IDENTIFICATION OF *Helicobacter pylori* IN SALIVA OF PATIENTS WITH AND WITHOUT GASTRITIS BY POLYMERASE CHAIN REACTION

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the degree of

MASTER OF DENTAL SURGERY

BRANCH IV

ORAL AND MAXILLOFACIAL PATHOLOGY

March 2009

CERTIFICATE

This is to certify that this dissertation titled **"IDENTIFICATION OF *Helicobacter pylori* IN SALIVA OF PATIENTS WITH AND WITHOUT GASTRITIS BY POLYMERASE CHAIN REACTION"** is a bonafide record of work done by **Dr. E.V.SOMA SEKHAR GOUD** under our guidance during the post graduate period 2006 - 2009.

This dissertation is submitted to THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY in ORAL AND MAXILLOFACIAL PATHOLOGY, BRANCH IV**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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ACKNOWLEDGMENT

This project would have been a distant dream without the grace of LORD SHRI VENKATESWARA, the Almighty. I profusely thank god, for his blessings and grace, without which my project would not have seen the light of the day.

I express my deep sense of gratitude and indebtedness to **Dr.K.Ranganathan***, MDS MS (ohio), Professor and Head, Department of oral and Maxillofacial Pathology, Ragas Dental College and Hospital for providing a very good research atmosphere and all the necessary facilities for the work. I owe him my heartfelt thanks for his constructive suggestions, untiring patience and strenuous effort in bringing out and finalizing the dissertation manuscript in a beautiful and a systematic way.*

I am greatly indebted to **Dr. Uma Devi.K.Rao***, Professor, Department of oral and Maxillofacial Pathology, Ragas Dental College and Hospital for her magnanimity, unstinted, kind and whole hearted help, constant encouragement, cooperation, valuable guidance, constant supervision, constructive criticism, support, and help throughout my postgraduate curriculum, and her advice in completion of this work.*

It gives me immense pleasure to thank gratefully and express my profound gratitude to **Dr.Elizabeth Joshua***, Professor for her able and erudite guidance, supervision at every stage of my work and constant monitoring during the course of my project. I sincerely thank her for*

her sustained and keen interest in my work and extending her wholehearted support in my efforts to complete my work

I record my sincere gratitude and thanks to Principal **Dr.S.Ramachandran** *Ragas Dental College and Hospital for providing a very good research atmosphere and all the necessary facilities for the work*

*I thank my Readers***, Dr.T.Rooban,** *and lecturers* **Dr.K.M.Vidya, Dr.Jayanthi.P, Dr.Deepu George, Dr.Lavanya.** *Department of oral and Maxillofacial Pathology, Ragas Dental College and Hospital*

I express my heartfelt regards ***to*** **Dr.Krishna sagaram, Dr.Tiruvadanam**, *Sundaravadanam Nursing Home*. **Dr.Selvarangam** *Selvarangam Nursing Home for helping me in collecting the samples from their hospitals their, moral support and the much-needed encouragement in the course of my project.*

I acknowledge with affection and thanks, the arduous efforts of my entire batchmates **Dr.Deepak, Dr.Kanwar, Dr.Mukur, Dr.Samyuktha, Dr.Vinod**. *I thank all my seniors and juniors for their constant help and support throughout my post graduation.*

I express my heartfelt regards to **Dr.Arati sagar** *who guided me to Sundaravadanam Nursing Home and Selvarangam Nursing Home for sample collection.*

I extend my sincere thanks to Research Assistant **Mrs.Kavitha.**

I express my heartfelt regards biostatistician **Mrs.Deepa,** *Lab technician* **Mr.Rajan** *for all the patience shown and the constant help they rendered in completion of this study.*

I express my regards and gratitude to my parents and family for their affection and constant inspiration through out my educational career.

CONTENTS

ABBREVIATIONS

µl	microliter
µg	microgram
ATP	adenosine tri phosphate
bp	base pairs
CTAB	cetyl trimethyl ammonium bromide
DNA	deoxy ribonucleic acid
DEPC	diethyl pyro carbonate
DU	duodenal ulcer
dNTP	deoxy nucleotide triphosphate
dATP	deoxy adenosine triphosphate
dTTP	deoxy thymidine triphosphate
dCTP	deoxy cytosine triphosphate
dGTP	deoxy guanosine triphosphate
EDTA	ethylene diamine tetra acetic acid
Et Br	ethidium bromide
FP	forward primer
g	gravity
GU	gastric ulcer
GC	gastric carcinoma
IAA	iso amyl alcohol
IPA	iso propyl alcohol
Kb	kilobase
L	liter
M	molar
MALT	mucosa associated lymphoid tissue
mM	millimolar
NaCl	sodium chloride

NUD	Non Ulcer Dyspepsia
PBS	phosphate buffer saline
PUD	peptic ulcer disease
RP	reverse primers
rpm	rotations per minute
rRNA	ribosomal ribonucleic acid
RUT	rapid urease test
SDS	sodium dodecyl sulphate
TE	tris EDTA
TBE	tris boric acid EDTA
TAE	tris acetate EDTA
Tris Hcl	tris hydrochloric acid
U	unit
UBT	Urea breath test
V	volts

GENES

babB	blood group antigen –binding adhesion gene
*cag*A-*glr* racemase	cytotoxin associated gene A glutamate
Cag PAI Island	cytotoxin associated gene Pathogenicity
Fla A&G	flagellin genes
Ice	induced by contact with epithelium gene
Oip	outer inflammatory protein gene
Ure A	ureas gene A
Ure B	ureas gene B
Ure C	ureas gene C (*glmM*)(phosphoglucosamine mutation gene)
Ure D	ureas gene D
Ure E	ureas gene E

Ure F	ureas gene F
Ure G	ureas gene G
Ure H	ureas gene H
Ure I	ureas gene I
Vac A	Vacuolating cytotoxin A gene
RAPD	Random amplified polymorphic DNA
Pic A&B	permitting induction of cytokines genes

Introduction

Helicobacter pylori is a poly-flagellated, spiral, gram negative, micro-aerophilic, bacterium[1], with 4-6 sheathed flagella that is involved in the pathogenesis of peptic ulcer diseases and is the main etiologic agent of duodenal ulcer and chronic atrophic gastritis. More than hundred *Helicobacter* species have now been identified, the majority being gastric organisms. *H. pylori* presence is not a disease in itself but a condition that affects the relative risk of developing various clinical disorders of the upper gastrointestinal tract.

Colonization with *H. pylori* always leads to infiltration of the gastric mucosa in both antrum and corpus with neutrophilic and mononuclear cells. This chronic active gastritis is the primary condition related to *H. pylori* colonization. Very little is known about the acute phase of infection and mostly come from reports of patients who ingested *H. pylori* or underwent procedures with contaminated gastric endoscopy apparatus.[2]

It has been found that, a close correlation exists between the level of acid secretion and the distribution of gastritis. This interaction is critical in the determination of outcomes of *H. pylori* infection. Approximately 95% of duodenal ulcers and 85% of gastric ulcers occur in the presence of *H. pylori* infection[3.] The gastric mucosa does not normally contain lymphoid tissue, but MALT nearly always appears in response to colonization with *H. pylori*. In rare cases, a monoclonal population of B cells may arise from this tissue and slowly proliferate to form a MALT lymphoma.

H. pylori is also related with coronary heart disease, dermatological disorders such as rosacea and idiopathic urticaria, autoimmune thyroid disease and thrombocytopenic purpura, iron deficiency anemia, Raynaud’s phenomenon, scleroderma, migraine, and Guillain-Barre´ syndrome. The mechanism by which it is

related is by chronic low-grade activation of the coagulation cascade, accelerating atherosclerosis, antigenic mimicry between *H. pylori* and host epitopes leading to autoimmune disorders[4].

Attempts to show saliva as an appropriate specimen for diagnosing *H.pylori* have not shown consistent result. This may be due to low detection rates of *H. pylori* in the salivary secretion and low detection power of the method used. The reason could be that environment present in the mouth affects the survival of these organisms due to increased oxygen tension prevalent in some areas. Another reason could be the high contamination load with other organisms of the micro flora which suppresses the growth of *H. pylori.*

The present study was designed to detect the presence of Helicobacter pylori in saliva of symptomatic and subjects selected randomly from our dental institution by using polymerase chain reaction technique.

Aims and Objectives

Aims and objectives

AIM - To identify the presence of *H.pylori* in saliva of patients with and without gastritis by Polymerase Chain Reaction method. (PCR)

OBJECTIVE - To evaluate the presence of *H.pylori* in saliva of.

1. Patients with symptoms of gastritis
2. All were asymptomatic at the time of study (four had previous history of gastritis treated)

HYPOTHESIS – Oral cavity is a reservoir of *H.pylori* and its presence correlates with clinical gastritis

Materials and Methods

Materials and Methods

Materials

A total of 20 patients presenting with various symptoms including epigastric pain, burning sensation in the stomach and attending an endoscopy clinic Sundaravadanam nursing home and Selvarangam nursing home, Chennai were enrolled for the study. In group 2, all were asymptomatic at the time of study (4 had previous history of gastritis treated (n=10). A detailed case history was taken. The study was approved by the IRB and patient consent obtained for all cases.

Endoscopy Biopsy and Saliva Specimens:

- The gastro intestinal endoscopy antral biopsy and saliva specimens were collected from 20 symptomatic patients with gastroduodenal disorders.
- PCR materials (Synergy chemicals) & Oligonucleotide Primers were obtained from Bangalore Genei Ltd, Bangalore, India.
- The general chemicals of analytical grade are from Owaisi medical college and hospital, Hyderabad.

