EXPRESSION OF CD44 ISOFORMS IN HELICOBACTER PYLORI MEDIATED GASTRIC CARCINOGENESIS

4.1 INTRODUCTION

Recent evidences suggest that the alteration in epithelial cell growth in *Helicobacter pylori*-colonised mucosa is dependent on specific host factors than bacterial factors. Nevertheless, the role of the host factors in the pathogenesis of *Helicobacter pylori* associated disease has been largely ignored.

4.1.1 Cell adhesion molecule CD44

CD44 is one of the major molecules mediating adhesion between epithelial cells which was originally described as a glycoprotein surface marker of T.lymphocytes. The hematopoietic form of CD44 (70 to 90kDa) in lymphoid cells is the standard unit of CD44 protein.

4.1.2 Normal domain structure of CD44

CD44 is a single-pass transmembrane glycoprotein consisting of four functional domains. The distal extracellular domain is the region primarily responsible for the binding of hyaluronan. The membrane-proximal extracellular domain is the primary site of alternative splicing of CD44 mRNA that produces the many isoforms of CD44. The CD44 transmembrane domain is fairly typical of most single-pass membrane
glycoproteins. However, site-directed mutagenesis studies as well as work with detergent-solubilized hyaluronan receptors suggest that associated lipids or accessory membrane proteins, interacting with this domain, modulate hyaluronan binding as well as CD44 interaction with the cytoskeleton. In most isoforms of CD44, a 70-amino-acid cytoplasmic domain or "tail" is also expressed. This cytoplasmic domain exhibits protein motifs that indicate a capacity for interaction with cytoskeletal proteins as well as the potential for intracellular signaling.

![Figure 4.1 Protein Domains of CD44](image)

The four principal protein domains of CD44 are shown in the diagram (Fig.4.1) namely (1) the distal extracellular domain (link protein-homologous domain), (2) the membrane proximal extracellular domain, (3) the transmembrane domain and (4) the intracellular cytoplasmic domain.
Also shown is an isoform of CD44 containing protein extensions within the membrane proximal extracellular domain (3 exons shown in yellow). These protein extensions are absent in CD44s, the most common isoform of CD44.

4.1.3 CD44Gene/mRNA

The CD44 gene consists of 20 exons (19 exons in earlier literature, exons 6a and 6b have been reclassified as exons 6 and 7, to make 20 exons in total), (Fig.4.2). Although a single gene located on the short arm of human chromosome 11 encodes CD44, multiple mRNA transcripts that arise from the alternative splicing of 12 of the 20 exons have been identified. The standard and most prevalent form of CD44 (termed CD44s) consists of a protein encoded by exons 1-5, 16-18, and 20 (the exons shown in dark blue in this Fig. Since this form is also the predominant form on hematopoietic cells, CD44s is also known as CD44H. CD44s exhibits the extracellular domains (exons 1-5 and 16), the highly conserved transmembrane domain (exon 18), and the cytoplasmic domain (exon 20). The 1482 bp of open reading frame mRNA for human CD44s results in translation of a polypeptide chain of ~37 kDa. Post-translational addition of N-linked and O-linked oligosaccharides contribute to the ~85-kDa molecular mass of the final CD44 protein as estimated by Knudson et al., (1996).
The exons that encompass the CD44 genes are numbered and shown in the diagram (Fig.4.2). The top line of numbering illustrates a numbering system that uses 19 exons for CD44. The bottom line is based on the current numbering system, which uses 20 exons for CD44. The exons used for CD44s, the most common isoform of CD44, are shown in dark blue. Turquoise-colored exons denote the variant exons that exhibit extensive rearrangements due to alternative splicing, resulting in a myriad of variant isoforms of CD44. Alternative splicing may also occur within exons encoding the intracellular domain of CD44. Either exon 19 (light blue) or exon 20 (dark blue) are utilized, resulting in a short cytoplasmic tail isoform of CD44 or the complete cytoplasmic domain isoform, respectively (Underhill, 1992).
CD44 isoforms are created by alternative splicing of the mRNA. The variable exons are numerically designated from $V_1$ to $V_{10}$. Potentially each V exon can encode a separate domain, in the CD44 protein. The reason for the existence of so many CD44 isoforms are not known. Different isoforms may bind to different ligands (Sunsy, et al., 1997). The varied structure and distribution of CD44 suggests that the molecule has a variety of functions like cellular adhesion, hyaluronate degradation, lymphocyte activation, lymphnode homing, myelopoiesis and lymphopoiensis, angiogenesis, release of cytokines and bacterial receptor function (Sneath, and Manghen., 1998).

