to LIGHT than to other HVEM ligands [16]. Trans-interaction of membrane HVEM-LIGHT transduces co-stimulatory signals, which leads to T-cell activation and inflammatory response [16, 48]. Consequently, sHVEM can play an immune inhibitory role via binding to LIGHT and blockade of its ligation with mHVEM, which is considered an immune escape mechanism of the tumor cell. In this regard, the findings of studies performed on rats indicated that the interaction of HVEM-LIGHT could decrease T-cell responses in autoimmune diseases [49]. Moreover, the soluble immune checkpoint receptors have been reported to be able to function as immune adjuvants or decoy receptors, and thereby, influence the efficacy of immunotherapy. In patients receiving ipilimumab treatment, elevated levels of soluble CTLA-4 were associated with better clinical outcomes [50]. In this respect, further investigations on the soluble forms of immune checkpoint receptors can improve the success rates and reduce the side effects of immunotherapies.

In the present study, a significant association was observed between the *H. pylori* and HVEM expression, as the presence of *H. pylori* in the tissue was associated with lower expression of HVEM protein. In other words, *H. pylori* might benefit from the down-regulation of the HVEM protein expression. *Helicobacter pylori* not only induced CD4+ T-cell responses toward Th-1 and Th-17 cells but also could modulate immune responses via altering the expression of immune checkpoint receptors in epithelial cells surface. In this regard, the results of studies have shown that Cag A and type IV secretion system in gastric epithelial cells cause the up-regulation of PD-L1 and down-regulation of inducible costimulator-ligand (ICOS-L)[28]. Therefore, it is possible to presume that *H. pylori* may modulate the BTLA/HVEM expression in gastric epithelial cells. Since BTLA has a low expression on Th2 cells, it can be inferred that the BTLA/HVEM pathway is more involved in regulating Th1 immune responses [51]. Given these findings and our result, it is possible that *H. pylori* maintained Th1 and Th17 responses in gastritis through reducing HVEM expression on the epithelial cells, and thereby, inhibited the interaction between the HVEM on epithelial cells and BTLA-expressing immune cells.

In summary, the findings of our study demonstrated the role of the BTLA/HVEM molecular network in the process of the development and progression of GC. Moreover, increased expression of BTLA, HVEM, and sHVEM in GC can be deliberated as a diagnostic factor, compared to NUD. Since BTLA/HVEM has a crucial role in the inhibition of immune responses against tumors, targeting the BTLA/HVEM signaling pathway could be suggested as a therapeutic approach for GC.

Declarations

Authors' contributions

All authors contributed to the study. Abolghasem Ajami and Mohsen Tehrani designed and conducted the research. Maryam Azarafza carried out the assays, contributed to data collection, and prepared the manuscript. Iradj Maleki and Mohammad Mehdi Ghaffari-Hamedani provided the samples. Reza Valadan helped in PCR optimization. Reza Alizadeh-Navaei contributed to data analysis and interpretation. Alireza Ghanadan helped in the evaluation of IHC results. Maryam Azarafza, Abolghasem Ajami, and Mohsen Tehrani prepared the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to appreciate all the patients, their families, departmental nursing, and staff from all units that participated in this study. They also express their gratitude to Dr. Omid Emadian (Department of Pathology, School of Medicine, Mazandaran University of Medical Sciences) and Hadi Hossein-Nataj for their support during IHC test setup and to Dr. Zahra Hosseinikhah and Saeid Taghiloo for their assistance in data analyses.

Conflicts of interest

The authors declare that there is no conflict of interest.

Funding

This research was financially supported by Mazandaran University of Medical Sciences, grant number MCBRC-MAZUMS-2843

Ethical approval

All procedures performed in this study were in accordance with the ethical standards of the Research Committee of Mazandaran University of Medical Science

Consent to participate

Written informed consent was obtained from all subjects before endoscopy/biopsy and blood sampling.