Methods

Isolation of *Helicobacter pylori*

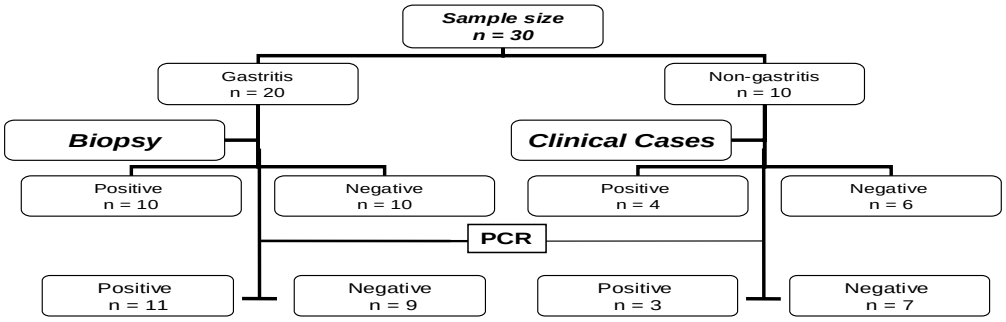
Collection and Transport of Specimens:

Biopsy: A total of 2 biopsies were collected from patients using fiber optic video gastroendoscope with precautions under sterile conditions by a gastroenterologist.

The forceps and endoscopes were decontaminated with 2% glutaraldehyde for 30 min. to prevent cross infection among patients.

Saliva: The saliva specimens were taken from all the 30 patients in sterile containers for molecular diagnostic tests before endoscopy in digestion buffer.

Flow chart displaying stratification of study subjects.



COLLECTION OF UNSTIMULATED SALIVA:

The patient was asked to sit in the chair with head tilted in front and instructed not to speak / swallow or do any head movements during the procedure. At the beginning, the patient was asked to swallow any saliva if present in the mouth. After this the patient was instructed to spit in the sterile graduated container every minute for 10 minutes. All salivary samples were collected between 7am to 8 am

Digestion buffer

- 100mM NaCl
- 10mM Tris-HCl
- 25mM EDTA

- 1%SDS

Isolation of Genomic DNA from Saliva Specimens by kit method

1. Collect fresh saliva in collection tube.

↓

2. Add 300µl saliva to a 1.5ml microcentrifuge tube.

↓

3. Add 3 times the sample vol of Real Biotech Corporation (RBC) lysis buffer and mix by inversion method.

↓

4. Incubate the tube for 5 min at room temperature.

↓

5. Centrifuge at 300 rpm for 2 min and discard supernatent.

↓

6. Add 100 µl RBC lysis buffer to resuspend the cell pellet.

CELL LYSIS:

7. Add 200µl GB buffer (commercially available and used for cell lyses) to tube and mix by vortexing. ↓

8. Incubate the mixture at room temperature for 10 min until the sample lysate is clear.During incubation invert the tube every 3 min.

↓

9. At this time, preheat required elution buffer (commercially available) (200µl per sample) in a 70° C waterbath (for DNA elution)

DNA BINDING:

10. Add 200µl of ethanol (96-100%) to sample lysate and mix immediately by vortexing for 10 seconds.

↓

11. Place a GD (a tube with a sieve) column in 2ml collecting tube.

↓

12. Apply the total mixture (including any ppt) from previous step to GD column.

↓

13. Close cap and centrifuge at full speed 13000rpm.

WASH:

14. Add 400 µl of W1 buffer (wash buffer, commercially availabe) into GD column.

↓

15. Centrifuge full speed 13000 rpm 30 sec.

↓

16. Discard the flow-through and place the GD column in 2ml collecting tube.

↓

17. Add 600 µl of wash buffer in GD column.

↓

18. Centrifuge at full speed 13000 rpm 30 sec.

↓

19. Discard the flow-through and place the GD column in 2ml collecting tube.

↓

20. Centrifuge at full speed 13,000 rpm for 3 min to dry column matrix.

DNA elution

21 Transfer dried GD column into clean 1.5 ml micro-centrifuge tube.

↓

22. Add 100 µl of preheated elution buffer into the centre of column matrix.

↓

23. Stand at room temperature for 3-5 min until elution buffer absorbed by matrix.

↓

24. Centrifuge 13000 rpm for 30 sec to elute purified DNA.

↓

25. Store at - 4 ° C.

Polymerase Chain Reaction

PCR was done using the genomic DNA isolated from the saliva and the biopsies of the patients as the template to detect the presence of the 16S rRNA gene in *H. pylori*.

Molecular diagnosis of *Helicobacter pylori*:

16S rRNA gene

The presence of *H. pylori* was diagnosed by 16S rRNA PCR amplification of the DNA. The Polymerase Chain Reaction (PCR) was carried out using 16S rRNA primers and the DNA isolated from the saliva specimens of the subjects. The amplified product gives a 534 bp band upon agarose gel electrophoresis using a 2 % gel at 120v.

16S rRNA primers:

Forward primer **5' – TAAGAGATCAGCCTATGTCC – 3'**

Reverse primer **5’ – TCCCACGCTTTAAGCGCAAT- 3’**

PCR Mixture

The PCR mixtures used were commercially available

PCR Conditions

The reaction conditions for the16S rRNA gene amplification with both the primers were optimized and are as follows: 40 cycles with denaturation at 94°C for 30 sec; annealing temperature at 56°C for 30 sec and synthesis at 72°C for 1min.

The reaction cycles are as follows:

95^0 C for 5 min (Initial denaturation step)

94^0 C for 30 sec

56^0 C for 30 sec 40 cycles

72^0 C for 1 min

72^0 C for 5 min (Final extension step)

Positive (ATCC 26695 strain) and negative controls are kept for the validation of the PCR performed. The negative control consists of the reaction mixture without the DNA template. The positive control contains the *H. pylori* DNA as the template.

Electrophoresis Conditions:

PCR products were visualized after electrophoresis at 120v on 2% agarose gel containing 15µl Ethidium bromide under UV light. The PCR product size was **534 bp**

Statistical analysis

Descriptive analysis for all the variables were done and the accuracy of the PCR technique was estimated using sensitivity, specificity, positive predictive value (ppv), and negative predictive value(npv)

Review of Literature

Review of literature

Jang L, Cherng P, Rong et al in March 1999[5] studied *H-pylori* in gastric biopsy specimen by 5 different PCR methods to determine which method is the most appropriate one to use, and compared the sensitivities and specificities. They found that for *ureC (phosphoglucosamine* (*glmM*))gene PCR is the most appropriate of the five different PCR methods examined for detection of *H. pylori* organisms.

Farhana K, Abid H, Irshad A et al in November 2004[6] compared the genomes of 10 *H. pylori* strains from Ladakh, North India. Molecular analysis was done to identify rearrangements within and outside the *cag* pathogenicity (*cag* PAI) and DNA sequence divergence in candidate genes. Analysis of virulence genes such as the *cag* PAI as a whole, *cagA*, *vacA*, *iceA*, *oipA*, *babB*, and the plasticity cluster revealed that *H. pylori* strains from Ladakh are genetically different and may be less virulent than the isolates from East Asian countries. Phylogenetic analysis based on the *cagA-glr*, enterobacterial repetitive intergenic consensus patterns, repetitive extragenic palindromic signatures, the *glmM* gene mutations, and several genomic markers representing fluorescent amplified fragment length polymorphisms revealed that Ladakh strains share features of the Indo-European and also the East Asian gene pools.

Song Q, Lange T, Spahr A et al in April 1999[7] studied the prevalence and distribution of *H. pylori* in oral cavity by nested PCR .They took saliva and plaque samples from 42 patients who were undergoing endoscopy and found that, *H. pylori* DNA was identified in dental plaque of 41 patients (97%) and in 23 saliva samples (55 %). They concluded that *H. pylori* were present in oral cavity of 97 % of tested samples and so they may belong to normal oral microflora.

Francesco F, Antonia R, Antonio G, et al in March 2002[8] studied to determine whether anti-CagA antibodies cross-react with antigens of normal and atherosclerotic arteries. To see this, they took 8 umbilical cord sections, 14 atherosclerotic artery sections, and 10 gastrointestinal tract sections and did immunohistochistry using polyclonal anti-CagA antibodies. They found that Anti-CagA antibodies cross-reacted with antigens of both normal and atherosclerotic blood vessels. So they concluded that the binding of anti-CagA antibodies to those antigens in injured arteries could have influenced the progression of atherosclerosis in CagA-positive *H pylori*-infected patients

Vaishnavi B, Vikram K, Nilakantan A et al in 2004[9] conducted a study to determine the prevalence of *H pylori* in patients with portal hypertensive gastropathy (PHG) and also to determine if it contributes to the severity of the disease. They selected 37 patients who presented with portal hypertensive gastropathy and looked for *H. pylori* by urease test and histology. They found that 16 of 37 were positive for *H pylori*, 27 patients had endoscopic evidence of mild PHG, 9 had moderate and 1 patient had evidence of severe PHG. The *H. pylori* status was 52%, 22%, and 0% in patients with mild, moderate, and severe gastropathy respectively indicating an inverse relationship of severity of PHG with *H. pylori* colonization. From this they concluded that PHG does not provide a favorable environment for the colonization of *H. pylori*

Dowsett S, Kowolik M et al in 2003[10] reviewed the evidence for a definitive role of the oral cavity in transmission of *H. pylori* and the role of the oral cavity as a reservoir for gastric *H. pylori* infection by culture and PCR. They concluded that there is increasing evidence for a role of the oral cavity in transmission of *H. pylori* . If the oral cavity is a

reservoir for gastric infection, in even a minority of individuals, this can be sufficient to warn a preventive approach that encompasses consideration of the oral reservoir

Dowsett S, Archila V, Segreto A et al in April 1999[11] studied the possible sources of *H. pylori* infection in an isolated, rural population in Guatemala. A total of 242 people in family units were included in the study. By serological test immunoglobulin G antibodies to *H. pylori* were detected. Overall, 58% of subjects were seropositive, with a positive relationship between mother and child ($P = 0.02$) and a positive correlation between the serostatuses of siblings. There was no association between serostatus and gastric symptoms. Oral *H. pylori* was detected from periodontal pockets of different depths and the dorsum of the tongue by nested PCR. 87 % of people had minimum one oral site positive for *H. pylori*, with majority of people having multiple positive sites. There was no association between periodontal pocket depth and the detection of *H. pylori*. Nested PCR was also used to detect *H. pylori* from beneath the nail of the index finger of each person's dominant hand. Overall, 58% of people had a positive fingernail result. With a positive relationship between fingernail and tongue positivity with this they concluded that oral carriage of *H. pylori* may play a role in the transmission of infection and that the hand may play a major role in transmission.