4.2 CD44 EXPRESSION IN NEOPLASIA

Earlier studies showed that CD44 is involved in cell attachment phenomenon such as binding of circulating lymphocytes to vascular endothelium and of sedentary epithelial and stromal cells to each other or to the intercellular matrix. Initial studies using Northern blotting (Stamenkovic et al., 1989; Stamenkovi, et al., 1991) suggested that tumor tissues contain additional, unusual CD44 transcripts relative to those present in corresponding normal tissues. Separate work in tumor cell lines (Birch, et al., 1991; Sy, et al., 1991). Woodman et al., (1996) have reported the increased expression of CD44s and variant isoforms v5, v6 and v9 in breast carcinoma cells. The expression of the CD44 isoforms v5, v7, v8 and most notably that of v6 were found to strongly correlate with tumors of squamous cell and bronchio-alveolar carcinoma origin (Tran, et al., 1997; Wimmal et al., 1997). A study using RT-PCR demonstrated the presence of CD44s and
v6 in all samples of normal cervix as well as those with invasive carcinomas (Shimaburkuro, et al., 1997). Colonic adenomas and carcinomas express a large range of CD44v isoforms (Goodison et al., 1997) Tumors of the gastric mucosa display increased expression of isoforms containing v8-10 and the expression of these has been correlated with invasion and metastasis (Yamaguchi et al., 1995). Some correlation with the expression of CD44v5 and v6 with histological subtype has been suggested, and these isoforms are also present in intestinal metaplasia of the stomach, a premalignant condition (Dammrich et al., 1995).

However, there are little or no data regarding a possible link between CD44 isoform expression and the clinical outcomes subsequent to Helicobacter pylori infection. Therefore, this study focus on the association of CD44 informs as a host factor in the pathogenesis of gastric diseases mediated by Helicobacter pylori.

4.3 WORKING HYPOTHESIS

The changes in the pattern of expression of a multi-functional gene in a single tissue of an organ are likely to disrupt essential epithelial-mesenchymal interactions and thus contribute to the progressive structural and functional disorganization characteristic of cancer. It is unclear whether overexpression of CD44 isoforms plays any functional role in the progression of the primary tumour from its earliest precursor to a large palpable tumour (Lesley et al., 1993; Matsumura and Tarin, 1992). Based on this, we
hypothesize that the expression of abnormal isoforms of CD44 induced by \textit{H. pylori} infection may contribute to the process of gastric carcinogenesis.

The purpose of this investigation was to obtain information for a better understanding of the molecular pathomechanism of gastric cancer in order to improve the diagnosis of premalignant and early malignant gastric lesions.

4.4 STUDY DESIGN

This study was carried out in two hundred gastric specimens, consisting of normal gastric mucosa (n=20), mucosa with chronic gastritis (n=63), atrophic gastritis (n=20), intestinal metaplasia (n=11) and gastric adenocarcinoma (n=86), in which the \textit{H. pylori} status have been analysed previously (Chapter II). Histopathological grading of specimens was carried out by haematoxylin and eosin staining according to revised Sydney System. The expression of CD44 isoform was studied at mRNA as well as protein level using RT-PCR and immunoblotting. The localization of CD44 isoform was also studied semi quantitatively by immunohistochemistry.

The data obtained were subjected to statistical analysis for the association of CD44 isoform between histological progression and \textit{H. pylori} status.
4.5 METHODOLOGY

Gastric biopsy samples were processed as given in the Chapter-II. From each case one biopsy was used to prepare frozen sections for histology and immunohistochemistry. 2 biopsies were used for immunoblotting analysis and one was used for RNA extraction and subsequent Northern blotting and RT-PCR.

