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# INCIDENCE OF GASTROESOPHAGEAL REFLUX DISEASE RELATE WITH HUMAN LEUKOCYTE ANTIGEN HLA-DRB1

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**Abstract:**

***Background:*** *Gastro Esophageal Reflux Disease (GERD) is characterized by various symptoms. There is evidence for a genetic component of gastro-esophageal reflux disease, confirmed by familial aggregation of the disease. The aim of the study was to investigate whether certain genes of human leukocyte HLA-DRB1 antigens are associated with (GERD).*

***Methods:*** *Patients and controls were prospectively recruited from the Gastroenterology department of Dhulikhel Hospital Kavre District, Nepal for one-year duration from March 2019 to March 2020. Sixty patients with a history of heartburn and indigestion were compared with 100 controls. All studied patients and control groups underwent endoscopic examination of the upper gastrointestinal tract, and their sera were analyzed for CagA Immunoglobulin G (IgG) antibodies to H. pylori. HLA-DRB1 genotyping was performed in both groups.*

***Results:*** *A total of 60 patients with erosive gastritis; GERD was assessed (grade II and III) along with 100 controls. There is a significant increase in H. pylori infection (p = 0.0001) in patients with GERD than in the control group. HLA-DRB1 * 15: 01 was significantly increased in patients with GERD compared to the control group and the increased frequency of HLADRB1 * 11: 01 in the control group compared to the group of patients.*

***Conclusions:*** *There is an association between HLA-DRB1 * 15: 01 in GERD and H pylori positive patients.*

**Keywords:** *HLA, GERD, H pylori.*

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## INTRODUCTION:

Gastro-esophageal reflux disease (GERD) is the end result of the involuntary reflux of gastric contents into the esophagus, causing heartburn and symptoms of acid regurgitation or damage to the esophageal tissue [1-2]. Therefore, esophagitis is considered a complication of GERD. GERD has two types; erosive esophagitis and non-erosive reflux disease (NERD) (endoscopic negative reflux disease). GERD is classified into four grades (A, B, C, and D) according to the Los Angeles classification [3-4]. Another classification was Savary-Miller who classified him into five classes (I, II, III, IV, and V). The prevalence of GERD symptoms ranges from 9% to 42%. Despite the high prevalence of GERD symptoms, its etiology is not yet fully understood [5-6]. Environmental factors are one of the important triggers of GERD. These include lifestyle factors such as weight, nutrition, alcohol consumption, smoking, taking non-steroidal anti-inflammatory drugs, and sleeping position [7-8]. Regarding genetic factors; there is evidence of a role for the genetic component in GERD as confirmed by familial aggregation of GERD symptoms. Other studies conducted in Sweden and the United Kingdom on monozygotic and dizygotic twins revealed a significant genetic contribution to the etiology of GERD [9]. In addition, ethnic differences in the prevalence of GERD between Western and Asian populations. All this evidence points to a genetic etiology of GERD in addition to environmental factors. Another genetic factor is the human leukocyte antigen (HLA) system, which is characterized by extensive polymorphism and is considered an excellent marker for population genetic analysis and disease association [10].

In this study, we investigate whether alleles of the human leukocyte HLA-DRB1 antigen, important for the immune response, may be a susceptibility locus for GERD.