Donald F, Chuanfu L, Nikhil P et al in July 1993[12] studied whether *H. pylori* can be grown in saliva samples of patients, who were positive for gastric biopsy samples by soluble-protein electrophoresis, restriction endonuclease DNA analysis, and southern blot hybridization. They founded that viable *H. pylori* could be cultured from saliva samples.

Qiang H, Jian P, Michael O et al in July 2002[13]studied on the development of a real-time quantitative (Q) PCR-based assay to measure *ureC* gene copy number to detect *H. pylori*, based on the fact that there is only one copy of the *ureC* gene per bacterium. Upon optimization of Light Cycler Q-PCR conditions, they obtained a standard curve with a linear range (correlation coefficient = 1) across six logs of DNA concentration. They were able to accurately quantify as few as 1,000 bacteria in their assay. Analysis of variance on 15 randomly selected clinical samples showed good reproducibility of this assay.

Reilly T, Poxon V, Sanders D et al in 1997[14] compared a new rapid whole blood test (Helisal rapid blood, Cortecs), two serum enzyme linked immunosorbent assays (ELISAs; Helico-G, Shield and Helisal serum, Cortecs), and a salivary assay (Helisal saliva, Cortecs), with slide biopsy urease, 13C-urea breath test, and histology. With 303 consecutive dyspeptic patients attending for gastroscopy, underwent 2 antral biopsies for histology, and one for rapid slide biopsy urease test for assessment of *H.pylori* status. Blood and saliva were also collected. Of the 303 patients, 100 underwent a 13C-urea breath test. They found that, out of 300 patients (median age 63,range 28–89) eligible for analysis, 137(46%) were gold standard positives, of which Helisal rapid blood identified 116, Helico-G 129, Helisal serum 130, and Helisal saliva 120; 137 (46%) were gold standard negatives of which the number falsely identified as positive was 30 by Helisal rapid blood, 45 by Helico-G, 41 by Helisal serum, and 41 by Helisal saliva. Sensitivities and specificities were: for the whole blood test 85% and 78% respectively; for Helico-G 94% and 67%, for Helisal serum 95% and 70%, and for Helisal saliva 84% and 70%.

Pascalis R, Del P, Nardone G et al in Feb 1999[15] conducted a study on salivary immunoglobulin G (IgG) immune response to *Helicobacter pylori* in 70 people by enzyme-linked immunosorbent assay (ELISA). Patients with a positive *H. pylori* culture showed higher antibody titers than subjects with no detectable *H. pylori*: the overall sensitivity and specificity of the test were 84% and 90% respectively. With this they concluded that the detection of salivary anti-*H. pylori* IgG antibodies may be used as an alternative to serum IgG detection when blood samples are not available in screening of patients with dyspepsia.

Julie P, Haim S, Thomas H et al in 1999[16] done a study to determine the route of transmission of *H. pylori* and also to see if *H. pylori* could be recovered from feaces, vomitus, and saliva of asymptomatic, infected adults by culture and Immunomagnetic separation PCR (IMS PCR). They took 16 asymptomatic *H. pylori* infected and 10 uninfected individuals. They found that all vomitus samples grew *H. pylori*. Saliva before and after emesis grew low quantities of *H. pylori* in 3 (18.8%) and 9 (56.3%) cases. All stool samples were negative by culture but in 5 infected subjects IMS PCR detected *H. pylori* 16s rRNA. They concluded that healthy *H. pylori* could be cultured from saliva vomitus and stools of infected persons.

Alejandra B, Marianella P, Mari A et al in 2002[17] studied to determine the presence of *H. pylori* DNA in the gastric antrum and dental plaque of a Venezuelan population by PCR and the relationship between this infection and the oral hygiene index. In this study they took 32 patients, attending for routine gastroscopy, and 20 asymptomatic subjects. Patients' supragingival plaque was tested by a PCR for a specific internal urease gene. Gastric antrum biopsies were taken for histological examination and PCR. *H. pylori* was

detected in antral samples from 24 (75%) of 32 patients, *H. pylori* was also detected in dental plaque samples of 12 (37.5%) of the 32 patients. In 7 (58%) of these 12 patients, *H. pylori* was identified in the gastric biopsy. 7 patients with chronic gastritis carried *H. pylori* in dental plaque and antral samples. Of these patients, 4 also had dysplasia and one had metaplasia. 3 subjects in the control group were positive by PCR. They said that there is no correlation between *H. pylori* infection, dental hygiene, dental caries, periodontal disease or use of dentures. They concluded that the oral cavity may be a reservoir for *H. pylori* infection and oral secretions may be a means of transmission of this organism.

Bruce A, Deanna K, Geraldine M et al in 2002[18] studied the relationship between *H. pylori* infection and abnormal periodontal conditions. In this study they used 4504 participants aged 20 to 59 years who underwent a periodontal examination and tested positive for *H. pylori* antibodies by ELISA. And found that *Periodontal* pockets with a depth of 5 mm or more were associated with increased odds of *H. pylori* seropositivity (odds ratio [OR]=1.47; 95% confidence interval [CI]=1.12, 1.94) after adjustment for sociodemographic factors. With this they concluded that poor periodontal health, with advanced periodontal pockets, may be associated with *H. pylori* infection in adults, independent of socio-economic status.

Fariborz M, Mehdi A, Amir H et al in 2005[19] studied on *H. pylori* in brush biopsy samples of oral aphthous ulcers by the polymerase chain reaction (PCR) method. Patients who were diagnosed as RAS were referred to the laboratory in Rasht city. Oral aphthous specimens were collected by toothbrush from the patients. Enzyme-linked immunosorbant assay (ELISA) was also done in all patients to determine IgG antibody.

They found that of the 50 patients with ages between 18 to 60 years (mean ±SD: 32.38±11.30), 26 patients (52%) had positive ELISA but *H. pylori* DNA was found in only one patient (2%). With this they concluded that, *H. pylori* DNA could not be found in the aphthous ulcers of these patients, even in patients with positive anti-*H.pylori* antibody (IgG), and these bacteria are not involved in recurrent oral aphthous ulcers.

MacKay W, Williams C, McMillan et al in 2003[20] did a study on to develop a noninvasive method to isolate *H. pylori* DNA. In this study a total of 30 children were included and were tested for gastric *H. pylori* colonization by using the 13C-urea breath test (UBT) and fecal samples, which were tested for *H. pylori* by using the HpSA fecal antigen test. They found that among 15 UBT-positive and 15 UBT-negative children, the positive and negative predictive values for the assay were 80 and 100% and concluded that feacal DNA purification followed by *H. pylori* PCR analysis is an effective method for *H. pylori* DNA isolation from the feaces of children.

Goosen C, Theron J, Ntsala M et al in 2002[21] studied to develop a heminested PCR assay based on the amplification of a specific internal region of the Phosphoglucosamine mutase gene (*glmM*) of *H. pylori* and evaluated the heminested PCR assay for the detection of *H. pylori* in saliva and dental plaque by comparison with an established PCR assay. In this they took oral specimens like dental plaque and sulcular fluid, with sterile toothpicks and filter paper, respectively, from 58 randomly selected, clinically healthy volunteers attending the Gastroenterology Unit at Pretoria Academic Hospital, Pretoria, South Africa. All oral specimens were cultured and DNA was isolated and a set of primers specific for the phosphoglucosamine mutase gene (*glmM*) of *H. pylori* produced a 765-bp fragment that was used as template for the heminested primer pair delineating a

496-bp fragment. They found that the heminested PCR assay was specific for detection of *H. pylori*, with no false-positive results, and that *H. pylori* had a low prevalence (approximately 3%) in specimens obtained from the oral cavity.

Anak I, Siriporn C, Sukanya L et al in 2003[22] studied the association of *H. pylori* and RAU (Recurrent aphthous ulceration) using, nested polymerase chain reaction (PCR), in 22 patients with RAU with ages ranging from 12-36 years. Samples were taken from the lesions on the dorsum of the tongue of each patient and also, from the dorsum of the tongue of 15 normal individuals with ages of 13-40 years who were used as controls. The results showed that one sample from a lesion (4.5%) and one sample from the tongue (4.5%) of two different patients with RAU were positive for *H. pylori*. In the control group, 3 samples (20%) were positive for *H. pylori*. With this they concluded that *H. pylori* do not play a role in the pathogenesis of RAU and the dorsum of the tongue may be a reservoir of *H. pylori.*

Patrizia S, Vittorio R, Roberto F et al in1998[23] studied

1) The delivery of VacA to cells,

2) The localization and fate of internalized toxin, and

3) The persistence of toxin inside the cell,

in cultured gastric epithelial cells. In this they used human gastric epithelial cells in culture and broth culture filtrate from a VacA-producing *H. pylori* strain The techniques used were neutral red dye uptake, ultrastructural immunocytochemistry, quantitative immunofluorescence, and immunoblotting. They found that

1) VacA is delivered to cells in both free and membrane-bound form (i.e., as vesicles formed by the bacterial outer membrane),

2) Localizes inside the endosomal-lysosomal compartment, in both free and membrane-bound form,

3) Persists within the cell for at least 72 h, without loss of vacuolating power and generally does not degrade into fragments smaller than, 90 kDa.