4.5.1 Northern Hybridisation

1μg of the extracted RNA was separated in the 1.3% formaldehyde gel and transferred to Hybond N+ (Amersham) Filters. Then the filters were probed with the specific biotinylated probes specific for CD44 V6 exons. The hybridization was developed using star detection kit (New England Biolabs) as per manufacture's protocol (see appendix for detailed protocol.)

Probe : 5' – GCA ACA GAT GGC ATG AGG GA-3'

4.5.2 RT-PCR analysis of CD44 isoform expression

A fresh biopsy sample was snap frozen in liquid nitrogen and RNA was extracted using standard TRIZOL protocol. The extracted total RNA was reverse transcribed to cDNA by using reverse transcriptase. PCR was performed on the cDNA using standard primers published already.
Primers

Forward 5'-GAC ACA TAT TGC TTC AAT GC-3'
Reverse 5'-GAT GCC AAG ATG ATC AGC CA – 3'

(For detailed protocols see Appendix).

4.5.3 Immunoprecipitation and Immunoblotting

The tissue lysate prepared from the biopsies were immunoprecipitated with CD44 isoform rat monoclonal antibody using protein A sepharose. The immunocomplex was then resolved in 12% SDS-PAGE and transferred to nitrocellulose membrane. The transferred protein was then hybridized using CD44 isoform rat monoclonal antibody and the expression was detected using antirat secondary antibody tagged with HRP. DAB substrate was used as a chromogen (see Appendix for detailed protocol).

4.5.4 Immunohistochemistry and immunofluorescence

The CD44 isoform protein expression was semiquantitatively analysed by immunohistochemistry and further confirmed by immunofluorescence analysis. The cryostat sections were used for the analysis. After airdrying and fixation in acetone, the tissue sections were treated with 1% NP 40 for membrane lysis and treated with CD44 isoform rat monoclonal antibody.
After appropriate incubation and washing, the sections were treated with HRP conjugated antirat secondary antibody (or) FITC tagged antirat secondary antibody in case of immunofluorescence. DAB chromogen was used in immunohistochemistry and counter stained with haemotoxylin and examined under light microscope. In case of immunofluorescence, the sections were directly analysed under Axioscope-II fluorescence microscope using appropriate filters (for detailed protocol see Appendix).

Sections of tonsilitis tissue was used as positive control. Section without primary antibody treatment was used as negative control.

4.5.5 Assessment of immunoreactivity

Immunoreactivity of CD44 isoform was graded based on the intensity of the brown DAB colour developed and the cells showing positivity. Atleast 10% cells in the field showing positivity was taken as significant CD44 isoform expression, 10-25% cells mild expression, 26-50% moderate, 51% and more were taken as intense expression Table – 4.1.

Table 4.1 Assessment of Immunoreactivity

<table>
<thead>
<tr>
<th>Immunoreactive cells</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10%</td>
<td>Negative</td>
</tr>
<tr>
<td>10-25%</td>
<td>Mild</td>
</tr>
<tr>
<td>26-50%</td>
<td>Moderate</td>
</tr>
<tr>
<td>51 and above</td>
<td>Intense</td>
</tr>
</tbody>
</table>
4.5.6  Insitu Hybridisation analysis for CD44v6 expression

Frozen sections were airdried and fixed as mentioned in immunohistochemistry. The sections were incubated over night with biotinylated probe specific for CD44v6 exon sequence. After incubation, the section were developed with streptavidin – Biotin – ALP Conjugation method. (See appendix for detailed protocol)

Probe : 5’ – GCA ACA GAT GGC ATG AGG GA-3’

4.5.7  Data analysis

χ² analysis were used to identify the association of CD44 isoform with histopathological grade and with H. pylori status. The expression of CD44 isoform was analysed using three techniques, RT-RCR and immunoblotting for qualitative and immunohistochemistry for semiquantification. The patients were considered to express CD44 isoform if they were detected positive by both RT-PCR and immuno blot.