## METHODS:

Patients and controls were prospectively recruited from the Gastroenterology department of Dhulikhel Hospital Kavre District, Nepal for one-year duration from March 2019 to March 2020. Demographics of all patients and controls were recorded. Written informed consent was obtained for this study from all patients and the control group. The study protocol was reviewed and approved by the Scientific and Ethical Committee. The patient group and the control groups were matched for sex and age. Sixty patients with a history of heartburn and indigestion at least three times a week for over three months and referred for upper gastrointestinal endoscopy and diagnosed with GERD were prospectively recruited. Exclusion criteria included patients with Barrett's esophagus and oesophageal varices, patients with secondary causes of gastroesophageal reflux, patients who were taking antacids, H2 blockers, proton pump inhibitors (PPIs), non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, history of eradication Helicobacter pylori, people with a history of gastrointestinal surgery, gastric ulcer and gastric cancer, or with systemic diseases that require chronic treatment. The 100 controls were all subjects who underwent upper gastrointestinal endoscopy for reasons other than reflux symptoms, Barrett's esophagus, or any form of indigestion and heartburn. This group included subjects with normal OGD and screened for anemia or fecal occult stool test positive for blood, unknown cause chronic diarrhea requiring a small intestine biopsy, irritable bowel syndrome, and screening for familial adenomatous polyposis. All examined patients and control groups underwent endoscopic examination of the upper gastrointestinal tract using a gastroscope: GIF-H260; Olympus, Tokyo, Japan and display screen; Olympus OEV-261H liquid crystal monitor; Olympus, Tokyo, Japan. The gastroesophageal junction was defined as the squamous junction and the proximal edge of the gastric folds. The endoscopic results of erosive esophagitis in the lower esophagus were classified using the Savary and Miller classification. All of them are either Grade II, which are confluent erosive or exudative lesions of the mucosa that do not cover the entire circumference of the esophagus, or Grade III, which are erosive or exudative lesions of the mucosa that cover the entire perimeter of the esophagus and lead to inflammation of the wall without stenosis (according to Savary and Miller, 1979). Histopathological examination was performed by taking samples of the gastric mucosa to confirm the diagnosis and presence of H pylori. Blood samples (3 ml) were collected in a standard tube. The separated sera were analyzed for CagA Immunoglobulin G (IgG) antibodies to H. pylori using an immunoassay (immunochromatography test) (ACON, USA). HLA class II genotyping (HLA-DRB1): 2 ml of venous blood was collected in EDTA containers for DNA extraction from human blood using a blood kit (QIAmp DNA blood Mini Kit, QIAGENINC - Germany). DNA concentration and purification product were assessed. DNA was verified by electrophoresis on a 2% agarose gel containing ethidium bromide and visualized under UV light. In the case of DRB1, site-specific and allele-specific amplification was performed in genomic patients as well as control DNA. DNA amplification and hybridization were performed using sequence-specific

oligonucleotide probes (SSOP) in HLA-DRB1 amplification and hybridization kits (SSOHLA type DRB1 plus and Mastermix for HLA Kits type DRB1 Amp plus-Innogenetics Belgium) from AutoLipa - 48 Inn genetics-Belgium-Belgium. The results were interpreted using LiRas software version 5.0 Inn genetics-Belgium. HLA-DRB1 frequencies were determined by direct counting. The frequency of each allele was compared between patients and controls using Fisher's exact chi-square test on the Mini Tab version. Software 3.0. The odds ratio (OR) was used in each comparison together with the 95% confidence interval (95% CI). The gene frequencies for both groups were calculated. A p value of less than 0.05 was considered statistically significant.

## RESULTS:

A total of 60 patients with erosive gastritis; GERD was assessed (grade II and III) along with 100 controls. The mean age of the patients was 45.67 ± 15.54, compared with 44 ± 15.22 in the control group. The male to female ratio was 1.0 in the patient group compared to 1.0 in the control group. 50% of them are GERD II (Fig. 1) and the rest are GERD III (Fig. 2) on endoscopic examination.