Maher T, Sharon M, Jane G et al in 1999[24] studied on the endoscopic biopsy pathology of Helicobacter pylori gastritis and compared bacterial detection by immunohistochemistry using a specific antibody with the Genta stain, to compare the relative costs of the 2 techniques. For this they took 100 cases of gastritis identified as positive for *H pylori* by Genta stain and 100 cases considered negative by the same technique and stained using an anti-*H pylori*–specific polyclonal antibody. They found out that Chronic active gastritis with lymphoid follicles was significantly associated with *H pylori* infection ($P < 0.0001$). The immunohistochemical method had sensitivity of 97% and a specificity of 98% compared with the Genta stain. Reagent costs were similar for both methods, but immunohistochemistry using an autoimmunostainer required less technical time and hence was less expensive than the Genta stain. With this they concluded that Immunohistochemistry using a specific antibody is an accurate and cost-effective method for *H.pylori* detection in gastric biopsies.

Britta B, Ragnar B, Bernhard J et al in 1998[25] studied to develop a PCR-based method to detect macrolide resistance and the virulence gene *cagA* in *H. pylori* within 24 h, to improve the lengthy process of culture-based procedures. Total DNA was prepared directly from stomach biopsy specimens. The procedure proved to be rapid and reliable and could be utilized for diagnostic purposes. For this biopsy samples were collected

from 69 patients (39 females and 30 males; mean age, 57 years) and concluded that this is rapid and reliable and could be utilized for diagnostic purposes.

Allaker R, Young K, Hardie J M et al in 2002[26] studied to find out the possible route of transmission in children. In this study they took 100 children attending for upper gastrointestinal endoscopy were tested for the presence of H. pylori and this organism was detected in antral gastric biopsies by the rapid urease test (13 patients), culture (13 patients), histology (15 patients) and PCR (20 patients). Gastric juice was positive for H. pylori in 3 patients by culture and 11 patients by PCR. The dental plaque from 68% of gastric biopsy-positive patients and 24% of gastric biopsy-negative patients was positive for *H. pylori* by PCR. H. pylorus in dental plaque was same as that of in the stomach. *H. pylori* was detected by PCR in the faeces of 25% of gastric biopsy-positive children sampled. With this they concluded that oral-to-oral transmission may be a possible mode of spread of *H. pylori* in children.

Patrizia D, Stefano B, Anna Rita et al in 1999[27] studied the rate of intra familial transmission of *Helicobacter pylori* infection in the general population and the role of a family's social background. In this they took 3289 residents, accounting for 416 families. It was found out that the overall prevalence of *H pylori* infection was 58%. Children belonging to families with both parents infected had a significantly higher prevalence of *H pylori* infection (44%) than children from families with only one (30%) or no parents (21%) infected ($P < 0.001$). Family social status was independently related to infection in children, with those from farming families showing an increased risk of infection compared with children of high class families (odds ratio 2.02, 95% confidence interval

1.16 to 3.49). With this they concluded that *H pylori* infection clusters within families belonging to the same population. Social status may also be a risk factor.

Mohamed N E, Magdy E M et al in 2005[28] the presence of *H.pylori* and, if detected, its potential prevalence in causing recurrent aphthous ulcers confined to mucosa associated lymphoid tissues of the pharynx. They did prospective, controlled clinical trial. In this study they took 146 patients with recurrent multiple aphthous ulcers of the oral cavity and pharynx and 20 normal control subjects. Patients were divided into group 1 (n=58), in which the ulcers were strictly limited to the lymphoid tissues, and group 2 (n=88), in which the ulcers were randomly distributed in the oral cavity and pharynx. *H. pylori* DNA was extracted from 3-mm-diameter tissue samples, and PCR amplifications were done for the 16S ribosomal RNA gene. They found, that in group one, 39 patients (67%) were positive for *H pylori* DNA, while in group two, 9 patients (10%) were positive ($P < 0.001$). It was not detected in any of the 20 control samples. With this they concluded that there could be a possible causative role for *H.pylori* in recurrent aphthous ulcers with a characteristic distribution and affinity to mucosa-associated lymphoid tissues of the pharynx.

Anna H, Rikka I, Vuokko L et al in 2004[29] studied the sensitivity of *H.pylori* to physiological concentrations of lactoperoxidase and its salivary substrate thiocyanate and different amounts of hydrogen peroxide (H2O2) in buffer and in human whole saliva and concluded that concentrations of lactoperoxidase and its salivary substrate thiocyanate, and different amounts of hydrogen peroxide do not seem to generate enough antibacterial property to effectively kill *H.pylori*

Czesnikiewicz M, Karczewska E, Bielanski W et al in 2004[30] studied the relationship of *H.pylori* in oral and gastric infections. They included 100 patients in the study. Gastric infection was determined by C-urea breath test UBT. In addition oral saliva and plaque were cultured. Of which *H.pylori* was present in 51 % of stomach, 54 % saliva and 48.3 % of samples from gingival pockets. The difference was not statistically significant. They concluded that oral cavity contamination with *H.pylori* occurs at similar degree to that in the stomach and there was no correlation between the occurrence of *H.pylori* in stomach and oral cavity.

Loster B W, Majewski S W, M Nikiewicz G et al in 2006[31] compared the bacterial culture from the oral mucosa to those from the gastric mucosa, by PCR. In this study 40 men (25-70 yrs) were induced and they found that the oral bacteria and bacteria from stomach are completely different, suggesting that *H. pylori* may be present only transiently in oral cavity and does not play major role in gastric *H. pylori* infection. Thus, oral cavity does not serve as bacterial reservoir to infect gastric mucosa.

Bee L N, Seng H Q, Marion A et al in 2003[32] studied the role of coccoid form in *H. pylori* infection by comparing the seroprevalences of spiral and coccoid forms in children with epigastric pain. In this study 489 children (mean age, 8.5 years) with epigastric pain participated. 599 schoolchildren of comparable ages, with no history of dyspepsia were used as controls. The seroprevalence of antigens prepared from both morphological forms were tested by ELISA, and they found out that 65 (13.3%) and 273 (55.8%) of 489 symptomatic children were seropositive for antigens of the *H. pylori* spiral and coccoid forms and, 7.0% of the control group had increased levels of immunoglobulin G antibodies against the spiral form, while 26.5% were positive for antibodies against the

coccoid form. With this they concluded that the seroprevalence of antigens of the *H. pylori* spiral and coccoid forms in children with epigastric pain was two fold higher than that in the control subjects.

Asish K , Dangeruta K, Jin Y et al in 2000[33] studied the differences between strains of East Asia and the West. For this they took adult ethnic Bengali patients of both sexes, age 21 to 65 Years. Endoscopy biopsies and gastric juice samples were taken. Two biopsies were taken for culture, 1 from the gastric antrum and 1 from the corpus, and were stored at 270°C in 0.5 ml of brucella broth (Difco) with 15% glycerol until culture. 2ml of gastric juice was also collected during endoscopy in some cases and stored frozen at 270°C until use. They employed culture and PCR techniques. It was found that 55 of the patients had peptic ulcer disease, and 23 had gastritis only. With this they postulated that-

- PCR tests indicated that 80 to 90% of Calcutta strains carried the *cag* pathogenicity island (PAI) and potentially toxigenic *vacAs1* alleles of the vacuolating cytotoxin gene (*vacA*), independent of disease status.
- This was higher than in the West (where *cag* PAI1 *vacAs1* genotypes are disease associated) but lower than in East Asia.
- In Calcutta the *iceA2* gene was weakly disease associated, where as in the west the alternative but unrelated *iceA1* gene at the same locus is weakly disease associated.
- DNA sequence motifs of *vacAm1* (middle region) alleles formed a cluster that was distinct from those of East Asia and the West, whereas the *cagA* sequences of Calcutta and Western strains were closely related.

- An internal deletion found in 20% of Calcutta *iceA1* genes was not seen in any of ~ 200 strains studied from other geographic regions and thus seemed to be unique to this *H.pylori* population.
- 2 mobile DNAs that were rare in East Asian strains were also common in Calcutta.

With the above findings they concluded that, *H. pylori* gene pools differ regionally.

Abdussalam A, Guilherme B , Edilberto N et al in 2001[34] studied the frequency of *iceA* alleles and *cagA* status in *H.pylori* strains. For this study they took 142 patients (62 children and 80 adults; 66 female; mean age, 30.0 years; age range, 3 to 78 years) with gastritis, duodenal ulcer, or gastric adenocarcinoma. *iceA* was identified in bacterium samples obtained from all patients.*iceA2* allele was detected in 118 (90.1%) and was associated with ulcer (P = 0.02) and with carcinoma (P = 0.001). *iceA2* amplicons of 229, 334, or 549 bp were detected and were more frequent in patients older than 7 years (P = 0.001). This gene was also more frequent in strains obtained from males (P = 0.02). *cagA* was more common in strains obtained from carcinoma (P =0.0008) and ulcer patients (P < 0.006). *cagA*-positive strains were more frequent in children older than 7 years (P < 0.003).

With the above findings they concluded that *iceA* should not be used as a reliable marker for predicting the clinical outcome of *H. pylori* infection.