4.6  RESULTS

4.6.1  RT-PCR

CD44 is encoded by a single gene that is composed of at least 20 exons. Depending on the combination of the variant exons, RT-PCR will yield amplified fragments of 498 bp if the sample expresses CD44s and 588-1632 bp if the sample contains variant CD44. A representative RT-PCR reaction products are shown in Plate 4.1a. All the H. pylori positive, chronic gastritis, gastric atrophy and intestinal metaplasia samples yielded amplified fragments
PLATE 4.1

.A RT-PCR analysis of CD44 isoforms expression. *H. pylori* positive specimens showing three transcripts >1000 bp, ~850 bp and ~498 bp in contrast to normal showing only one transcript at ~850 bp. Cancer specimens also showing the same CD44 mRNA expression pattern as *H. pylori*; positive.

.B The quality and abundance of the cDNA were confirmed using β-actin specific PCR.

.C Proteins were electrophoresed on SDS - PAGE (5% and 10%) and transferred to nitrocellulose membrane and incubated with anti CD44 MAb followed by a second anti rat peroxidase coupled antibody, visualised by diaminobenzidine staining.
M-100bp ladder 1. Normal Stomach  2. Chronic Gastritis  3. Atrophic Gastritis
  4. Intestinal Metaplasia  5. Adeno carcinoma
Figure 4.3 Expression of CD44 isoform in the study population

- Normal n=20
- Gastritis n=63
- Atrophy n=20
- Intestinal metaplasia n=11
- Adenocarcinoma n=86

Histopathological Stages

- CD44 isoform negative
- CD44 isoform positive
of 498 bp, ~850 bp and >1000 bp, which indicates the transcriptional expression of CD44s and two more isoforms in infected stages, whereas normal samples showed only one transcript at ~850 bp. Gastric adenocarcinoma showed the same CD44 expression pattern as *H. pylori* infection. Differential expression of CD44s was also seen between infected cases and normal. The active *H. pylori* infected chronic gastritis patients showed increased expression of CD44s when other *H. pylori* positive atrophy and metaplasia patients showed comparatively low expression of CD44s.

### 4.6.2 Immunoblotting

The samples were also examined by immuno blotting and Immunofluorescence to confirm the RT-PCR results with protein expression and its localization in cells. *H. pylori* infected chronic gastritis, atrophic gastritis, metaplasia and gastric adenocarcinoma showed the expression of three CD44 protein isoforms, 80-95 kDa, 110-150 kDa and 200-220 kDa, whereas normal patients showed only the 110-150 kDa isoform. A representative immunoprecipitation and Immuno Blot results are given in the plate 4.1c and 4.1d.

### 4.6.3 Expression of CD44 isoforms in relation to Histological Stages of Gastric Carcinogenesis

Out of 200 cases analysed for the expression of CD44 isoforms by RT-PCR & WB, only 44 cases (22%) were negative for CD44 isoform while the remaining 156 cases (78%) were expressing CD44 isoform. In that, out of 20 normal patients 17/20 (85%) were negative for CD44 isoform and only 3
Figure 4.4 Association of CD44 isoform expression with \textit{H. pylori} infection
PLATE 4.2

Immunohistochemical analysis of CD44

(A) Normal stomach mucosa (20 x magnification)

(B) Chronic gastritis (20 x magnification)

(C) Gastric Atrophy (20 x magnification)

(D) Intestinal Metaplasia (20 x magnification)

(E) Adeno carcinoma (20 x magnification)
PLATE-4.2

Immunohistochemical Expression of CD44 in various stages of *Helicobacter pylori* Mediated Gastric Carcinogenesis

A

B

C

D

E
PLATE 4.3

**Immunofluorescence analysis of CD44**

(A) Normal stomach mucosa (20 x magnification)
(B) Chronic gastritis (20 x magnification)
(C) Gastric Atrophy (20 x magnification)
(D) Intestinal Metaplasia (20 x magnification)
(E) Adeno carcinoma (20 x magnification)
PLATE-4.3

Immunofluorescent Analysis of CD44 in various stages of *Helicobacter pylori* Mediated Gastric Carcinogenesis
cases (15%) were expressing CD44 isoforms. The expression in gastric preneoplastic lesions of chronic gastritis, atrophic gastritis, intestinal metaplasia were 53/63 (84%), 20/20 (100%) and 11/11 (100%) respectively. In gastric adenocarcinoma out of 86 patients 69/86 (80%) showed the CD44 isoform expression. These results indicate the steady increase in the expression of CD44 from normal to intestinal metaplasia. When the data were analysed for association with progression, significant association between CD44 isoforms and histopathological progression was observed. p<0.0001 $\chi^2 = 56.6$

Fig.4.3.