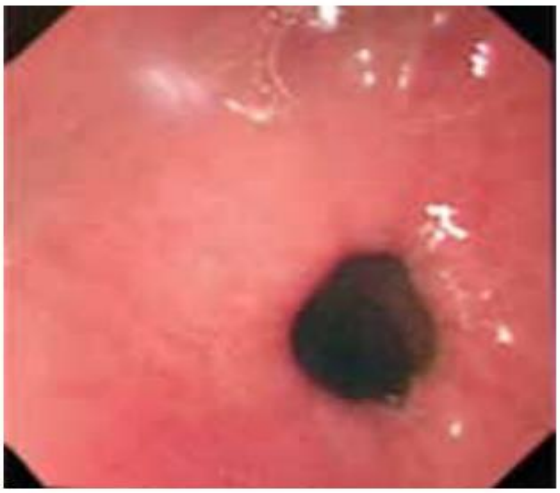

**Figure 1:** Erosive esophagitis GERD Grade II by Endoscopy.

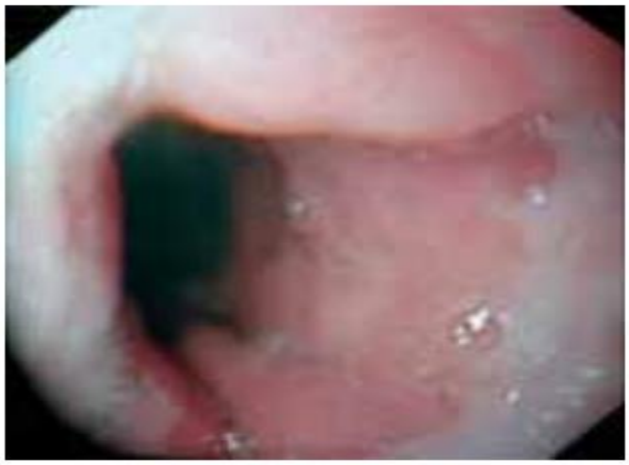

**Figure 2:** Erosive esophagitis GERD Grade III by Endoscopy.

There is a significant increase in H. pylori infection (p = 0.0001) in patients with GERD than in the control group. Odd rate (OD) = 18.00 with 95% CI = from 7.727-41.926. Relative Risk = 6.666, which indicates a relationship between H. pylori and disease as shown in Table I.

**Helicobacter pylori in GERD (Grade II and III) patients compared with control group**

| H. pylori Cag A+ | GERD + Patients | | GERD–Control | | P- value | Odd ratio | RR Relative |
|---|---|---|---|---|---|---|---|
| Status | No. | % | No. | % | | 95% CI | risk |
| H. pylori Positive | 40 | 66.66 | 10 | 10 | 18 | | |
| H. pylori Negative | 20 | 33.33 | 90 | 90 | 0.0001 | 7.727-41.926 | 6.666 |
| Total | 60 | | 100 | | | | |

The distribution of HLA * DRB1 polymorphism was studied in the control group and patient groups in the Bangladeshi population. The observed and expected phenotypes of all loci for the patient group, as shown in Table 4, were in good agreement with the Hardy-Weinberg equilibrium as shown in Table V. Control and patients were typed to identify the DRB1 * allele using a methodology based on -SSOP). There was an increased frequency of HLADRB1 * 11: 01 in the control group compared to the patients group (p = 0.0001, odds ratio = 0.141, 95% CI: 0.055-0.358). Other alleles, such as HLA-DRB1 * 15: 01, were significantly increased in GERD patients compared to controls (p = 0.004, odds ratio = 3.833, 95% CI: 1.513-9.708) as shown in Table II.

**Table-II**

Frequencies of HLA-DRB1 alleles in patients with GERD disease compared with control group

| HLA-DRB1 alelles | Patients with GERD disease, N=60 | | Control group, N=100 | | Odd ratio 95 % confidence interval | P – value |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| 01: 01 | 3 | 5 | 6 | 6 | 0.824 (0.198-3.426) | 0.790 |
| 01: 02 | 3 | 5 | - | - | NA | NA |
| 02: 01 | - | - | 10 | 10 | NA | NA |
| 03: 01 | 9 | 15 | 10 | 10 | 1.588 (0.605-4.164) | 0.346 |
| 03: 02 | - | - | 14 | 14 | NA | NA |
| 03: 17 | 3 | 5 | 2 | 2 | 2.578 (0.418-15.897) | 0.307 |
| 03: 39 | 9 | 15 | - | - | NA | NA |
| 03: 40 | 3 | 5 | - | - | NA | NA |
| 04: 02 | 13 | 21.66 | 25 | 25 | 0.829 (0.386-1.770) | 0.631 |
| 04: 03 | 1 | 1.66 | 1 | 1 | 1.678 (0.103-27.333) | 0.716 |
| 04: 05 | 1 | 1.66 | - | - | NA | NA |
| 05: 01 | - | - | 4 | 1 | NA | NA |
| 06: 01 | - | - | 6 | 6 | NA | NA |
| 07: 01 | 15 | 25 | 18 | 18 | 1.518 (0.699-3.298) | 0.291 |
| 07: 08 | 3 | 5 | - | - | NA | NA |
| 08: 31 | 3 | 5 | 6 | 6 | 0.824 (0.198-3.426) | 0.790 |
| 10: 01 | - | - | 6 | 6 | NA | NA |
| 11: 01 | 6 | 10 | 44 | 44 | 0.141 (0.055-0.358) | 0.0001 |
| 12: 01 | 3 | 5 | 5 | 5 | 1.00 (0.230-4.343) | 1.00 |

The highest frequency of genotypes in patients with GERD was 15: 01, which is 0.065, while in the control group 11: 01, or 0.117, as shown in Table III.

