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Association Between Selected Human Leukocyte Antigen Alleles and *Helicobacter pylori* Infection Among Dyspeptic Patients

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Abstract

Background: *Helicobacter pylori* has been identified as a group I carcinogenic bacterium that infects the gastric mucosa leading to gastritis, peptic ulcer disease, lymphoma, and gastric cancer. Pathogenesis of *H. pylori* depends on the virulence of the strain, host immune response, and modulating factors like smoking and diet.

Objectives: This study aimed to assess the association between selected human leukocyte antigen (HLA) alleles including HLA-DQA1*0102, HLA-DQA1*0103, and HLA-DQB1*0301, and the presence of *H. pylori* infection and disease severity among dyspeptic patients.

Materials and Methods: Gastric tissue samples were collected from 100 dyspeptic patients, who underwent upper gastrointestinal endoscopy at a tertiary care hospital. Presence of HLA alleles was confirmed using polymerase chain reaction (PCR). *Helicobacter pylori* infection was determined using PCR and Histology. The histological interpretation was done according to the 'Sydney classification.' Statistical analysis was done with SPSS version 22.

Results: Respective percentages of HLA-DQA1*0102, HLA-DQA1*0103, and HLA-DQB1*0301 were 39%, 31%, and 20%, respectively. Of the 25 samples positive for *H. pylori* infection, 56% (14/25), 36% (9/25), and 12% (3/25) were positive for HLA-DQA1*0102, HLA-DQA1*0103, and HLA-DQB1*0301 alleles respectively. Considering the association with *H. pylori* infection, only HLA-DQA1*0102 showed significant association ($P = .044$). No significant association was found between the HLA alleles and the histological severity among the *H. pylori* infected patients.

Conclusion: Investigation of immunogenetic factors contributing to susceptibility or resistance to *H. pylori* infection in Sri Lanka can provide an insight into understanding the risk of developing severe pathological complications among patients.

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Background

Helicobacter pylori is a bacterial pathogen which infects the gastric mucosa leading to gastritis, peptic ulcer disease, gastric cancer, and lymphoma.[1] The outcome of *Helicobacter* infection depends on multiple factors including diversity of *H. pylori* strains, host or environmental factors, and duration of infection.[2] Progression of the infection can lead to gastric atrophy, decreased gastric acid secretion, and gastric cancer.[3]

The human leukocyte antigen (HLA) class II encodes highly polymorphic cell surface molecules and is involved in antigen binding and presentation to T helper cells. The polymorphism of these HLA genes results in a diversity of immune responses of individuals to antigens, thereby making that individual more susceptible or resistant to infection.[4] Immunogenetic analysis indicates a positive or negative association of HLA-DQ alleles with *H. pylori* infection, gastritis, and gastric cancer.[5] The HLA allele frequency varies among different races and populations.[6] Therefore, it is important to investigate the association of HLA alleles with *H. pylori* infection and development of gastric complications in a given population. In this study,

we intended to investigate the association between selected HLA alleles and *H. pylori* infection among a group of dyspeptic patients in Sri Lanka.

Material and Methods

The study was a cross-sectional, descriptive study carried out among 100 dyspeptic patients who were required to undergo endoscopy at a tertiary care hospital. Two biopsy specimens were collected from antrum of each patient during endoscopy for further investigations. The samples were transported to the Department of Microbiology and the Department of Pathology in a state university in Sri Lanka.

Selection of Participants

Patients referred to the endoscopy unit at a tertiary care hospital for routine endoscopy procedure with symptoms of dyspepsia were recruited to the study. The selected patients were above 18 years of age and not taking antibiotics for two weeks prior to the endoscopy. Patients less than 18 years of age, mentally unstable patients, and those currently taking antibiotics were excluded from the study.

Histology of Biopsy Specimens

Biopsy specimens were subjected to histopathological examination for presence of *H. pylori* and the histological severity. Biopsy specimens were fixed with formalin and embedded in paraffin wax for preparation of 4-micron thick sections. Tissue sections were stained with hematoxylin and eosin and Giemsa stains as described previously.[7] Specimens were examined by an investigator and severity was graded according to the updated Sydney system.[8]

Polymerase Chain Reaction and Human Leukocyte Antigen Genotyping

The second biopsy specimen was used for DNA extraction using the QIAamp DNA mini kit (Qiagen, Germany) following the manufacturer's instructions. The presence of *H. pylori* was determined by polymerase chain reaction (PCR) using primers targeting *glmM* gene as described previously.[9] PCR was carried out to identify the presence of HLA-DQA1*0102, HLA-DQA1*0103, and HLA-DQB1*0301 genes using PCR specific primers as described in Table 1.[10]

All PCR reactions were performed in 0.2 mL tubes using Flexigene thermal cycler (version 31.04). PCR for HLA-DQA1 genes was performed in a 50 µL reaction mixture consisting of 0.5X buffer (Go Taq Flexi DNA polymerase kit, Promega) with 2 mM $MgCl_2$ (Go Taq Flexi DNA polymerase kit, Promega), 0.2 mM each of dATP, dCTP, dGTP and dTTP (Promega), 1 µM of each forward and reverse primers, 1.25 U of Go Taq Flexi DNA polymerase (Promega) and 2 µL of template. The PCR conditions mentioned in Ota et al, 1991, was followed with 5 minutes of initial denaturation at 94°C, 35 cycles, and 7 minutes of final elongation at 72°C.[11]

