International Journal of Research and Review
www.ijrrjournal.com E-ISSN: 2349-9788; P-ISSN: 2454-2237

*Original Research Article*

# The Study of Proportion and Molecular Characterisation of *Helicobacter pylori* in Dyspeptic Patients in Sri Manakula Vinayagar Medical College and Hospital

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## ABSTRACT

**Background**: *Helicobacter Pylori* is the colonized in humans over 100,000 years, the prevalence of H.Pylori in different in various parts of the world. Complications of the organism are varies. This study helps in proportion of H.pylori in the patients attending in tertiary hospital.
**Aims:** 1. The study of proportion of Helicobacter pylori in dyspepsia.
2. Molecular characterisation of Helicobacter pylori in dyspeptic patients in Sri Manakulavinayagar Medical College and Hospital.
**Methodology:** A Hospital based cross section study conducted in Department of General Medicine. The study period was about one and half year after obtaining the ethical committee approval. The study population is of 95 who are came with the complaint of dyspepsia diagnosed by Rome category, patients underwent upper gastro endoscopy tissue sample obtained sent for histopathology staining, Urease test, PCR test.
**Results:** Among the study participants, histopathology report for H.pylori positive for 19 out of 95 participants, percentage 20.0%.Urease report is positive for 22 out of 95 participants, percentage 23.16%.Among the histopathology positive for H.pylori in study participants, PCR for VacA Gene is positive in 10 out of 19 study participants, percentage 52.63%.
P valve = 0.001 in Histopathology report, P valve = 0.003 in Urease report, P valve = <0.01 in PCR report. P value <0.05 will be considered statistically significant.
**Conclusions:** H.pylori infections cause gastro-duodenal ulcers, carcinoma, MALT lymphoma. Identifying and treating the infection is important. Patients with VacA gene positive in this study can be treated with four drug regimens.

***Keywords:*** Helicobacter pylori, dyspepsia, Histopathology test, Urease test, PCR test.

## INTRODUCTION

H. pylori is a gram-negative bacillus that has naturally colonized humans for at least 100,000 years. [1] The prevalence of this infection varies world widely as low as 10 percent in developed western nations to higher than 80 per cent among the indigent populations of many developing countries it is estimated that more than 20 million are affected in India. [2]

A working group of the World Health Organization's International Agency for

Research on Cancer concluded that H. pylori is a group I carcinogen in humans. [3] Dyspepsia is a common symptom. Dyspepsia is defined as upper abdominal or retrosternal pain or discomfort referable to the proximal alimentary tract. [4]

The manifestations of H. pylori infection include gastritis, gastric atrophy, duodenal ulcer disease, gastric ulcer disease, primary gastric B-cell lymphoma, gastric adenocarcinoma, iron deficiency anemia and vitamin B12 deficiency. [5]

## MATERIALS AND METHODS

**Study design**:
A hospital based cross sectional study

**Duration of study:**
18 months

**Study Population**:
The Patients who attended the outpatient and admitted as inpatient in the department of general medicine

**Sample:**
The patient who came with the complaint of dyspepsia, upper gastro intestinal endoscopy done and tissue sample taken from gastric antrum, lesser & greater curvature of the stomach, and body of the stomach.

**Sample size:**
Sample size calculated to be 95
Calculated by formula = $4*p*q/d^2$
P = 65% from the previous study [7]
Q=100-p, d = 5% of absolute precision
4*65*35/25=95

**Inclusion criteria**:
Dyspepsia, defined as upper abdominal pain or discomfort more than 2 months associated with any of the following:

- Bothersome postprandial fullness
- Early satiation
- Epigastric burning
- Persistent or recurrent pain or discomfort centered in the upper abdomen

(Above the umbilicus)

- Not relieved by defecation or associated with the onset of a change in stool
- Frequency or stool form (i.e., not irritable bowel syndrome)
- Relief / aggravation with food intake Before meals or when hungry
- Nausea or vomiting
- Abdominal bloating or distension
- Anorexia and weight loss (more than 3 kg)
- Night pain. [6]

**Exclusion criteria:**

- Pregnant and lactating females.
- patients who had a history of gastric surgery,
- Chronic intake of steroids,
- Patients who had an active infection requiring current antimicrobial therapy,
- Had taken antimicrobial agents within 2 weeks prior to endoscopy, or
- Had active gastrointestinal bleeding.