4.6.4 Association of CD44 isoform expression with *H.pylori* infection

Out of 57 *H.pylori* negative patients, 29 cases 29/57 (51%) were positive for CD44 isoform expression and 28 cases 28/57 (49%) were not expressing CD44 isoform. Out of 143 *H.pylori* positive cases only 16 cases 16/143 (11%) were negative for CD44, while the remaining 127/143 (88.8%) were expressing CD44 isoform. A highly significant association was found between *H.pylori* infection and CD44 expression. p<0.001 and $\chi^2 = 34.17$. Fig.4.4.

4.6.5 Immunohistochemistry and Immunofluorescence

Protein localization study by Immunostaining showed strong expression of CD44 on the glandular epithelial cells of all the *H.pylori* infected samples. The expression pattern was homogenous and infected epithelium mostly showed circumferential, linear, cell membrane staining. No difference in staining intensity was evident between *H.pylori* infected
Figure 4.5 Immunohistochemical expression of CD44 isoform in different Histopathological Stages

**Histopathological Stages**

- Normal n=20
- Gastritis n=63
- Atrophy n=20
- Intestinal metaplasia n=11
- Adenocarcinoma n=86

Legend:
- ■ Negative
- □ Mild
- ▼ Moderate
- ▣ Intense
Figure 4.6 Immunohistochemical expression of CD44 isoform in relation to H. pylori infection.
precancerous lesions and gastric adenocarcinoma. The expression of CD44 in the normal patients appeared relatively weak. (Plate 4.2 and 4.3).

*H. pylori* infected gastric precancerous lesions and gastric adenocarcinoma samples strongly expressed CD44 isoforms when compared to normal samples.

### 4.6.6 Immunohistochemical localisation of CD44 and the histopathological progression

The data for CD44 immunoreactivity among the 5 histopathological stages are given in Fig 4.5. It was observed that out of 20 normal samples, 17 were negative for CD44 (85%). In chronic gastritis cases, only 6 (9.5%) were negative for CD44, 22 (34.9%) were stained mild, 31 (49%) stained moderately and 4 (6%) intensely stained. In atrophy, 8/20 (40%) were stained moderately and 12/20 (60%) were stained intensely. In intestinal metaplasia, all the samples showed intense staining. In gastric cancer patients (*n*=86) 11.6% were stained negative, 28% stained mild, 42% stained moderate and 12% were stained intense. When the data were analysed for the association between expression and histopathological grade, highly significant association was observed. \( p < 0.0001 \) and \( \chi^2 = 134.07 \), Fig.4.5.

### 4.6.7 Association of CD44 immunoreactivity and *H. pylori* infection

The immunoreactivity of CD44 in relation to *H. pylori* status was analysed in the study population. Table 4.5 explains the data analysis.
Figure 4.7 Expression of CD44v6 in the study population

![Bar chart showing expression of CD44v6 in different histopathological stages.](chart)

- Normal n=20
- Gastritis n=63
- Atrophy n=20
- Intestinal metaplasia n=11
- Adenocarcinoma n=86

Histopathological Stages

- CD44v6 negative
- CD44v6 positive
Among the 57 *H. pylori* negative cases 25 (44%) were negative while the remaining 14/57 (25%) were mild, 16/57 (28%) were moderate, 2/57 (3.5%) stained intense. In the 143 positive cases, only 14/143 (10%) negative, while the remaining 46/143 (32%) were mild, 59/143 (41.3%) moderate and 24/143 (17%) intensely stained with CD44 and thus highly significant association between CD44 expression and *H. pylori* status was observed p<0.0001 and $\chi^2 = 32.46$ Fig.4.6.