Marinko M, Zarko B, Mirjana N et al in 2005[35] studied the role of proton pump inhibitors in patients of unsuccessful eradication of *H pylori*. For this they took 335 patients with gastritis (142 males and 193 females, mean age 51.3 years, between 18 and 83 years). All patients underwent upper gastrointestinal endoscopy with histopathologic

examination and they found that there is no significant difference in resolution of Intestinal metaplasia by using different PPI between the groups of eradicated and noneradicated patients (P<0.4821 and P<0.4388, respectively).

Leisa M, Anthony P, Andrew C et al in 2004[36] studied on the role of TLRs in the recognition of *H. pylori*, *H. felis*, and *H. hepaticus*. Here they used normal human monocytes and macrophages, transfected cell lines, and genetically deficient animals; they showed that TLR2 is an important cytokine signaling receptor for *H. pylori*, *H. felis*, and *H. hepaticus*. Further, they said that although the lipopolysaccharide (LPS) of *H. pylori* is recognized by TLR4, the major TLR for intact *Helicobacter* bacteria is TLR2 and not TLR4.

Tiwari S K, Khan A, Manoj G et al in 2007[37] studied on developing a multiplex PCR assay for diagnosing and specific identification of virulent *H. pylori* strains and their main virulence genes cagA, cagE, cagT, vacA and hrgA. For this study they used Genomic DNA from 82 gastric tissues. Multiplex PCR assay was able to detect all the five target genes in 81.7 % and deletions in one or more loci among 18.3%. Genotype cagT +ve／ hrgA +ve／ cagA +ve／ cagE +ve／ vacAs1 +ve was predominant in this study population(67.07%). hrgA, cagT, cagE and cagA genes were present in 100%,92.7%, 85.4% and 81.7% respectively. The vacAs1 subtype had higher prevalence frequency in patients with overt gastrointestinal disease (78.57%) than with GERD (gastro-esophageal reflux disease) and NUD (nonulcerdyspepsia) (50%). With the above findings they concluded that multiplex PCR assay developed here was able to genotype *H. pylori* isolates based on the main virulence genes.

Ahmed K S, Khan A, Ahmed I et al in 2006[38] studied the routes of transmission of

H. pylori. For this study, they took saliva and biopsy samples of 400 people and did PCR. With this, they found out that the *H. pylori* in saliva and biopsy samples increased with age. It was found more commonly in the saliva and biopsy samples among males (64 % and 60 %, respectively) than females (53.3 % and 64 %). 71.6 % and 73.5 % of those who consumed municipal water acquired *H. pylori* compared to a lesser proportion (12.6 % and 12.6 %) of those who consumed boiled or filtered water. They also found that subjects who have home-cooked food (57.1 % and 57.7 %) showed a lower prevalence of *H. pylori* in saliva and biopsy samples, compared to those (80 % and 88%) who frequently ate out. With the above findings they concluded that transmission of *H. pylori* also takes place through the consumption of food prepared under unhygienic conditions. Consumption of municipal tap water also has a high impact in the transmission of *H. pylori*.

Tiwari S K, Khan A, Ahmed K S et al in 2005[39] studied on standardizing a cost effective, non-invasive method for the rapid diagnosis of *H. pylori* in salivary secretion of infected patients with gastric disorders using 16S rRNA PCR analysis. For this study they took 100 patients (65 men and 35 women) with a mean age of 48.4 (range 21 to 73) years. The patients were classified at the time of endoscopy into two groups, those having gastric diseases (n=80), and those with no evidence of mucosal ulcer and gastritis (n=20) i.e. normal study. With this they found that 72 (90 %) of the symptomatic group and 10 asymptomatic subjects were infected with *H. pylori* in the stomach by PCR amplification of biopsy DNA obtained from each subject. *H. pylori* DNA was identified in the saliva of 70 (87.5 %) symptomatic patients and 12 (60%) asymptomatic control patients. With the above findings they concluded that increased number of organisms identified in saliva

indicate that saliva of the infected person can serve as a reliable non-invasive alternative to detect the presence of *H. pylori* infection.

Goodwin C, Armstrong A, Chilvers T et al in 1989[40] studied on identification of a bacterium. "*Campylobacter pyloridis*," or "*Campylobacter pylori*" but is now named *Helicobacter* pylori in recognition of the fact that this organism is distinct from members of the genus *Campylobacter*. The genus *Helicobacter* belongs to the subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*. This family also includes the genera *Wolinella, Flexispira, Sulfurimonas, Thiomicrospira*, and *Thiovulum*. The genus *Helicobacter* consists of 20 recognized species, with many species waiting for recognition. Members of the genus *Helicobacter* are all microaerophilic organisms and in most cases are catalase and oxidase positive, and many but not all species are also ureases positive. *Helicobacter* species can be subdivided into two major classes,

1. The gastric *Helicobacter* species and
2. The Enterohepatic (nongastric) *Helicobacter* species.

Kuipers J, Uyterlinde M, Pena A et al in 1995[41] studied on patients with intact acid secretion, *H. pylori* in particular, colonize the gastric antrum, where few acid-secretory parietal cells are present. Patients, in whom acid secretion is reduced, have a more even distribution of bacteria in antrum and corpus, and bacteria in the corpus are in closer contact with the mucosa, leading to a corpus-predominant pangastritis.

Eidt S, Stolte M and Fischer R et al in 1994[42] studied on MALT lymphoma patients are *H.pylori* positive, and found out that *H. pylori*-positive subjects have a significantly increased risk for the development of gastric MALT lymphoma. A major predictor for response appears to be the presence of a t(11;18) (q21;q21) translocation.

Results

. The study population comprised of patients with symptoms of gastritis (group 1) (n=20) and the randomly selected group (group 2) (n=10)

In the (group1) 20 cases of clinical gastritis, 10(50%) were positive for H. pylori by endoscopy biopsy under modified Giesma stain and 10 (50%) were negative for the stain.

Of the 10 endoscopy biopsy positive for H. pylori, 8 cases were PCR positive for *H.pylori* in saliva and 2 were negative. Of the 10 endoscopy biopsy negative cases, 3 were PCR positive for *H. Pylori* in saliva and the rest 7 were negative.

In randomly selected (group2) 10 cases were taken of which 6 were not clinically symptomatic of gastritis and 4 were symptomatic for gastritis. Out of 6 gastritis negative, 3 were PCR positive for *H.pylori* and out of 4 gastritis positive, 3 were PCR positive for *H.pylori*.

In group 1 there were 14 males and 6 females. In group 2 there were 6 females and 4 males. The mean age of the patients in group 1 was 39.43 (SD = ±10.01) in males and 52.33 (SD = ±19.83) in females. In group 2, there were 4 males and 6 females. The mean age of the patients in group 2 was 27.75 (SD = ± 2.06) in males and 27.83(SD = ±4.54) in females.

The saliva sample from all the patients in group 1 and group 2 was collected as per the protocol as stated in methodology. By using PCR the samples were subjected for detection of *H.pylori* using specific primers which has been shown in gel documentation picture.

The gel consisted of 18 wells. The counting was done from left side as DNA ladder was put in the second well. From left side in well numbers 3, 4, 5, 6, 7 , 14 , 15 , 16, samples number 4,6,10,12,13,14,16 were loaded.

Figure 1 shows Gel image of 16S rRNA amplification of *H. pylori* DNA isolated from the gastritis patients. In lane 1 and lane 2,100 bp DNA ladder was added. Lanes 4, 6, 7, 15, and 16 shows 16S rRNA amplification of *H. pylori* DNA isolated from saliva of patients. Lane 17 shows the amplification of positive control (ATCC 26695). Lane 18 is the negative control.

Figure 2 shows Gel image of16S rRNA amplification of *H. pylori* DNA isolated from the gastritis patients. In Lane 2: 100 bp DNA ladder was added. In Lanes 3, 7, 8, 10, 12 and 14 16S rRNA amplification of *H. pylori* DNA isolated from saliva of patients was seen. Lane 17 shows the amplification of positive control (ATCC 26695). Lane 18 is the negative control

Figure 3 shows Gel image shows 16S rRNA amplification of *H. pylori* DNA isolated from the normal controls. In the lane 2 100 bp DNA ladder was added. Lanes: 6, 7, 8, 9, 10, 11 shows 16S rRNA amplification of *H. pylori* DNA isolated from saliva of normal controls. Lane 17 positive control (ATCC 26695). Lane 18 is negative control.

Of all the H. pylori, positive samples, the mean age was 35.3 ± 11.8 years while the negative samples age was 41.5±16.3 years. Of the 17 PCR positive subjects, 7 were females and the rest 11 were males.

There were bands corresponding to 534 bp size at the level above 500 bp ladder in correspondence with the level of band for positive control - ATCC which identified the presence of *H. pylori.* Histological examination by the modified Giemsa of the biopsy

section showed the presence of *H. pylori* in 10 cases (50%) of the total 20 symptomatic subjects. Out of the 10 clinically asymptomatic subjects who were taken as controls, the rate of detection with PCR was 60% (6/10). This organism was also detected in saliva samples of 11 (55%) of the 20 patients who had active gastric disease proven by histology, whereas saliva samples from 6 (60%) subjects in the control group of 10 were found to be positive by PCR.

Sensitivity of PCR in detection of *H. pylori* in saliva sample of patients who had clinical symptoms of gastritis and diagnostic biopsy as positive was 80%. The specificity of PCR in detection of *H. pylori* in saliva sample of patients who had clinical symptoms of gastritis and diagnostic biopsy as positive was 70%. The positive predictive and negative predictive values of PCR in detection of *H. pylori* in saliva sample of patients who had clinical symptoms of gastritis and diagnostic biopsy as positive were 72.7% and 77.7% respectively.

We also estimated the sensitivity, specificity, positive predictive value and negative predictive value of PCR for *H. pylori* detection from salivary samples of both study groups. They were 58%, 50%, 82.35 % and 23% respectively.