**Data Collection Tool:** Proforma Attached

**METHODS**

1. Dyspepsia patients are selected and procedure was explained to the patient after getting the consent send for upper GI endoscopy, tissue sample taken from gastric antrum , lesser and greater curvature, body of the stomach.
2. Tissue sample put in formalin solution sent for staining: Haematoxylin and Eosin Staining and Slow Giemsa Staining. Positive sample for Helicobacter pylori sent for PCR technique for VacA gene sensitivity. [7]
3. **PCR detection of vacA genotypes:** DNA from the biopsy samples will be isolated using Qiagen- QIAmp DNA Mini Kit (Cat.No. 51304), following the manufacturer's instructions. DNA will be stored at 40C until used.

PCR will be performed using the following primer set, targeting the 678 bp intermediate region of vacA gene,
VacA-1.SE
CAATCGTGTGGGTTCTGGAGC,
Forward

VacA-3.AS
GCCGATATGCAAATGAGCCGC,
Reverse [described by Monstein et.al, 2002]
2.5µl of each forward and reverse primer (10µm), 5µl of template DNA will be added to a 25 µl of 1X PCR master mix and 6µl of PCR water. Amplification will be done in BIO-RAD T100 Thermal Cycler with 35 cycles of initial denaturation at 930C for 5 minutes, cycle denaturation at 930C for 1 minute, annealing at 580C for 1 minute, cycle extension at 720C for 1 minute, final extension at 720C for 5 minutes and hold at 40C for 5 minutes. The resulting product will be viewed in 1% agarose gel electrophoresis with 100bp DNA marker.

**Agarose Gel Electrophoresis:**

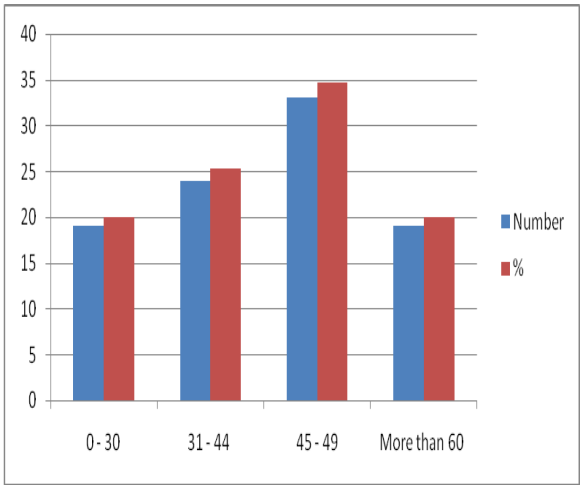
Exactly 1g of agarose will be added in 100ml of 1X TAE buffer and heated in a microwave oven for 2-3 minutes to get a clear solution. When the agarose solution has cooled down to a tolerable warm temperature, ethidium bromide (EtBr) will be added to get a final concentration of approximately 0.5µg/ml. agarose solution will be poured carefully into the gel casting tray after placing the comb in position. The gel tank will be filled with the tank buffer (1X TAE).The comb will be removed carefully without damaging the wells. Pre run of the gel for 5 minutes at 50V will be done. 10µl of the PCR product will be added to each well
And 10µl of 100bp DNA marker will be added in one well. Electrophoresis will be run for 20 minutes at 50V.

DNA Sequencing will be done for all the positive samples and compared with the reference sequences available in the NCBI database [https://blast.ncbi.nlm.nih.gov/Blast.cgi].
Phylogenetic tree will be constructed and sequence heterogenecity among the vacA genotypes will be calculated.
4. A valid informed consent in patient's native language will be obtained after explaining to the patient about the nature of study and any queries from the patients will be cleared.
5. The study will be conducted after ethical committee clearance.