**Table-III**

Genotypes frequencies of HLA-DRB1 alleles in patients with GERD disease and control group

| HLA-DRB1 alleles | Patients with GERD disease, N=60 Gene frequency | Control group, N=100 Gene frequency |
|---|---|---|
| 01: 01 | 0.013 | 0.016 |
| 01: 02 | 0.013 | ------ |
| 02: 01 | ----- | 0.026 |
| 03: 01 | 0.039 | 0.026 |
| 03: 02 | ------ | 0.006 |
| 03: 17 | 0.013 | ------- |
| 03: 39 | 0.039 | ------- |
| 03: 40 | 0.013 | 0.036 |
| 04: 02 | 0.057 | 0.065 |
| 04: 03 | 0.005 | 0.003 |
| 04: 05 | 0.005 | ------- |
| 05: 01 | ------ | 0.011 |
| 06: 01 | ------ | 0.016 |
| 07: 01 | 0.06 | 0.047 |
| 07: 08 | 0.013 | ------- |
| 08: 31 | 0.013 | 0.016 |

**Table-IV**

Observed and expected numbers and percentages of HLA-DRB1 alleles in patients with GERD disease

| HLA-DRB1 alelles | Patients with GERD Observed, N=60 | | Patients with GERD Expected, N=100 | |
|---|---|---|---|---|
| | No. | % | No. | % |
| 01: 01 | 3 | 5 | 3.09 | 5.15 |
| 01: 02 | 3 | 5 | 3.09 | 5.15 |
| 02: 01 | ------ | ------ | ------ | ----- |
| 03: 01 | 9 | 15 | 9.17 | 15.28 |
| 03: 02 | ----- | ------ | ----- | ------ |
| 03: 17 | 3 | 5 | 3.09 | 5.15 |
| 03: 39 | 9 | 15 | 9.17 | 15.28 |
| 03: 40 | 3 | 5 | 3.09 | 5.15 |
| 04: 02 | 13 | 21.66 | 13.28 | 22.13 |
| 04: 03 | 1 | 1.66 | 1.19 | 1.98 |
| 04: 05 | 1 | 1.66 | 1.19 | 1.98 |
| 05: 01 | ------ | ------ | --- | ---- |
| 06: 01 | ------ | ------ | --- | ------ |
| 07: 01 | 15 | 25 | 15.09 | 25.15 |
| 07: 08 | 3 | 5 | 3.09 | 5.15 |

## DISCUSSION:

The erosive type of gastroesophageal reflux disease (GERD) predisposes to the onset of Grade IV Barrett's esophagus, leading to esophageal adenocarcinoma. Each abnormal cell expresses new antigens as a result of multiple genetic alterations that are associated with inflammation or transformation of cells that are recognized by helper T cells or T cytotoxic cells presented by human leukocyte antigen (HLA) class I or class II molecules[10-11]. The HLA system is a highly polymorphic system and an excellent marker for population genetic analyzes and disease association studies. HLA molecules play a key and important role in regulating the immune response. In this study, HLA-DRB1 * 15: 01 was significantly increased in patients with GERD compared to controls (p = 0.004, odds ratio = 3.833, 95% CI: 1.513-9.708). Thus, this allele is considered a predisposing factor to GERD, while HLADRB1 * 11: 01 is a protective factor because there is an increased frequency of HLADRB1 * 11: 01 in the control group compared to the patient group (P = 0.0001, odds ratio = 0.141 , 95% CI: 0.055-0.358). The expression of HLA-DR antigens is more complex. The squamous epithelium of the esophagus is deficient in HLA class II expression, as is the epithelium of the lungs, stomach and breasts. The increased expression of class II may be due to H pylori infection, which accounts for 66.66% of GERD patients, and patients with GERD have a significant increase in H. pylori infection (p = 0.0001) than controls. HLA class II antigens appear in pathological conditions such as inflammation, infection, tumor transformation, and autoimmunity. Another study found an association between Barrett's esophagus in Asians, particularly Native Americans, and HLA-B7; strengthening the genetic component of gastroesophageal reflux disease. Ethnic differences in the prevalence of GERD with familial aggregation suggest a possible genetic component of GERD in addition to environmental factors, eg Helicobacter pylori infection, abdominal obesity and metabolic syndrome[11-13]. The HLA-B07 gene, common in South Asian and Caucasian populations but not in Eastern populations, and the high prevalence of H. pylori in South Asians and the resulting atrophic gastritis and hypochlorhydria may partially alleviate this genetic predisposition to the disease. Another study found an increase in the prevalence of GERD with gastrointestinal malformations in children, which supports the genetic component of gastroesophageal reflux disease[14-15].

## CONCLUSION:

Larger samples and different ethnic populations should be genotyped to further confirm this association and to identify possible additional risk factors at the human leukocyte antigen locus.

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