Diagnostic test values for PCR

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Biopsy + gastritis	80 %	70%	72.7 %	77.7 %
clinical gastritis + PCR	58 %	50 %	82.35 %	23%

Tables and Graphs

Table 1 **Distribution of Gender among the Study Group**

	Male		Female	
		%		%
GROUP 1 (n=20)	14	70%	6	30%
GROUP2 (n=10)	4	40%	6	60%

GROUP 1: Patients with symptoms of gastritis

GROUP 2 Post graduate students who were randomly recruited in this study from our dental institution

Graph 1

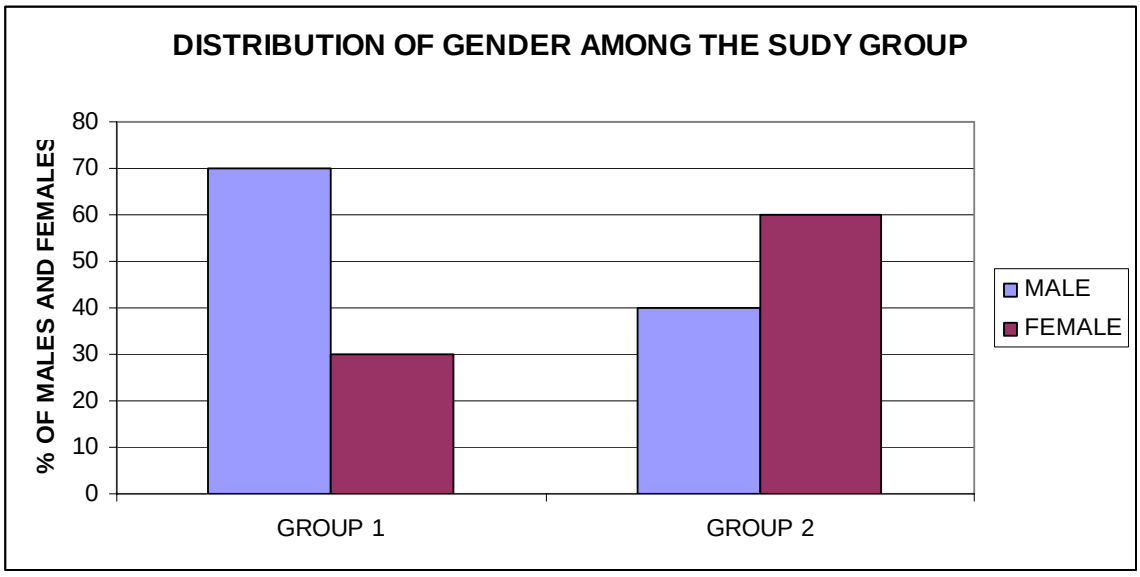


Table2 Correlation of *H.pylori* PCR in saliva (G1) and subjects asymptomatic at the time of study (4 had previous history of gastritis treated) (G2)

		Gastritis	
		Positive	Negative
PCR	Positive	14	3
	Negative	10	3
	Total	24	6

Graph 2 Correlation of *H.pylori* PCR in saliva (G1) and subjects asymptomatic at the time of study (4 had previous history of gastritis treated) (G2)

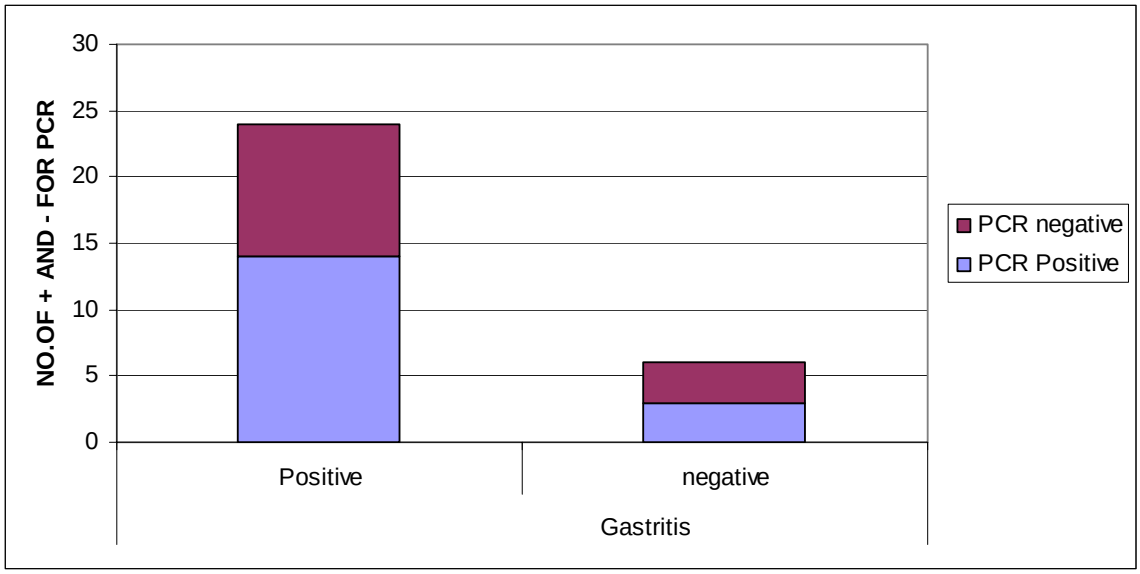


Table 3 Relation between the results of gastric biopsy and PCR detection of *H.pylori* in Saliva (G1)

		Biopsy	
		Positive	Negative
PCR	Positive	8	3
	Negative	2	7
Total		10	10

Graph 3

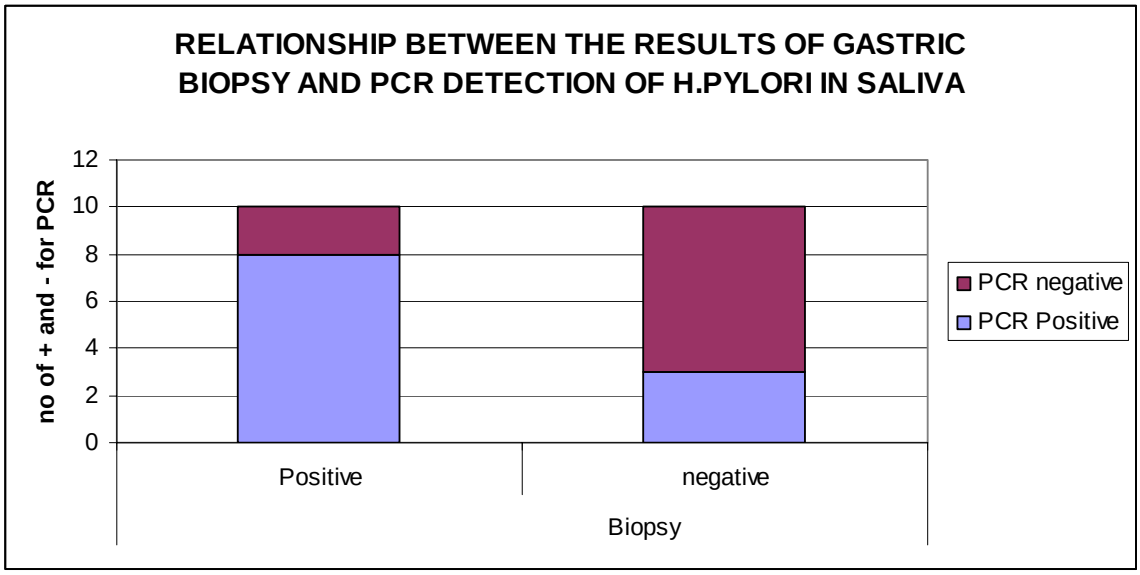
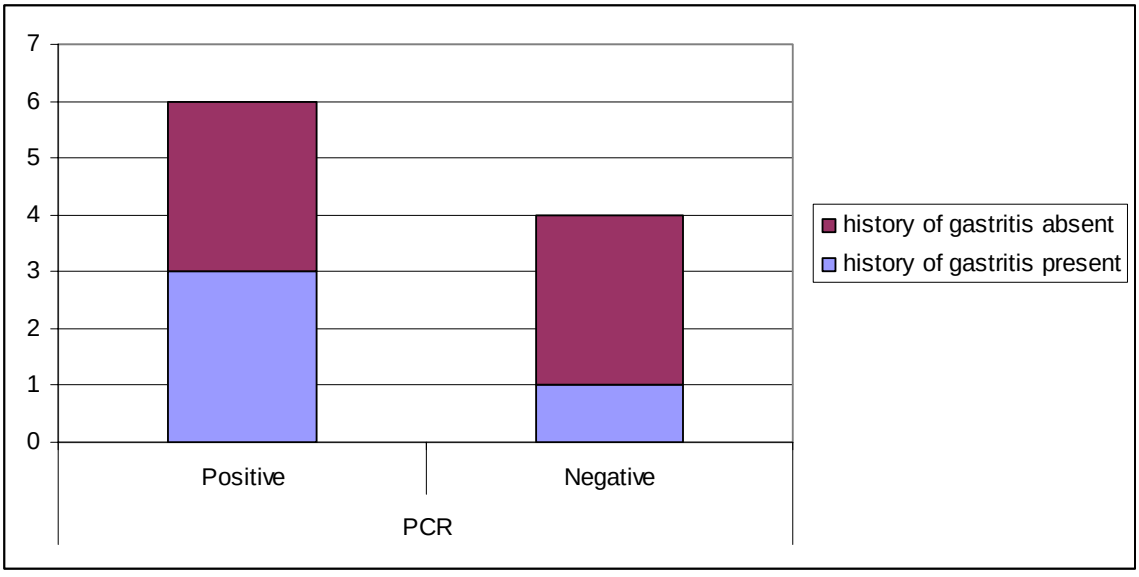