4.6.8 Analysis of the Expression of CD44v6 mRNA using Northern hybridization and insitu hybridization

Biotinylated probe specific for variant exon v6 sequence was used, to analyse the expression of CD44v6 in the study population using Northern blotting and *in situ* hybridization. The visible chemiluminescent band reaction and the prominent cytoplasmic staining of BCIP/NBT substrate was considered positive for CD44v6.

4.6.9 Expression of CD44v6 in different histopathological stages

The data of CD44v6 expression in relation to histopathological stages are expressed in the Fig. 4.7. There are distinct differences in the expression of CD44v6 between the histopathological stages. However, the probe did not show the reactivity in the normal samples. In case of chronic gastritis, 50 /63 (79%) were positive for CD44v6 while the remaining 13/63 (20.6%) were negative. In atrophy only 2/20 (10%) were negative and the, remaining 18/20 (90%) were positive for CD44v6. All the cases of Intestinal metaplasia expresed CD44v6. In gastric cancer cases, 62/86 (72%) were
**PLATE 4.4**

Northern hybridization Analysis of the expression of CD44v6

<table>
<thead>
<tr>
<th>Lane</th>
<th>Stage</th>
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<tbody>
<tr>
<td>(1)</td>
<td>Normal stomach mucosa</td>
</tr>
<tr>
<td>(2)</td>
<td>Chronic gastritis</td>
</tr>
<tr>
<td>(3)</td>
<td>Gastric Atrophy</td>
</tr>
<tr>
<td>(4)</td>
<td>Intestinal Metaplasia and</td>
</tr>
<tr>
<td>(5)</td>
<td>Adenocarcinoma</td>
</tr>
</tbody>
</table>
1. Normal Stomach  2. Chronic Gastritis  3. Atrophic Gastritis
4. Intestinal Metaplasia  5. Adeno carcinoma
PLATE 4.5

In situ analysis of CD44v6 Expression

(A) Normal stomach mucosa (20 x magnification)
(B) Chronic gastritis (20 x magnification)
(C) Gastric Atrophy (20 x magnification)
(D) Intestinal Metaplasia and (40 x magnification)
(E) Adenocarcinoma (40 x magnification)
PLATE-4.5

*In situ* Analysis of CD44v6 in various stages of *Helicobacter pylori* Mediated Gastric Carcinogenesis
Figure 4.8 Association of CD44v6 expression with H. pylori infection
positive for CD44v6 and the remaining 24/86 (28%) were negative. \( p<0.0001 \)
\[ \chi^2 = 58.5 \] Fig. 4.7.

When the CD44V6 expression was analysed in relation to \( H. pylori \) infection, only 30/153 (21%) were not expressing CD44V6, while the remaining all positive samples showed CD44v6. \( p<0.001 \) and \( \chi^2 = 17.51 \) Fig.4.8. These results suggest that there is a close association between \( H. pylori \) infection and CD44v6 expression.

4.7 DISCUSSION

Several groups simultaneously reported the presence of spiral bacteria in patients with chronic gastritis and peptic ulceration and now it is recognized and accepted that, \( Helicobacter pylori \) infection is the most frequent cause of gastritis, peptic ulcer disease and gastric adenocarcinoma. The development of gastric carcinoma involves a multistep process from chronic gastritis towards atrophy, intestinal metaplasia and finally to dysplasia. It has been shown that atrophic gastritis and intestinal metaplasia significantly increase the risk of gastric carcinoma. \( H. pylori \)-induced gastritis is significantly more pronounced in gastric carcinoma patients Stolte and Meining \textit{et al.}, 2000 and Asaka \textit{et al.}, (1995) have indicated that \( H. pylori \) infection provides the environment suitable for the development of gastric cancer in the form of atrophic gastritis, gastric atrophy and intestinal metaplasia. The molecular mechanism of gastric adenocarcinoma induced by \( Helicobacter pylori \) in humans remains unclear. We therefore propose that the host factors governing clinical outcome subsequent to \( Helicobacter pylori \) infection may play a vital
role in *Helicobacter pylori* pathogenesis and in the development of gastric cancer.

