

The Expression of CD74 Molecule in *H.pylori* Infected Gastric Mucosal Tissue

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Abstract

Background: *Helicobacter pylori* cause gastric inflammation. Recent interest has been focused on the role of CD74 (the class II MHC-associated invariant chain expressed on the surface of gastric epithelial cells) as an adhesion molecules used by *H.pylori* that may contribute to the proinflammatory immune response seen during infection.

Objective: The aim of this study was to detect the CD74 mucosal expression in *H.pylori* infected patients and compare it with uninfected patients.

Patients and Methods: Sixty-four patients' age mean (34± 1.7) years (14-66 years) who underwent upper gastrointestinal endoscopy because of gastrointestinal complaints, were studied.

A number of both invasive and non-invasive diagnostic tests were used for the diagnosis of *H. pylori* infection, as well as immunohistochemical study of biopsy

specimens to detect the CD74 mucosal expression.

Results: After the diagnosis of *H.pylori* infection, patients were grouped as *H. pylori* positive, (n=47) and *H. pylori* negative (n=17). According to immunohistochemical study of biopsy specimens, the expression of CD74 was observed in infected subjects, and there was a significant difference in the CD74 expression ($p= 0.005$) between infected and uninfected patients.

Conclusion: According to immunohistochemical study of biopsy specimens an overexpression of CD74 was observed in infected subjects

Keywords: *Helicobacter pylori*; CD74; gastric epithelial cells; Immunohistochemistry (IHC)

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Introduction

Helicobacter pylori infection provokes a vigorous humoral and cellular immune response in humans, but the organism is rarely eliminated from the gastric mucosa and infection persists lifelong in the absence of treatment⁽¹⁾. *H. pylori* colonize the human stomach and is usually found either as an extracellular pathogen in the gastric mucosa or tightly attached to the cells of the gastric epithelium. Colonization by *Helicobacter pylori* always causes chronic gastritis and leads to the development of severe gastroduodenal diseases such as peptic

ulcers, gastric adenocarcinoma, or Lymphoma of the mucosa associated lymphoid tissue (MALT)^(2,3). Although the mucosal colonization of *H. pylori* induces a mixed Th1/2-mediated mucosal cytokine milieu^(4,5) and the generation of *H. pylori*-specific T- and B-cell clones, the inflammatory response is not sufficient to eradicate the organism from its host^(5,6). The chronic immune response induced could afford a colonization advantage for the bacteria by providing improved availability of adhesion places. An example of this is the resulting increase in class II major histocompatibility complex (MHC) and CD74, induced by IFN- γ and IL-8 that are used as receptors by *H. pylori*^(7,8,9). The CD74 chain was thought to function mainly as an MHC class II chaperone, which promotes an endoplasmic reticulum (ER) exit of MHC class II molecules, directs them

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to endocytic compartments, prevents peptide binding in the ER, and contributes to peptide editing in the MHC class II compartment⁽¹⁰⁾. Class II MHC and invariant chain expression was believed to be restricted to classical antigen-presenting cell (APC); but during inflammation, other cell types including human mucosal epithelial cells, have also been reported to express class II MHC molecules. These cells have a high-level expression of surface CD74, which is polarized to the apical surface⁽¹¹⁾. However, in addition to its function as a chaperone molecule, CD74 was shown to have a role as an accessory signaling molecule.

Beswick *et al.*⁽¹²⁾ studied in details binding of the *H. pylori* urease A and B subunits to class II MHC and the class II MHC-associated invariant chain CD74. Consequently, the suggestion of the role for CD74 in gastric epithelial cell interaction with *H. pylori* leading to NF- κ B signaling results in IL-8 secretion, and that CD74 plays an important role in these events.

Patients and Methods

Patients:

A total of 64 patients (41 females and 23 males), aged between 14 and 66 years (34 ± 1.7 years), were screened for this study. Patients attended the Gastroenterology Unit at AL-Kadhimiya Teaching Hospital in Baghdad from 1st April to 1st October 2007, because of recurrent abdominal pain and other gastrointestinal complaints. All patients filled a questionnaire sheet with regard to their general health and were excluded if they had been previously treated for *H. pylori* infection and usage of non steroidal anti-inflammatory drugs (NSAIDs); also Patients with actively bleeding peptic ulcer disease were excluded, as this is a well recognized cause of a false-negative urease test

⁽¹³⁾. The study was approved by the ethics committee of the Hospital.

Determination of *H. pylori*

Endoscopic examination was performed under local pharyngeal anesthesia, during which three biopsies were obtained from grossly inflamed areas of the antrum. One biopsy was used for Ultra Rapid Urease test (URUT) and slide impression smear, while the other biopsy specimens were fixed with 10% buffered formalinized saline, for preparation the paraffin embedded tissue blocks to histological evaluation and Immunohistochemical staining tests (IHC). In addition, blood samples were aspirated from each patient after the endoscopy.