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Tables

Table.1

Demographic and major clinical and paraclinical findings of study subjects

Variable		Non-ulcer dyspepsia	Intestinal metaplasia	Gastric cancer
Study sample (n)	Male	22	10	48
	Female	10	9	15
Age (year)	Mean±SD	46.56±16.60	57.36±15.53	67.17±9.78
Age (Range)		19-85	21-82	37-89
Tumor grade	G1	-	-	6
	G2	-	-	29
	G3	-	-	28
Tumor stage[a]	I	-	-	6
	II	-	-	16
	III	-	-	14
	IV	-	-	19

[a] Tumor stage information of some patient not available

Figures

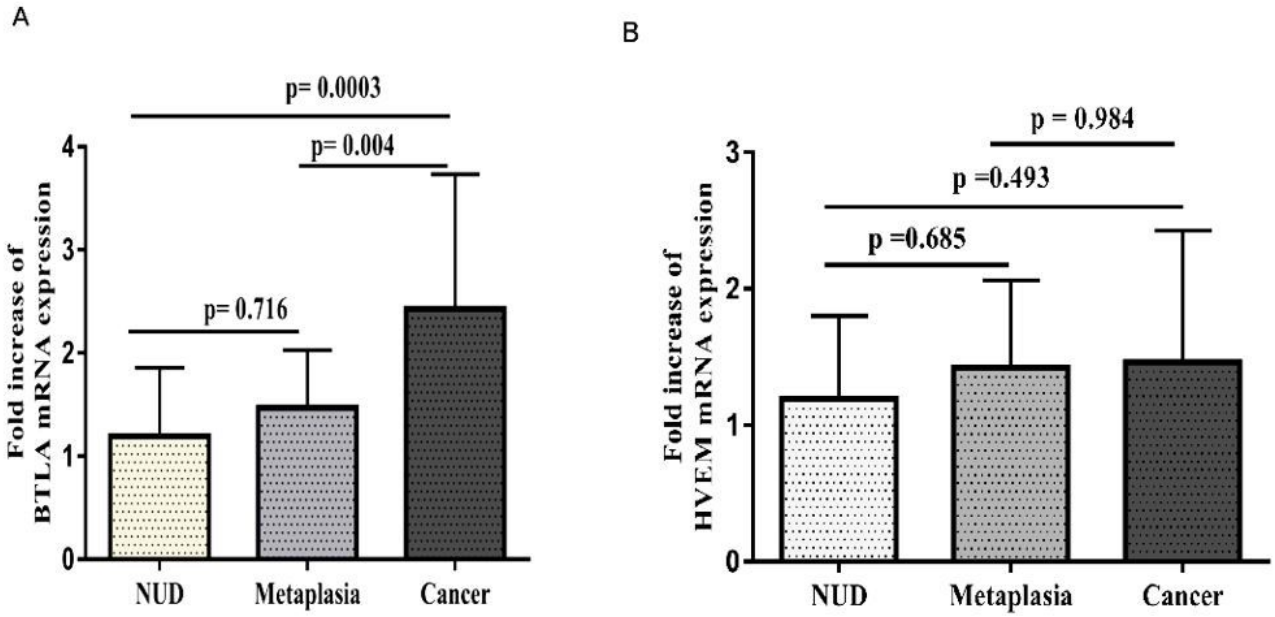

Figure 1

mRNA expression of B- and T-lymphocyte attenuator and herpesvirus entry mediator in gastric biopsies (a) Fold increase of BTLA mRNA in gastric biopsies obtained from cases with gastric cancer, intestinal metaplasia, and non-ulcer dyspepsia as the control group. (b) Fold increase of HVEM expression in patients with gastric cancer patients, intestinal metaplasia, and non-ulcer dyspepsia. Horizontal bars represent Mean ± SD. P-values of < 0.05 were considered significant.