For the HLA-DQB1*0301, the PCR was performed in a 50 µL reaction mixture which consisted 1X buffer (Go Taq Flexi DNA polymerase kit, Promega) with 1.5 mM $MgCl_2$ (Go Taq Flexi DNA polymerase kit, Promega), 0.2mM of each of dATP, dCTP, dGTP, and dTTP (Promega), 0.5 µM of forward and reverse primers, 1.25 U of Go Taq Flexi DNA polymerase (Promega), and 2 µL of template. Optimized PCR conditions were 94°C for 5 minutes for initial denaturation, 35 cycles of 94°C for 1 minutes, 55°C for 1 minute and 72°C for 2 minutes, and a final elongation step of 72°C for 7 minutes.

The PCR products were visualized in 1.5% agarose gel and observed for the specific amplicons for HLA-DQA*0102 (149 bp), HLA-DQA1*0103 (172 bp), and HLA–DQB1*0301 (129 bp), respectively.

Statistical Analysis

Statistical analysis was done with SPSS version 22 (SPSS, Inc., Chicago, Illinois, USA) to determine the association of each selected HLA type with the *H. pylori* infection and severity of the disease, and chi-square test was used to determine the *P* value. The level of significance was set at $P < .05$.

Results

Out of 100 dyspeptic patients enrolled in the study, 65 were female while 35 were male. Most of the patients (68/100) were between 36-65 years of age. In this population, 81/100 (81%) had mild chronic gastritis while 13/100 (13%) had moderate chronic gastritis and 2/100

Table 1. Primer Sequences Used for the Identification of *glmM*, HLA-DQA1*0102, HLA -DQA1*0103, and HLA –DQB1*0301

Gene	Primer	Primer Sequence	Product Size (bp)	References
glmM gene	glmM-F	AAGCTTTTAGGGGTGTTAGGGGTTT	294	6
	glmM-R	AAGCTTACTTTCTAACACTAACGC		
HLA*-DQA1*0102	F	CATGAATTTGATGGAGATGAGC	149	7
	R	ATGATGTTCAAGTTGTGTTTTGC		
HLA*-DQA1*0103	F	ACGGTCCCTCTGGCCAGTT	172	7
	R	ATGATGTTCAAGTTGTGTTTTGC		
HLA*-DQB1*0301	F	GACGGAGCGCGTGCGTTA	129	7
	R	CTGTTCCAGTACTCGGCGT		

Abbreviation: HLA, human leukocyte antigen.

(2%) showed severe chronic gastritis on examination of the gastric biopsy. Other variables including atrophy, metaplasia, and dysplasia, evaluated under the modified Sydney criteria, were negative in all specimens.

Histological examination revealed the presence of *H. pylori* in 13 biopsy specimens while 22 patients were positive by PCR. A total of 25 patients were confirmed as *H. pylori* positive by either PCR or histology. A majority of patients (14/25, 56%) were in the 46-65 (55.5±9.5) age group. Among the *H. pylori* positive patients, 18/25 (72%), 5/25 (20%), and 2/25 (8%) had mild, moderate, and severe chronic gastritis, respectively. Considering the 25 positive samples, 56% of the infected were females. However, there was no significant association of *H. pylori* infection with gender ($P = .276$).

Genotype Frequency of HLA-DQA1 and HLA-DQB1 Alleles Among the Helicobacter pylori Positive and Negative Patients

The frequencies of alleles HLA-DQA1*0102, HLA-DQA1*0103, and HLA-DQB1*0301 were 39%, 31%, and 20% respectively out of the 100 biopsy specimens analyzed. Of these patients, 25 were positive for both HLA-DQA1*0102 and HLA–DQA1*0103 and 4 were positive for all 3 alleles.

Out of the 25 who were positive for *H. pylori* by either histology, PCR, or both, 56% (14/25) were positive for HLA-DQA1*0102, 36% (9/25) were positive for HLA–DQA1*0103, and 12% (3/25) for HLA–DQB10*0301 as described in Table 2. A significant association was seen between HLA-DQA1*0102 and *H. pylori* infection ($P = .044$) in this population while no significant association was seen for the other two alleles investigated (Table 2).

Majority of the patients had mild chronic inflammation as shown in Table 3. No significant association between HLA alleles examined and the severity of the inflammation was observed among the *H. pylori* positive population.

Discussion

In this study we investigated the association between HLA alleles, HLA-DQA1*0102, HLA-DQA1*0103, and HLA–DQB1*0301, and *H. pylori* infection among a group of dyspeptic patients in Sri Lanka. The highest allele frequency was observed for HLA-DQA1*0102 in the current population and more than 50% of patients who were positive for *H. pylori* were also positive for this allele. The other two alleles did not have a significant association with *H. pylori* infection in this population.