**INSTRUMENTATION:**
1. Staining of the biopsy sample done through
Haematoxylin and Eosin Staining
Slow Giemsa Staining
2. DNA isolation done using Qiagen-QIAmp DNA Mini Kit (Cat.No. 51304**)**
3. Amplication will be don through BIO-RAD T100 Thermal Cycler

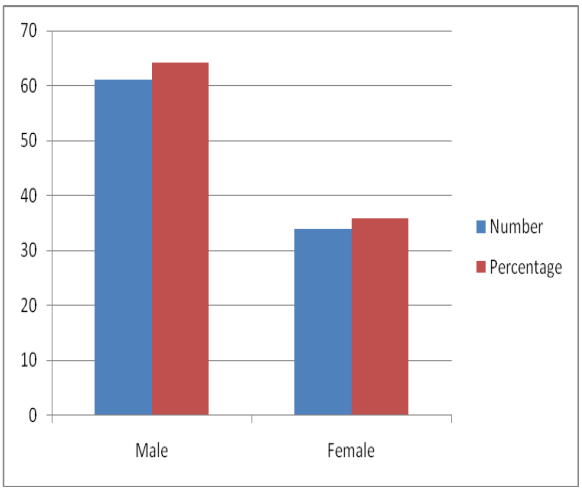
**Statistical analysis**:
Data entry will be done in Microsoft Excel, analysis will be done using software SPSS version 24.0
Basic description of study variables will be carried out using proportion, median, range, mean ± standard variation based on type of variables.
Association between clinical features and risk factors with the presence of H. pylori will be done using Chi-square test
Comparison of continuous variables between H. pylori positive and H.pylori negative group will be done using unpaired 't' test.
P value <0.05 will be considered statistically significant. Al test will be two sided.

## RESULTS




**Bar chart 1.Distribution of study participants by age**

Study participants in this group mostly come in the age group of 45-59 years which is the most common age of dyspepsia




**Bar chart 2. Gender distribution of study participants**

Most of study participants are male, percentage 64.21%and female, percentage 35.70%.

**Table 1: Endoscopy findings among the study participants:**

| Endoscopy findings | Number | Percentage |
|---|---|---|
| Antral erosion | 23 | 24.2 |
| Congestive gastropathy | 43 | 45.3 |
| Distal esophagitis | 4 | 4.2 |
| Duodenal erosion | 3 | 3.2 |
| Duodenal nodule | 1 | 1.05 |
| Duodenal ulcer | 5 | 5.3 |
| Gastric erosion | 4 | 4.2 |
| Gastro duodenal erosion | 10 | 10.53 |
| Normal | 2 | 2.11 |
| **Total** | **95** | **100** |

In this study, participants underwent endoscopy appearances are taken into account, percentage are made in which the congestive gastropathy is the most common presentation in this study with the percentage of 45.3%, the least common duodenal nodule is 1.05% percentage other common appearances such as duodenal ulcer is 5.3% percent, gastric erosion is 4.2%.

**Table-2: Frequency of Histopathology report among the study participants:**

| Histopathology report | Number | Percentage |
|---|---|---|
| **Positive** | 19 | 20.0 |
| **Negative** | 76 | 80.0 |
| **Total** | **95** | **100** |

Among the study participants, histopathology report for H.pylori positive for 19 out of 95 participants, percentage 20.0%.

**Table-3: Frequency of Urease report among the study participants:**

| Urease report | Number | Percentage |
|---|---|---|
| **Positive** | 22 | 23.16 |
| **Negative** | 73 | 76.84 |
| **Total** | **95** | **100** |

Among the study participants, urease report is positive for 22 out of 95 participants, percentage 23.16%.