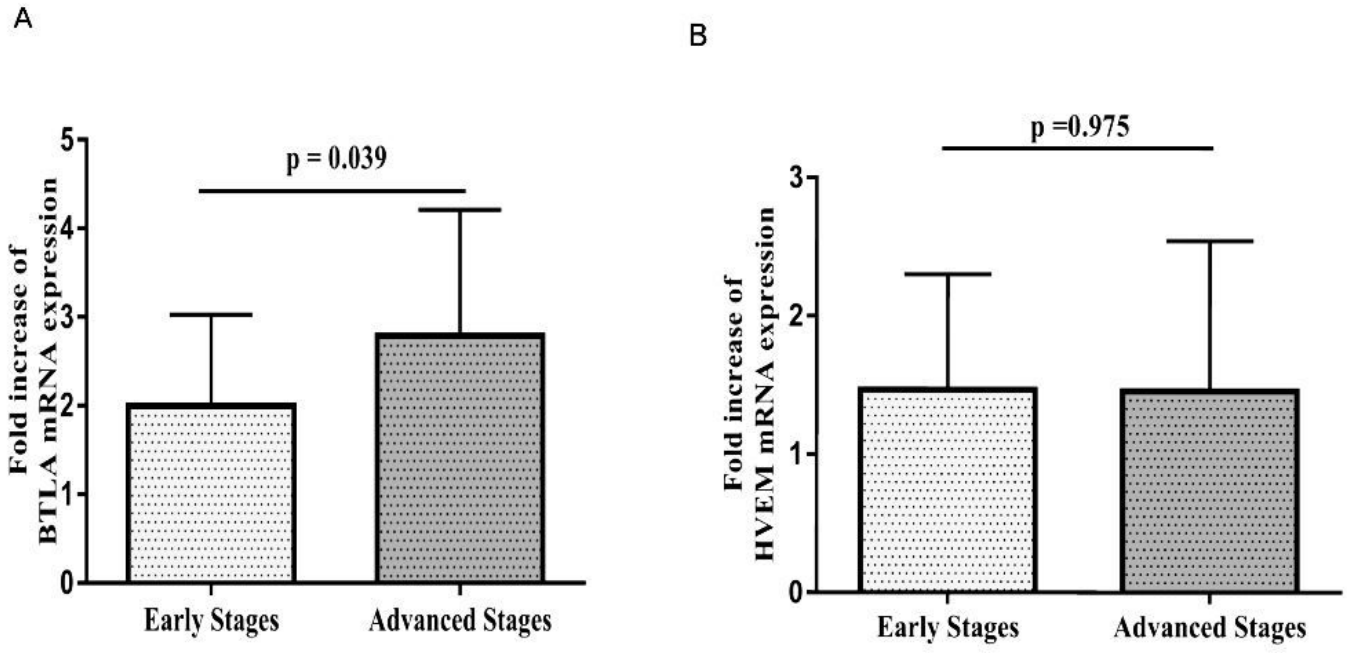

Figure 2

HVEM and BTLA mRNA expression in early and advanced stages of gastric cancer Patients with gastric cancer were divided into two groups of early (I and II) and advanced stages (III and IV). (a) BTLA mRNA indicated a higher expression in advanced stages, compared to early stages (P=0.039). (b) HVEM mRNA expression did not show significant up-regulation in advanced stages than in the early stages (P=0.9). Horizontal bars represent Mean ± SD. P-values of < 0.05 were considered significant.

