Prospective study on Association of Helicobacter pylori infection in Colorectal Cancer

A DISSERTATION SUBMITTED TO THE TAMILNADU
DR.M.G.R MEDICAL UNIVERSITY CHENNAI

In partial fulfilment of the Regulations for the award of Degree of

M.S. DEGREE EXAMINATION BRANCH I –

GENERAL SURGERY

Reg.No.221711121

DEPARTMENT OF GENERAL SURGERY

MADURAI MEDICAL COLLEGE MADURAI,

MAY 2020

BY THE GUIDE OF THE DEPARTMENT

This is to certify that the dissertation entitled "***Prospective study on Association of Helicobacter pylori infection in Colorectal Cancer IN GRH, MADURAI***" submitted by **Dr.PUSHPARAJ T.S** to Tamil Nadu Dr. M.G.R Medical University, Chennai , done in partial fulfilment of the requirement of the award of MS Degree Branch – I (General Surgery) is a bonafide research work carried out by him under direct supervision and guidance from August 2018 to August 2019 in the Department of General Surgery, Madurai Medical College.

Place: Madurai

Date:

Dr.J.AMUTHAN DLO., MS.,

Unit Chief,

Department of General Surgery

Madurai Medical College,

Govt. Rajaji Hospital,

Madurai-625020.

CERTIFICATE

BY THE HEAD OF THE DEPARTMENT

This is to certify that the dissertation entitled "***Prospective study on Association of Helicobacter pylori infection in Colorectal Cancer IN GRH, MADURAI*** " submitted by **Dr.PUSHPARAJ T.S** to Tamil Nadu Dr. M.G.R Medical University, Chennai , done in partial fulfilment of the requirement of the award of MS Degree Branch – I (General Surgery) is a bonafide research work carried out by him under direct supervision and guidance from August 2018 to August 2019 in the Department of General Surgery, Madurai Medical College.

Place: Madurai

Date:

DR.A.M.SYED IBRAHIM MS.,
Professor and Unit Chief,
Head of the Department
Department of General Surgery
Madurai Medical College,
Govt. Rajaji Hospital,
Madurai-625020.

CERTIFICATE BY THE DEAN

This is to certify that the dissertation entitled ***Prospective study on Association of Helicobacter pylori infection in Colorectal Cancer IN GRH, MADURAI"*** is a bonafide research work done by **Dr.PUSHPARAJ T.S,** Post Graduate Student, Department of General Surgery, MADURAI MEDICAL COLLEGE AND GOVERNMENT RAJAJI HOSPITAL, MADURAI, under the guidance and supervision of **DR.J.AMUTHAN DLO;MS,** Professor , Department of Surgery, MADURAI MEDICAL COLLEGE AND GOVERNMENT RAJAJI HOSPITAL, MADURAI.

Place : Madurai
Date :

Dr.K.VANITHA MD., DCH.,
DEAN
MADURAI MEDICAL COLLEGE

DECLARATION BY THE CANDIDATE

I, **DR.PUSHPARAJ.T.S** hereby declare that this dissertation entitled "***Prospective study on Association of Helicobacter pylori infection in Colorectal Cancer IN GRH, MADURAI***" is a bonafide and genuine research work carried out by me in the Department of General Surgery, Madurai Medical College during the period of August 2018 To August 2019. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, diploma to any other University, Board either in India or abroad. This is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of regulations for the award of M.S. degree (Branch I) General Surgery course.

Place : Madurai
Date **:**

Dr. PUSHPARAJ T.S
Post graduate
MS General Surgery,
Madurai Medical College,
Madurai-625020.

ACKNOWLEDGEMENT

I take this opportunity to extend my gratitude and sincere thanks to all those who have helped me complete this dissertation.

I am extremely indebted and remain forever grateful to my guide, **Dr.J.AMUTHAN DLO.,MS.,** Professor of Surgery, for his constant able guidance and constant encouragement in preparing this dissertation and also throughout my post – graduate course.

It gives me immense pleasure to express my deep sense of gratitude and sincere thanks to my beloved assistant professors Dr. **T.VANITHA MS.,DA , Dr . A.SUGANYA MS , Dr. P.VANITHA MS., D.G.O for** their guidance and encouragement and support during my postgraduate course.

I thank the respected Dean of Madurai Medical College and Govt. Rajaji Hospital, **Prof.Dr.VANITHA MD.,DCH**, for permitting me to conduct this study in the Department of General Surgery of the Govt.Rajaji Hospital, Madurai.

I thank my parents for being a constant source of encouragement throughout my career . I also would like to thank my patients for having consented to be a part of this study. Last but not the least I would like to thank the almighty without whom this would not be possible.

SL. NO.	TABLE OF CONTENTS	PAGE NO.
1.	**INTRODUCTION**	**1**
2.	**REVIEW OF LITERATURE**	**3**
3.	**OBJECTIVES**	**21**
4.	**METHODOLOGY**	**22**
5.	**RESULTS**	**29**
6.	**TABLES**	**36**
7.	**FIGURES**	**56**
8.	**DISCUSSION**	**62**
9.	**SUMMARY & CONCLUSION**	**71**
10.	**BIBLIOGRAPHY**	**75**
11.	**ANNEXURES**	
	Consent Forms	
	Proforma	
	Key for Master chart	
	Master chart	
	Ethics Committee Certificate	
	Plagiarism Verification Certificate	

INTRODUCTION

INTRODUCTION

Colorectal cancers hold a major burden of cancer and cancer-related deaths in the world. Colorectal cancers were studied extensively for their association with environmental and dietary factors, and gut microflora. As these include modifiable risk factors there is a potential for their role in primary prevention of colorectal cancers. Helicobacter pylori (H. pylori) being highly prevalent in general population, any evidence of its role in colorectal carcinomas will warrant early screening and eradication of this risk factor.