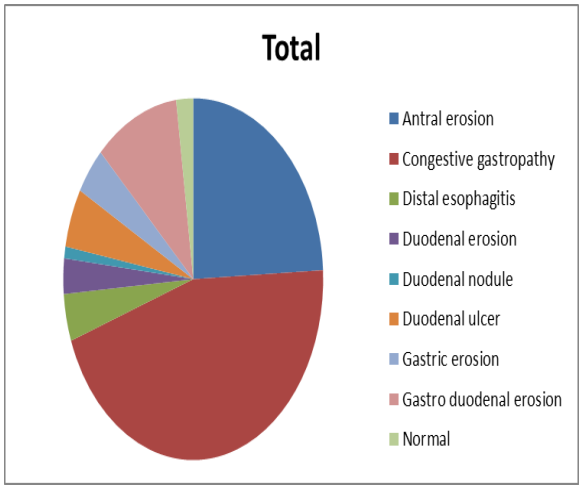
**Table-4: Frequency of PCR report among the histopathology report positive for H.pylori:**

| PCR report | Number | Percentage |
|---|---|---|
| **Positive** | 10 | 52.63 |
| **Negative** | 9 | 47.37 |
| **Total** | **19** | **100** |

Among the histopathology positive for H.pylori in study participants, PCR for VacA Gene is positive in 10 out of 19 study participants, percentage 52.63%.

**Table 5: Comparison of Endoscopy findings with HPE, PCR, Urease findings: (n=95)**

| Endoscopy findings | Total | HPE Positive N (%) | Urease Positive N (%) | PCR Positive N (%) |
|---|---|---|---|---|
| Antral erosion | 23 | 3 (13.04) | 4 (17.4) | 1(4.4) |
| Congestive gastropathy | 43 | 2 (4.7) | 3 (7.0) | 0(100.0) |
| Distal esophagitis | 4 | 3 (75.0) | 3(75.0) | 3 (75.0) |
| Duodenal erosion | 3 | 1 (33.3) | 1 (33.3) | 0 (0.0) |
| Duodenal nodule | 1 | 1 (100) | 1 (100.0) | 1(100.0) |
| Duodenal ulcer | 5 | 2 (40.0) | 2 (40.0) | 2 (40.0) |
| Gastric erosion | 4 | 2 (50.0) | 2 (50.0) | 1 (25.0) |
| Gastro duodenal erosion | 10 | 4 (40.0) | 5 (50.0) | 2 (20.0) |
| Normal | 2 | 1 (50.0) | 1 (50.0) | 0 (0.0) |
| **P Value** | | **0.001** | **0.003** | **<0.01** |




Among the study participants, P value =0.001 in Histopathology report, P value =0.003 in Urease report, P value =<0.01 in PCR report. P value <0.05 will be considered statistically significant.

**HPE vs PCR**

**1. Sensitivity and specificity (HPE vs PCR )**

| | PCR + | PCR - | Total |
|---|---|---|---|
| HPE+ | 10 | 9 | 19 |
| HPE - | 0 | 76 | 76 |
| Total | 10 | 85 | 50 |

| | |
|---|---|
| Sensitivity : | 100% |
| Specificity : | 89.41% |
| Positive predictive value : | 52.6% |
| Negative predictive value : | 100% |
| Diagnostic Accuracy: | 90.53% |

**2. SENSITIVITY AND SPECIFICITY (Urease vs PCR)**

| | PCR + | PCR - | Total |
|---|---|---|---|
| Urease+ | 10 | 12 | 22 |
| Ureas - | 0 | 73 | 73 |
| Total | 10 | 85 | 95 |

| | |
|---|---|
| Sensitivity : | 100% |
| Specificity : | 85.88% |
| Positive predictive value : | 45.45% |
| Negative predictive value : | 100% |
| Diagnostic Accuracy: | 87.37% (79.21 ,92.62 ) |

## DISCUSSION

Patient attending the tertiary care hospital with complaints of dyspepsia for more than 2 months. Basic investigation such as complete blood count, random blood glucose, renal function test, liver function test, investigation for procedure such as HIV, HbsAg. [8] During upper gastro endoscopy, tissue sample taken from antrum, greater and lesser curvature of stomach sent for histopathology staining such as Haematoxylin and Eosin Staining and Slow Giemsa Staining, urease test, PCR test. [9]

From the previous studies, the patients who came with the complaints of dyspepsia diagnosed by Rome criteria. the results shows more the two third positive in histopathology staining test and 40-60% are Vac A gene positive among the histopathology positive patients. [10]

The H. pylori World Health Organization's International Agency for Research on Cancer concluded that H. pylori is a group I carcinogen in humans.