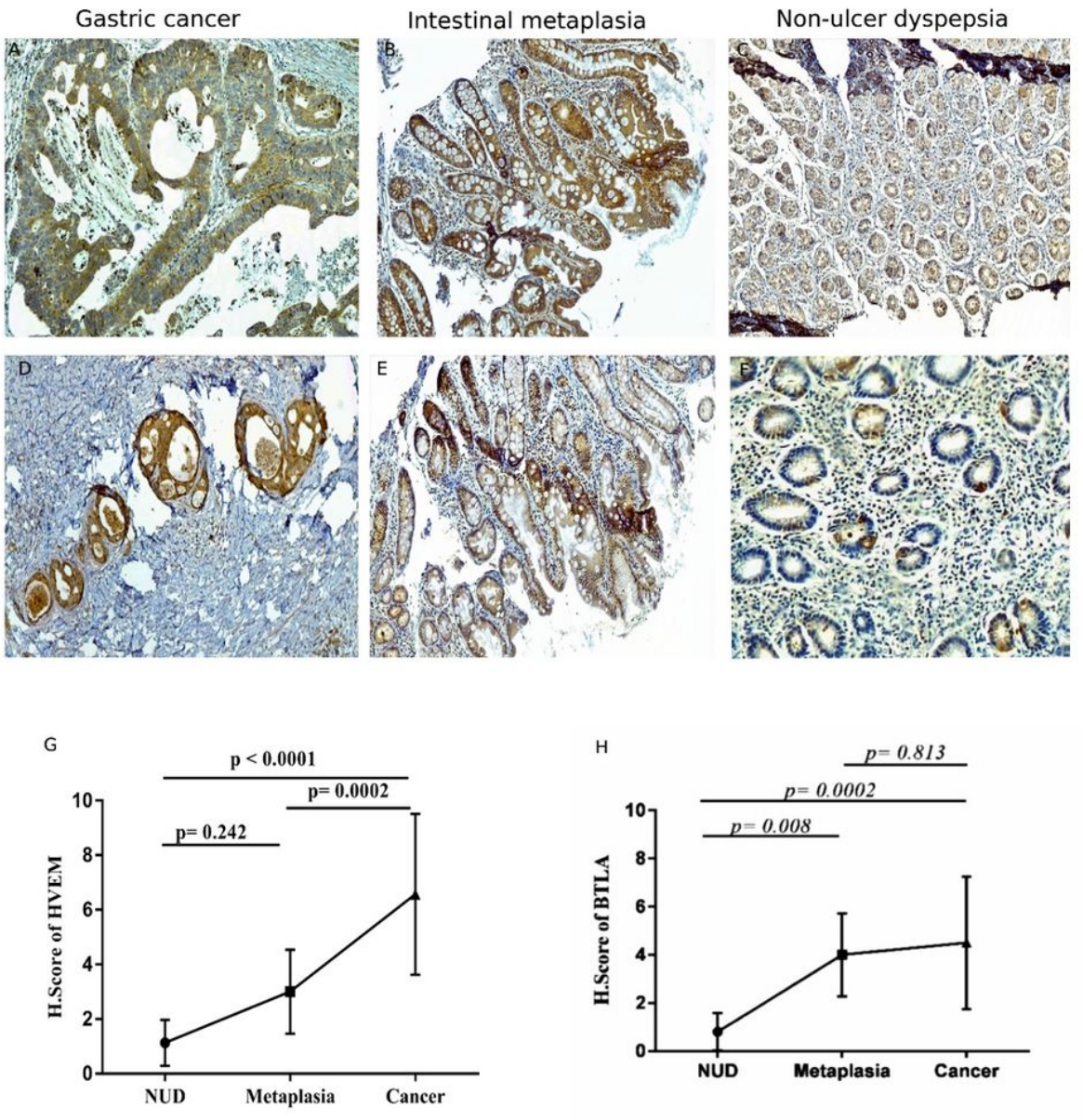

Figure 3

Immunohistochemistry analysis HVEM and BTLA in non-ulcer dyspepsia, intestinal metaplasia, and gastric cancer biopsy tissues (A) IHC showed strong HVEM immunostaining in the cytoplasm of gastric cancer samples, moderate staining in intestinal metaplasia(B), and weak or no staining in non-ulcer dyspepsia samples(C). (G) The quantitative H-score system of immunohistochemistry results indicated significantly higher HVEM expression in gastric cancer tissues, compared to intestinal metaplasia and non-ulcer dyspepsia groups (P=0.0002 and P<0.0001, respectively). Patients with intestinal metaplasia did not show higher HVEM expression than the non-ulcer dyspepsia group (P=0.242). (D) BTLA

immunostaining showed strong cytoplasmic expression in gastric cancer samples, moderate staining in IM (E) and weak staining in NUD samples (F). (H) The quantitative H-score system of immunohistochemistry results indicated higher BTLA expression in gastric cancer (p=0.0002) and intestinal metaplasia patients (P=0.0002 and P=0.008, respectively), compared to the non-ulcer dyspepsia group. BTLA expression was not significantly different in the gastric cancer and intestinal metaplasia groups (P=0.813). Error bars represent Mean ± SD. P-values of < 0.05 were considered significant. Magnifications: ×100.