The host genetic factors, namely HLA alleles, contributing to *H. pylori* infection has been studied by several groups. Studies done by Azuma et al[12] and Magnusson et al[13] suggest a decreased risk of *H. pylori* infection among Japanese and Swedish patients expressing HLA-DQA1*0102. Our results contrast with these studies as we observed a significant positive association of this allele with *H. pylori* infection among the Sri Lankan dyspeptic patient population. Reported studies by Santolaria et al[14] and Kunstmann et al[15] however was not able to determine any association of HLA-DQA1*0102 and *H. pylori* infection among Spanish and German populations. These differences reported may be due to the geographical variation in the allele distribution among the populations in various studies.

In the current study, no significant association of HLA–DQA1*0103 or HLA-DQB1*0301 with *H. pylori* infection was observed. Wang et al reported that HLA-DQA1*0103 and HLA-DQB1*0301 were associated with susceptibility to *H. pylori* infection among East Asian population while

Table 2. Association of Selected HLA Alleles With *Helicobacter pylori* Infection

Allele	*Helicobacter pylori*		*P* Value	Odds Ratio	95% CI
	Positive (n = 25)	Negative (n = 75)			
HLA*-DQA1*0102 (n = 39)	14	25	0.044	2.545	1.010-6.414
HLA*-DQA1*0103 (n = 31)	9	22	0.533	1.355	0.521-3.525
HLA*-DQB1*0301 (n = 20)	3	17	0.248	0.465	0.124-1.745

Abbreviation: HLA, human leukocyte antigen.

Table 3. Association of HLA Alleles With Chronic Inflammation Among the *Helicobacter pylori* Positive Patients

Allele	Severity of Inflammation			Total (n = 25)
	Mild	Moderate	Severe	
HLA-DQA1*0102	44% (n = 11)	8% (n = 2)	4% (n = 1)	14
HLA-DQA1*0103	28% (n = 7)	4% (n = 1)	4% (n = 1)	9
HLA-DQB1*0301	12% (n = 3)	-	-	3
HLA-DQA1*0102 and HLA-DQA1*0103	28% (n = 7)	4% (n = 1)	4% (n = 1)	9
HLA-DQA1*0102 and HLA-DQB1*0301	8% (n = 2)	-	-	2
HLA-DQA1*0103 and HLA-DQB1*0301	4% (n = 1)	-	-	1
All three alleles	4% (n = 1)	-	-	1

Abbreviation: HLA, human leukocyte antigen.

no such significant association was observed among the European population.[16] The allele HLA-DQA1*0103 was suggested to be significantly increased in MALT lymphoma patients compared to non-ulcer dyspepsia patients who were either *H. pylori* positive or negative. After *H. pylori* eradication, in patients who carried HLA-DQA1*0103-DQB1*0601 the lymphomas regressed completely.[17] However, in the current study no significant positive association could be found between *H. pylori* infection and HLA-DQA1*0103 allele.

The HLA-DQB1*0301 has been reported to be positively associated with gastric cancer with a decreased risk for *H. pylori* infection[18] which suggested that the association of HLA-DQB1*0301 with gastric cancer was not through increased susceptibility to *H. pylori* infection. Still, controversy exists regarding HLA-DQB1*0301 allele, as several studies have shown that the presence of DQB1*0301 may have a protective role.[6,19]

In this study the majority of patients had mild chronic gastritis. *H. pylori* is known to induce an inflammatory immune response with infiltration of neutrophils and other inflammatory cells in the gastric mucosa. Inflammation and pathogenesis is further enhanced due to pathogen mediated generation of reactive oxygen and reactive nitrogen species.[2,20] The severity of the gastric inflammation depends on several factors including the virulence of the bacterium, the host immune response, host genetic factors, and environmental factors.[2] The bacterium has several mechanisms such as hypo-inflammatory lipopolysaccharides, and molecular mimicry,[20] by which it can evade immune recognition and persist in the gastric epithelium with minimal pathology.

Previous studies indicate that the role of host genetic factors in determining the susceptibility to *H. pylori* infection varies among different countries. This can be due to not only the different distribution of HLA allele frequencies but also the other contributing factors such as diet, host genetic polymorphisms, and other environmental factors. Therefore, it is important to investigate the role of host genetic factors in different geographical locations to understand the risk factors for infection and disease progression in those populations. Out of the *H. pylori* infected patients, a majority had mild chronic inflammation regardless of the HLA allele. Therefore, it is possible that the selected HLA alleles do not play a major role in the severity of disease in this patient population.

Authors' Contributions

Data acquisition, analysis, interpretation, drafting of the manuscript and statistical analysis was carried out by PSA and NLU. CPG, MMW, DW, KS, BSe and NF were responsible for the study concept and design, critical revision of the manuscript, administrative, technical and material support and study supervision.

Conflict of Interest Disclosures

Non.

Ethical Approval

Written informed consent was obtained from patients before sample collection and ethical approval for the study was obtained from the Ethical Review Committee of the University of Sri Jayewardenepura, Sri Lanka (No: 14/15).

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