Variant CD44 has received attention as a cell surface molecule expressed mainly on leukocytes but also expressed on epithelial cells and cells of mesodermal and neuroectodermal origin. This receptor can be structurally and functionally considered as one of the most variable surface molecules. Alternative splicing of variant exons as well as post transcriptional modifications (i.e., glycosylations) enrich the CD44 repertoire, which in turn may increase the optimal functions of the molecule. CD44 isoform molecules are preferentially expressed on epithelial malignant lesions and some of them have recently been regarded as promising tools for the improvement of diagnostic accuracy and for predicting the unfavourable outcome of several neoplastic diseases (Naor *et al.*, 1997). Further more, the possibility of interfering with specific functions mediated by these molecules has been also proposed as the basis for a new therapeutic approach for inhibiting tumor progression (Wittig *et al.*, 1998). The clinical significance of the expression of CD44 isoforms is not very well known in gastric cancer. Several studies have shown that splice variants are overexpressed by aggressive non-Hodgkin's lymphoma (Koopman *et al.*, 1993) as well as in human colorectal carcinoma (Joensuure, *et al.*, 1993) lung (Fox *et al.*, 1993) esophageal (Gotoda, *et al.*, 2000), uterine (Shimabukuro *et al.*, 1997) and gastric carcinoma (Yamaguchi *et al.*, 2002).

The correlation between CD44 expression and prognosis was possible because, CD44 isoforms are expressed in most cell types, but not in
the normal gastric mucosa (Mayer et al., 1993). CD44 isoforms are expressed only when there is atrophic gastritis with a leukocyte infiltration. However, there is no data regarding a possible link between CD44 isoforms and the clinical outcome subsequent to *Helicobacter pylori* infection. To examine this point, molecular and immunochemical analysis of CD44 isoform expression on the *Helicobacter pylori* positive preneoplastic and neoplastic lesions of stomach was performed.

In this study, biopsy specimens of chronic gastritis, atrophic gastritis, intestinal metaplasia and gastric carcinoma were obtained from patients confirmed with *H. pylori* infection. Overexpression of CD44 isoforms was found in *Helicobacter pylori* infected chronic gastritis, atrophy, metaplasia and also in adenocarcinoma. mRNA and protein expression study confirmed the over expression of CD44s and some of its variant isoforms in the *Helicobacter pylori* induced pathological conditions. Normal patients showed only one high molecular weight protein at 100-200 kDa and ~850 bp transcript in western blot and RT-PCR unlike the infected cases which showed three isoforms. Results of CD44 isoform expression in gastric adenocarcinoma are consistent with previous reports (Harn et al., 1995). Surprisingly *H. pylori* infected early stage like chronic gastritis showed similar CD44 isoform pattern as in gastric adenocarcinoma.

The CD44v6 expression in the gastric biopsies was analysed using Northern hybridisation and insitu hybridisation to exploit the role of CD44v6 in gastric pathogenesis induced by *H.pylori*. Moreover, CD44v6 was not expressed in normal tissue without *H.pylori* infection and abundant expression
was noticed only in the *H. pylori* infected preneoplastic and neoplastic lesions. A highly significant statistical association was obtained between CD44v6 expression and histopathological progress as well as with *H. pylori* status.

The expression of the CD44 isoform in different clinical stages positive with *Helicobacter pylori* was also evaluated. The *H. pylori* infection induced expression of CD44 isofroms were persistent upto malignant stage. Earlier reports suggest that CD44 expression may be controlled by ras, Rho family GTPases as well as by protein kinase C. Overexpression of oncogene c-H-ras expression in the *Helicobacter pylori* positive precancerous and cancerous lesion of stomach was reported earlier (Suganuma *et al.*, 2001). Transfection studies on NIH3T3 fibroblasts have shown that overexpression of the oncogenic mutants of H-ras can result in enhancement of CD44 cleavage accompanied by the promotion of CD44 mediated cell migration (Kawano *et al.*, 2000). In conclusion, this study clarifies the association between *H. pylori* infection and gastric carcinogenesis. The high expression of CD44 isoforms in *H. pylori* infection unveils some aspects of host parasite interaction. The expression of carcinogenic potential variant CD44 induced by *H. pylori* infection thus plays an important role in pathogenesis.