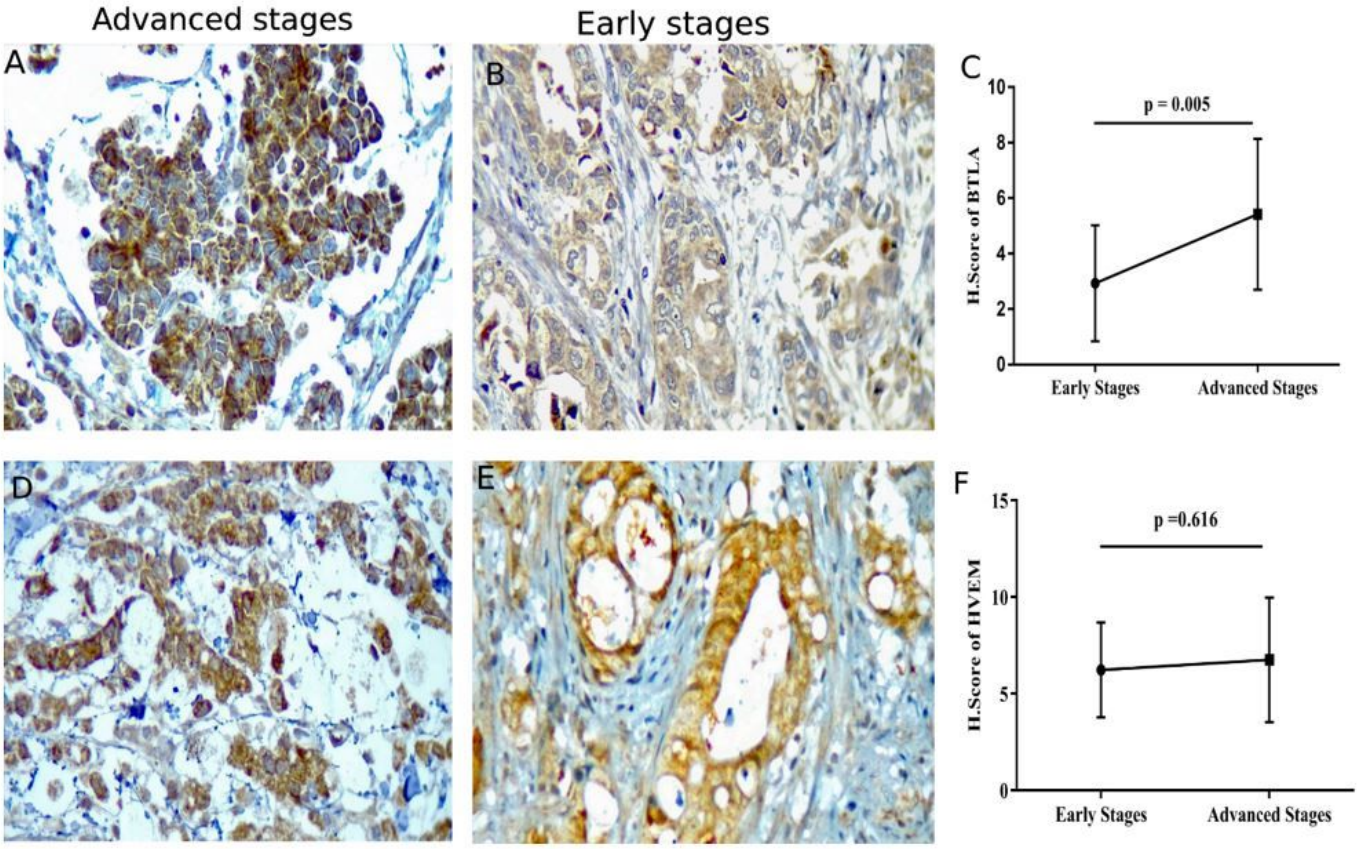

Figure 4

HVEM and BTLA protein expression in early and advanced stages of gastric cancer (a) Immunohistochemistry staining and quantitative H-score system indicate higher expression of BTLA in advanced stages, compared with early stages (P=0.005). (b) HVEM protein expression and semi-quantitative H-score system did not show a significant increase in advanced stages, compared to early stages (P=0.616). Error bars represent Mean ± SD. P-values of < 0.05 were considered significant. Magnifications: ×400.