Table 4 Distribution of group2 subjects based on gastritis symptoms and PCR reports

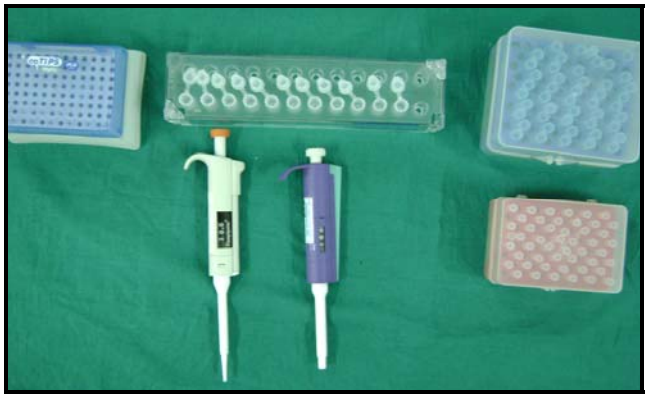
		PCR		Total
		Positive	Negative	
GASTRITIS	Present	3	1	4
	Absent	3	3	6
Total		6	4	10

Graph 4 Distribution of group2 subjects based on gastritis symptoms and PCR reports



Photographs

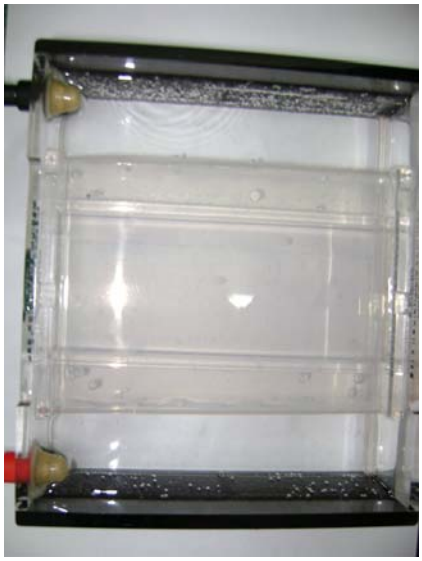
Armamentarium Used In DNA Isolation



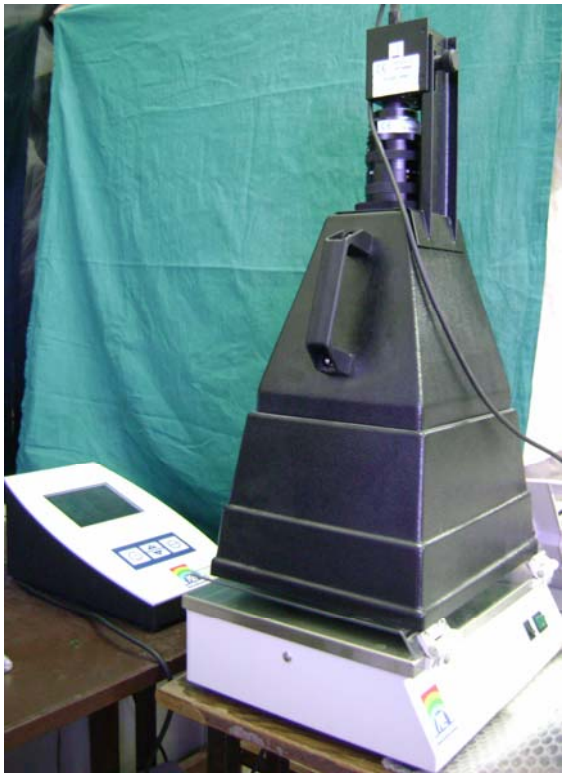
PCR TIPS, PIPETTES, 1.5 ML EPENDORF TUBES.

PCR GRADIENT THERMOCYCLER

ARMAMENTARIUM IN GEL PREPARATION AND VISUALISATION



GEL BOAT



GEL DOCUMENTATION SYSTEM

Figure 1

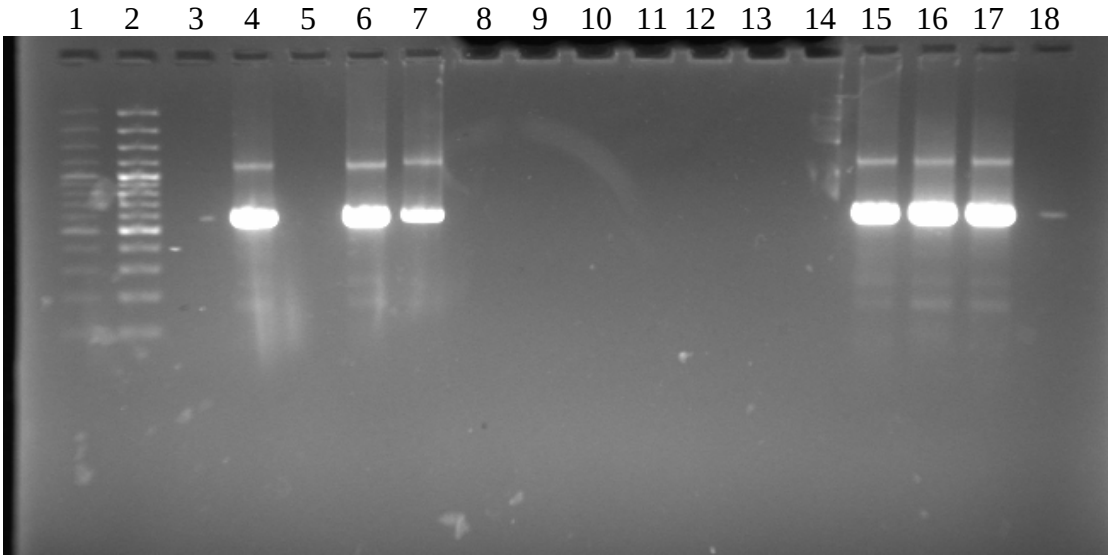


Fig 1 Gel image shows 16S rRNA amplification of *H. pylori* DNA isolated from the patients. Product size: 534 bp.

Lane 1 : 100bpmolecular weight marker
Lanes 4, 6, 7, 15, and 16: 16S rRNA amplification of *H. pylori* DNA isolated from saliva of patients,
Lane 17 : positive control (ATCC 26695),
Lane 18 : negative control

Figure 1:Lane 4,6,7,15 and 16 corresponds to 16S rRNA amplification fo H.pylori DNA isolated from saliva samples of gastritis patients (group1) with sample number 1,2,5,7,8 as in Annexure 1

Figure 2

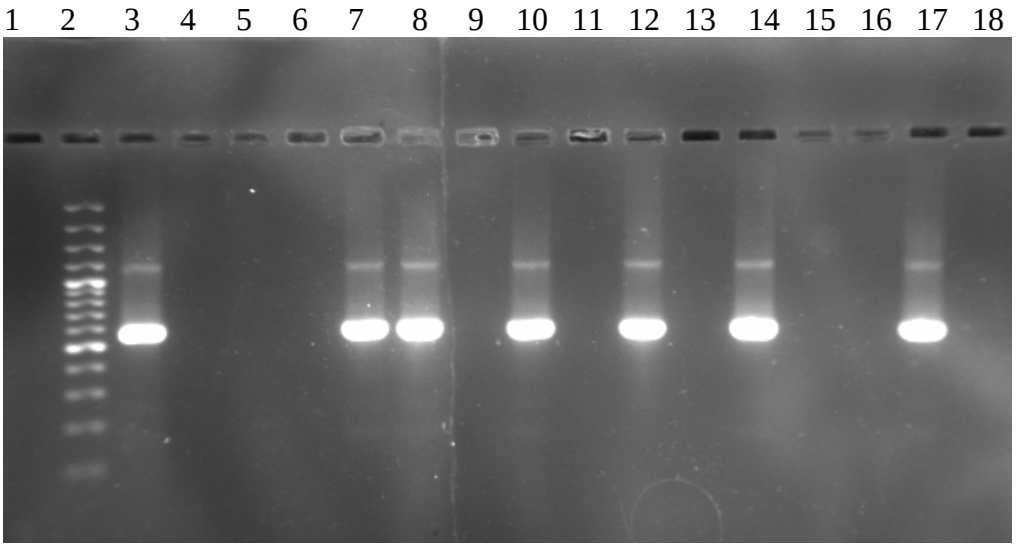


Figure 2 Gel image shows 16S rRNA amplification of *H. pylori* DNA isolated from the patients. Product size: 534 bp.