A number of invasive URUT Test, slide impression smear) and non-invasive (anti-*H. pylori* IgG ELISA Test) diagnostic tests were used for the diagnosis of *H. pylori* infection according to⁽¹⁴⁾.

Immunohistochemical Analysis of CD74

Immunohistochemistry was performed using the labeled streptavidin-biotin (LSAB) immunostaining method and the four-micrometer-thick, formalin-fixed, paraffin embedded serial sections of all biopsies were de-paraffinized and Re-hydrated. For antigen retrieval, pretreatment was performed by microwave heating in Glyca solution (BioGenex's U.S. Cat. No HK167-5K) for 5 min. on high power (~700 watts). Peroxidase block then incubation of each one with Mouse anti-Human CD74 (mouse monoclonal antibody, C2430-01E, dilution 1: 4, USBiological) was conducted at 37 °C for 1 hour and followed by phosphate-buffered saline washing. Positive immunohistochemical reactions were revealed using DakoCytomation LSAB 2 System-HRP Code K0673 (DakoCytomation, USA), using

immunohistochemistry detection kit as chromogen substrate. Tissue section were counterstained with hematoxylin and mounted with DPX. In negative controls, the primary antibody was omitted.

Slides were examined by light Microscope; the expression of CD74 was measured as the same scoring system used by Beswick *et al.* ⁽⁹⁾. High expression of the examined tissue was graded as 2 when > 30% of epithelial cells stained positive for CD74, and low expression was graded as 1 when < 30% of epithelial cells stained positive for CD74.

Results

According to the non-invasive and invasive diagnostic methods used for the diagnosis of *H. pylori* infection; a significant difference was noticed ($P < 0.05$) between positive and negative *H. pylori* infected patients. Accordingly, Patients were grouped as *H. pylori* positive (n= 47; 73.4%) and *H. pylori* negative (n=17; 26.5%).

Increase CD74 expression on Gastric Epithelial Cell in Biopsy Samples during *H. pylori* Infection:

In order to study the expression of CD74 molecules, staining was done by using anti-CD74 clone LN-2. As seen in Figure (1), there was a marked increase in CD74 staining of epithelial cells during *H. pylori* infection. In this study, 64 patient biopsies were examined for CD74 expression. Of the 64 patients, 47 were positive for *H. pylori* infection, and as determined by staining of the 47 biopsies, 44(93.6%) were found to have high CD74 expression and 3 out of 47 (6.4%) were found to have low CD74 expression (Table-1), suggesting there is also a link between inflammation, which is a hallmark response to *H. pylori* infection, and CD74 expression. Of the 17 samples from negative *H. pylori* patients, 13 (76.5%) had low CD74 expression and 4 (23.5%) had high CD74 expression. The results revealed that there was a highly significantly difference ($p=0.0001$) between *H. pylori* positive and negative groups.

Table 1: The CD74 expression on gastric epithelial cells in patient biopsy samples in the presence or the absence of *H. pylori*:

CD74 expression on gastric epithelial cells ($P=0.0001^*$)	<i>H. pylori</i> positive		<i>H. pylori</i> negative	
	No.	%	No.	%
Low expression	3	6.4	13	76.5
High expression	44	93.6	4	23.5