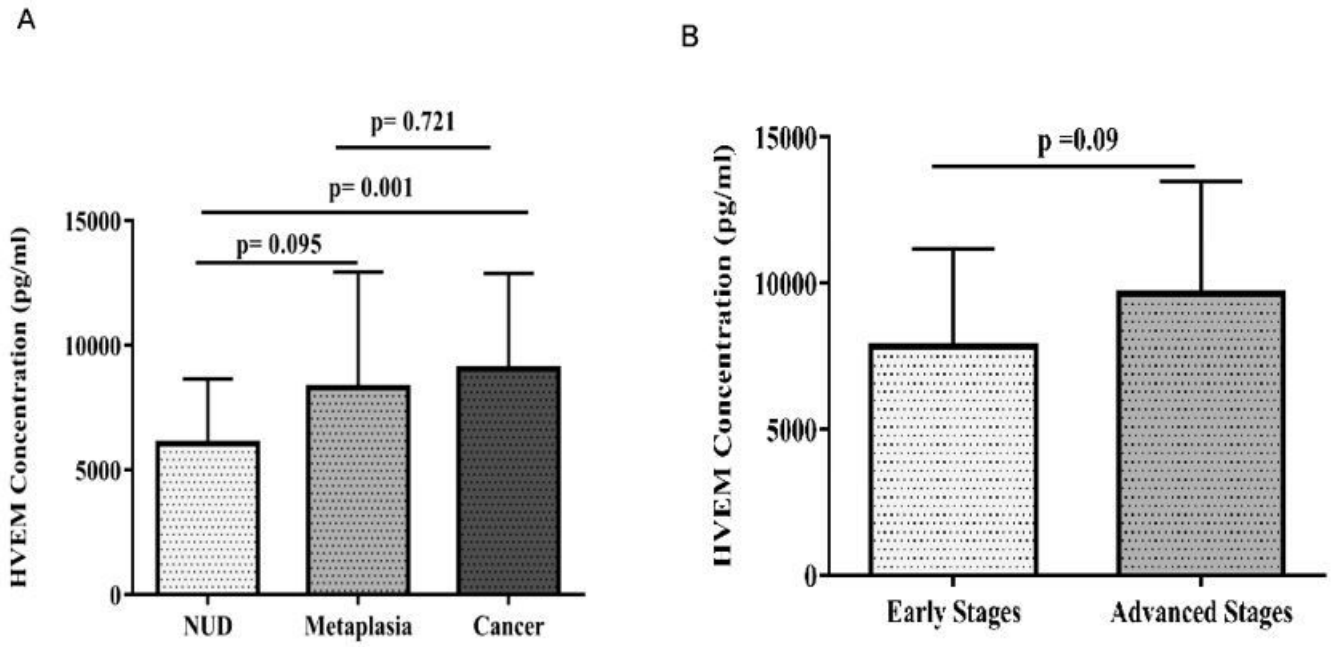

Figure 5

Concentrations of soluble HVEM in sera of patients in non-ulcer dyspepsia, intestinal metaplasia, and gastric cancer groups (a) Serum concentration of sHVEM in non-ulcer dyspepsia, intestinal metaplasia, and gastric cancer groups. (b) Serum concentrations of sHVEM in early and advanced stages of gastric cancer. The Y-axis shows the Mean ±SEM value. P-values less than 0.05 were considered significant. Horizontal bars represent Mean ± SD.