Lane 2 : 100bpmolecular weight marker
Lanes 3, 7, 8, 10,12 and 14: 16S rRNA amplification of *H. pylori* DNA isolated from saliva of patients,
Lane 17 : positive control (ATCC 26695),
Lane 18 : negative control

Figure 2:Lane 3,7,8 ,10, 12, and 14 corresponds to 16Sr RNA amplification fo H.pylori

DNA isolated from saliva samples of gastritis patients(group1) with sample number

9,13,14,16,18,20 as in annexure 1

Figure 3

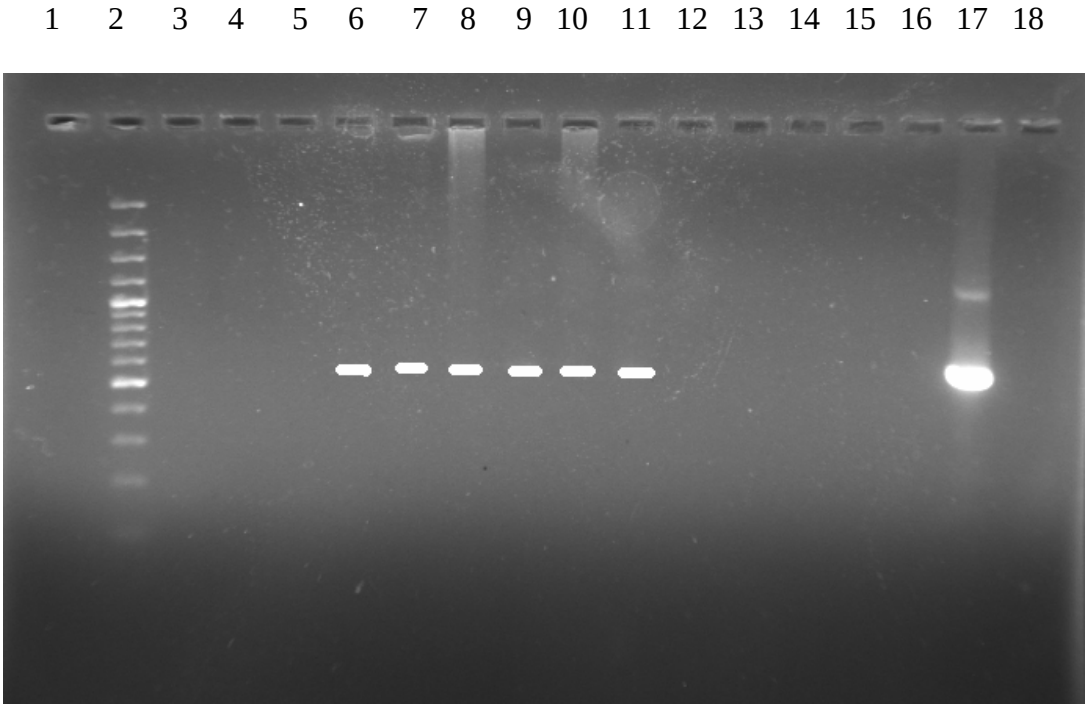


Figure 3: Gel image shows 16S rRNA amplification of *H. pylori* DNA isolated from the group 2 Product size: 534 bp.

Lane 2	: 100bpmolecular weight marker
Lanes: 6,7,8,9,10,11 are	: 16S rRNA amplification of *H. pylori* DNA isolated from saliva of normal controls
Lane 17	: positive control (ATCC 26695),
Lane 18	: negative control

Figure 3:Lane 6,7,8,9,10,11 corresponds to 16S rRNA amplification fo H.pylori DNA

isolated from saliva samples of group 2 with sample number 4,5,6,7,8,9 as in annexure 1

Discussion

This study has shown that in 56.67 % (17 cases out of 30) of study population, *H. pylori* was diagnosed with the help of saliva samples which was comparable with the results obtained from biopsy /histopathological analysis of the gastric tissues of the infected subjects. Further, the presence of *H. pylori* was also diagnosed, using saliva, in asymptomatic subjects who were later found to have a history suggestive of gastritis at some point of time in the past 6 months.

At present there are a number of diagnostic tests - both invasive and non-invasive tests available for the diagnosis of *H. pylori* infection. They have varying sensitivity and specificity along with specific limitation in clinical applications. Urease-based biopsy tests require invasive and traumatic endoscopy and are not reliable in cases where patients had used proton pump inhibitors priorly. Histological examination follows endoscopy with its accuracy dependent on the method used to collect/ transport specimen, stain selected and on the pathologist's skill. Serology is relatively inexpensive but is not reliable in determining the presence of active infection, which is important for clinical interpretation, making diagnosis as well as determining prognosis.

Our successful amplification and detection of *H. pylori* DNA directly from saliva samples in the majority of infected subjects indicates that this approach is non-invasive, feasible and reliable with a sensitivity of 82.35% and specificity of 23.08%. Gel images (fig1 and fig2) also exhibited bands at 1000 bp ladder, which could be due to primer dimer. This demonstrates that it has true potential in aiding the diagnosis/ estimating the prognosis of patients with active H. pylori infection. This indicates that *H. pylori* when isolated from gastric mucosa most probably also could have been observed in salivary

secretion and when isolated from salivary secretion, is not indicative of the gastric colonization by *H. Pylori*.

Earlier attempts made in the past to prove saliva as a better specimen for estimating *H. pylori* did not yield satisfactory data for which low detection rates of *H. pylori* in the salivary secretion, low detection power of the method used and lesser number of viable organism have been suggested[39]. There were attempts made to detect *H. pylori* by PCR from saliva and dental plaques that showed low rates of detection[43.] On the other hand, Weiss et al in comparative study of the sensitivity, specificity, and predictive value of PCR of formalin-fixed biopsies showed that the 16S rRNA gene of *H. pylori*, has a high accuracy in demonstrating the presence of *H. pylori* in gastric biopsy specimens. In our study, *H. pylori* DNA was identified by PCR in the saliva of 56.67% (17 cases out of 30) of patients, with 11 cases proven with gastric symptoms in group 1 (n = 20) and 3 subjects with history of gastric symptoms in group 2 (n = 10, 4 having history of gastritis). Among the 10 controls, we observed 60% positivity for *H. pylori* in the saliva and 4 of them confirmed that they had occasional symptoms of gastritis. These results indicate that the clinical symptoms of gastritis are not a reliable indicator of oral *H. Pyroli* status.

H. pylori DNA were not identified in the saliva of 10 of the study population who had clinical symptoms or proven gastritis as evident from biopsy and history. The reason for this failure to identify *H. pylori* DNA in the saliva of the 10 samples is unknown. However, *H.pylori* was also identified by PCR assay in saliva specimens from 3 control patients whose history did not give a clear picture of gastritis. The relationship between gastric symptoms and *H. pylori* DNA in saliva, however, is unclear. It could be possible

that the oral cavity is the initial site of infection. *H. pylori* may persist in low numbers in the oral cavity of these subjects for a long time without colonizing the stomach or gastric lining. There are reports with supportive[44] evidence for the presence of *H. pylori* in the oral cavity. These observations along with our findings suggest that the oral cavity could be a reservoir of *H. pylori* infection and oral secretion may be an important means of transmission of *H. pylori*.

As the presence of *H. Pylori* in saliva is comparable to the presence in gastric mucosa in our study, it could be said that its presence in saliva is a reliable marker of gastric mucosal immunity status. However studies involving genotypic status of oral *H. pylori* and gastric *H. pylori*, if found similar, would prove that oral cavity as a gate way of this infection and saliva as a means of transmission. Such studies have been carried out and none of them could prove that both of them are of the same strain. In contrast[45] that same strain was present in both sites, in an isolated study.

The results of our study indicate that *H. pylori* DNA is demonstrated accurately using PCR (11 cases) in contrast to the existing standard biopsy (n= 10 cases). In 5 cases, the histopathology did not correlate with the PCR result. This ambiguity may be due to low detection power of the stains used, the pathologist skills and lesser number of viable organisms existing in tissue to be demonstrated histopathologically.

The results of our study indicate that analysis of saliva for *H. Pylori* may potentially be useful sample and saliva could serve as an effective valuable, noninvasive specimen to diagnose/ monitor the prognosis in comparison to existing diagnostic modalities.

Summary and Conclusion

1. 30 subjects were enrolled for the study.
2. 20 cases were symptomatic gastritis, of whom 10 had biopsy proven *H.pylori* infection.
3. In group 2, all were asymptomatic at the time of study (n =10) (four had previous history of gastritis treated).
4. The mean age of male patients in group1 was 39.43 ± 10.01 and female patients was 52.33 ±.19.83.
5. The mean age of male subjects enrolled in group 2 was 27.75 ± 2.06 and female patients was 27.83 ± 4.54
6. When biopsy was taken as gold standard, PCR of saliva showed sensitivity of 80%, specificity 70%, positive predictive value 72.2% and negative predictive value 77.7% respectively.
7. When clinical gastritis was used as standard for comparison with PCR of saliva, the sensitivity was 58%, specificity was 50%, positive predictive value was 82.35 % and negative predictive value was 23% respectively.

In conclusion, the results of our study indicate that PCR analysis of saliva is useful for identification of *H. Pylori.* Saliva could serve as an effective, valuable, noninvasive specimen to diagnose / monitor the prognosis of *H. Pylori* infection in comparison to existing diagnostic modalities. However, the exact sensitivity and specificity of saliva based PCR to diagnose *H. pylori* needs to be demonstrated in further studies involving larger samples.

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Annexure

MASTER CHART OF PATIENTS

S.No	Sample number	Age	Gender male	Biopsy report positive/ negative	Pcr report positive/ negative	Special
1	4	39	male	negative	negative	
2	6	36	female	positive	positive	
3	10	27	male	negative	negative	
4	12	72	female	positive	positive	
5	13	48	female	negative	positive	difference in results of biopsy n pcr
6	14	58	female	positive	negative	difference in results of biopsy n pcr
7	16	40	male	positive	positive	
8	18	32	male	negative	positive	difference in results of biopsy n pcr
9	20	25	female	positive	positive	
10	21	35	male	positive	negative	difference in results of biopsy n pcr
11	28	75	female	negative	negative	
12	32	58	male	negative	negative	
13	35	47	male	positive	positive	
14	38	28	male	positive	positive	
15	39	40	male	negative	negative	
16	42	27	male	negative	positive	difference in results of biopsy n pcr
17	45	47	male	negative	negative	
18	58	35	male	positive	positive	
19	67	57	male	negative	negative	
20	77	40	male	positive	positive	

MASTER CHART OF CONTROLS

S.no	Sample.no	Age	Gender Male	Pcr Positive	gastritis
1	1	30	female	Negative	absent
2	2	24	female	Negative	absent
3	3	25	female	Negative	absent
4	4	25	female	Positive	absent
5	5	36	female	Positive	absent
6	6	28	Male	Positive	present
7	7	30	Male	Positive	present
8	8	27	female	Positive	present
9	9	28	Male	Positive	absent
10	10	25	Male	Negative	present