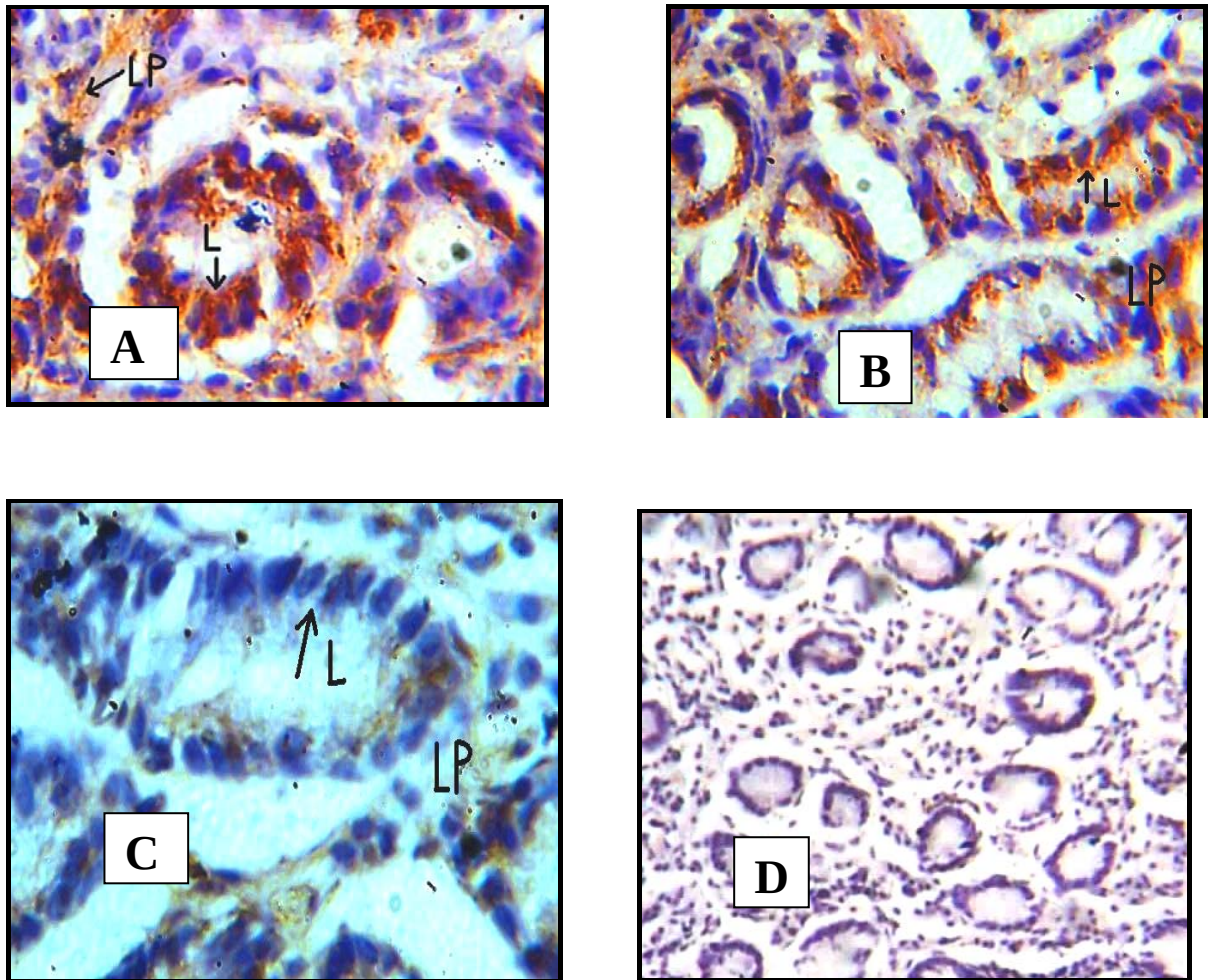


Figure 1: Immunohistochemical staining (IHC) in formalin fixed paraffin embedded antral biopsies from *Helicobacter pylori* infected (A and B) and uninfected (C) for the expression of the invariant chain CD74 . The sections were stained by DAB chromogen (brown) and counterstained with Hematoxylin (blue). (A and B) CD74 shows increased expression by epithelial cells in samples positive for *H. pylori*; compared with similar biopsies from uninfected biopsies (C); (D) Negative control section. Arrow points to intense apical staining on epithelial cells. L = lumen; LP = lamina propria.

Discussion

The variety of functions and signaling leading to immune responses that have recently been implicated in CD74 suggest that there is much more to be revealed about the functions of this molecule.

In this study, immunohistochemistry examination was performed on gastric biopsies from patients infected with

H.pylori to examine the expression of CD74. Interestingly, the expression of CD74 was evident in uninfected tissue, but the infected tissue showed a marked increase in CD74 expression with a highly significantly difference ($p = 0.0001$) between *H.pylori* positive and negative cases. The inflamed tissue also had other cells in the lamina

propria that expressed CD74. Specific staining using anti-CD74 clone LN-2 antibodies showed that the highest density of CD74 expression occurred along the apical side of the cells and faint staining was detected at the basolateral side. These results indicate a correlation between *H. pylori* infection and CD74 expression. There was a highly significant association between the high expression of CD74 in infected and uninfected patients. These results could be explained in the light of other studies, Beswick and colleagues^(8, 9) investigated the interaction of *H. pylori* with the class II major histocompatibility complex (MHC)-associated invariant chain (CD74), which found to be highly expressed by gastric epithelial cells, and they suggested a role for CD74 in gastric epithelial cell interaction with *H. pylori* leading to NF- κ B signaling that results in IL-8 secretion. Moreover, Bacterial binding was increased when CD74 surface expression was increased by IFN- γ treatment or by fibroblast cells transfected with CD74, while binding was decreased by CD74 blocking antibodies, enzyme cleavage of CD74, and CD74-coated bacteria. *H. pylori* was also shown to bind directly to affinity-purified CD74 in the absence of class II MHC. Increased CD74 expression by cells that showed increased IL-8 production in response to *H. pylori*, and agents that block CD74 decreased these responses. Therefore, adherence of the bacteria to the gastric mucosa is one of the initial steps of *H. pylori* infection and is an important virulence factor. Many different *H. pylori* adhesins have been identified⁽¹⁵⁾ implying that adherence is a multifactorial process.

In conclusion, the present results show that in *H. pylori* positive biopsy specimens an overexpression of CD74 were observed in infected patients.

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