The infection primarily involves the upper gastrointestinal tract causing progressive inflammation typically these inflammatory changes are silent but clinical manifestations seen after a long latent period. [11]

The manifestations of H. pylori infection include gastritis, gastric atrophy, duodenal ulcer disease, gastric ulcer disease, primary gastric B-cell lymphoma, gastric adenocarcinoma, iron deficiency anaemia, and vitamin B12 deficiency

Dyspepsia is a common symptom among the patient with H.pylori infections.

The risk of infection is related to the sanitary practice. Contaminated water is often the primary mode of transmission in rural areas. [12]

Higher socio-economic status the risk of infection correlates with the level of household hygiene. The outcome of an H. pylori infection reflects a complex interplay of environmental, host and bacterial factors including the virulence of the infecting bacterial strain. [13]

Profiling of the H. pylori gene pool serves as a surrogate marker for population migration and demographic studies, thus constituting the so-called "geographic genomics". [14] Genetic variation within bacterial populations can provide information relating to their evolution. However, it is rare that this variation can

provide a window into their hosts' evolution.

The evolutionary forces of natural selection and random genetic drift which includes founder effects may have each helped shape the gene pool of H. pylori. [15]

Genetic diversity implies a lack of population wide selection for just one or a few universally most fit H. pylori genotypes. Some of this may reflect preferential transmission within families and among people in close contact but not in large epidemics

DNA fingerprinting can distinguish any given isolate from most others and because of the; 3 to 5% DNA sequence divergence typically found in essential genes from unrelated strains. [16] This mutational diversity is enhanced by a rich history of inters train recombination.

The most probable place for genetic recombination is human gastric mucosa and it is possible that during the long-term colonization the H. pylori strains may undergo adaptive changes and eventually become significantly different from the ancestral genotype

The presence of mosaic genes' 4 in particular implies genetic recombination in vivo: one strain acquires another and swaps it into its chromosome.DNA from this mixing of genetic elements which has been likened to reproduction sexual in higher animals [17]

This toxin is encoded by the vacA gene, which is present in virtually all H. pylori strains. Recently, the existence of different allelic variants in two parts of this gene has been described

vacA s1a strains are associated with greater antral mucosal neutrophil and lymphocyte infiltrates than type s1b or s2 strains. vacA type m1 strains are associated with greater gastric epithelial damage than type m2 strains. Duodenal ulcer disease appears to be more prevalent in patients infected or colonized with type s1a strains than in patients colonized with type s1b and s2 strains. [18]

Among the study participants, Histopathology report positivity is less compare to the previous study but PCR report positivity correlation with pervious study. Histopathology report for H.pylori positive for 19 out of 95 participants, percentage 20.0%. Urease report is positive for 22 out of 95 participants, percentage 23.16%. Among the histopathology positive for H.pylori in study participants, PCR for VacA Gene is positive in 10 out of 19 study participants, percentage 52.63%.

P value = 0.001 in Histopathology report, P value = 0.003 in Urease report, P value = <0.01 in PCR report. P value <0.05 will be considered statistically significant.

**Limitations:** pylori infection causes chronic superficial gastritis in the stomach (The term gastritis use to describe not only histologically but also to describe endoscopic appearances and even symptoms but these features does not correlate with microscopic findings or even with the presence of H. pylori

The study was conducted in small amount of people may be conducted in a larger scale. Good proportion of H.pylori infection can be seen.

An additional reason for this difference might be because another set of primers, including PAI empty site primers was used in this study.

## CONCLUSION

H. pylori plays a significant role in causing symptoms of functional dyspepsia. Treatment with quadruple drug regimen brings a significant long-term improvement in the symptoms and prevents gastric carcinoma.

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How to cite this article: Sankar MKU, Yogesh S, Shenbagaram K et.al. The study of proportion and molecular characterisation of *helicobacter pylori* in dyspeptic patients in Sri Manakula Vinayagar Medical College and Hospital. International Journal of Research and Review. 2019; 6(5):219-225.

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