H. pylori is known to be associated with a large spectrum of gastric and extra-gastric conditions. H. pylori has been recognized as a class I human carcinogen by the International agency for cancer research (2). There are recent reports on the role of H. pylori in the promotion of tumour growth in extra-gastric organs(1), of which its role in colorectal neoplasm is gaining interest.

The pathogenic role of H. pylori in the development of colorectal malignancies is not clear (2). A possible mechanism described attributes it to the expression of the cytotoxin-associated gene (CagA) by the H. pylori strains (3). CagA strains result in the development of chronic atrophic gastritis which further leads to hypergastrinemia. Hypergastrinemia through a reverse feedback mechanism is known to facilitate the development of colorectal cancer (3-5). Moreover, hypochlorhydria

induced by the chronic atrophic gastritis also results in the overgrowth of microflora like B. fragilis and E. faecalis which are implicated in the colorectal cancer progression^). Alternatively, the inflammatory response mediated damage to the colorectal epithelium induced by H. pylori may also promote the development of colorectal neoplasia(l).

The correlation between H. pylori and colorectal malignancies, however, remains controversial. A higher seroprevalence of H. pylori has been reported in people with colorectal malignancy in various studies (7-11). A study by Strofilas et al demonstrated an association between H. pylori and colorectal neoplasia as statistically not significant, however, the same study reported a statistically significant association between hyperqastrinemia) and lymph node metastasis(12). .

There is a death of studies correlating the role of H. pylori in the development of colorectal neoplasia from Asia. The direct etiological association of H. pylori in colorectal malignancy, hence, can neither be supported nor rejected and requires more clinical studies to confirm its association^ 3). Hence this study is being carried out to evaluate the association of H. pylori and colorectal malignancy in our population.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Helicobacter pylori is a spiral gram-negative bacillus with unipolar flagella. It is a ubiquitous microaerophilic organism which was associated with humans for hundreds of years. It survives a highly acidic environment in gastric mucosa and colonizes the mucosa. H. pylori is found in all age groups, with more prevalence in older age group. It is transmitted from person to person by oral-oral or faecal-oral routes, contaminated water or iatrogenic through endoscopy(14).

H. pylori is highly prevalent in general population, as much as one-third in North America and Europe and half of the population in South America, South and East Europe and Asia(14). Studies conducted in East Asia demonstrated a seroprevalence of 55%-64%(15) An estimated seroprevalence was 58% in developed countries and 74% in developing countries in middle-aged adult population^ 5). A systemic review of prevalence throughout the world was done involving 37 studies and 22 countries with data collected from 1968 to 2011 by Barbara Peleteiro et.al(16). It showed a high prevalence in Central/ South America and Asia. Higher prevalence was observed in low socioeconomic status, poor hygiene and overcrowded regions. Improvement in these general conditions will reduce its burden on the population.

Its pathogenetic potential for gastritis and gastroduodenal ulceration was discovered and demonstrated by Nobel Prize winners Marshall and

Warren for the enormous and dedicated contribution. Since its discovery, inthe past 50 years, robust studies were done throughout the world to understand the pathogenesis and its role in different disorders.

H. pylori has multiple pathogenetic factors of which one of the majorly studied factors is CagA pathogenicity island which produces a highly immunogenic protein called CagA. Almost all strains in Asia and Africa have CagA whereas about one-third of the population in Europe and North America are negative for CagA.

DIAGNOSIS OF HELICOBACTER PYLORI INFECTION

Different diagnostic modalities are available like serologic tests, histopathological examination, urea breath test and rapid urease test. Serologic tests don't differentiate present and past infections, whereas others do. Rapid urease test and histology represent colonic burden while others represent the gastric colonization of H pylori. Both chronic and active infections may contribute to pathogenesis by different mechanisms (13).

Diagnostic tests can be classified into invasive and non-invasive methods based on the requirement for upper Gl endoscopy (17).

Specimen obtained with endoscopy can be subjected to following tests:

1. Rapid urease test (RUT)
2. Culture
3. Histology

4. Molecular methods including polymerase chain reaction (PCR) and fluorescence in vivo hybridization (FIVH)

Other non-invasive methods include:

1. Serology
2. Stool antigen test
3. Urea breath test
4. Ammonia breath test
5. H. pylori saliva antigen test

Histology

Haematoxylin and eosin stain detects the bacteria in biopsy specimen with sensitivity and specificity of 69-93% and 87-90% respectively (18). This test has a high rate of false-positive results because the distribution of bacteria is not uniform, and it may not be helpful in case of a low bacterial load. Also, this stain may not differentiate between luminal debris and bacteria.

Culture

Culture is the most specific test with sensitivity and specificity of 90% and 100% respectively. Antibiotics should be stopped at least 28 days prior to the test (19).It allows for analysis of pathogen type and resistance pattern (20).

Rapid Urease Test (RUT)

RUT gives rapid and reasonably accurate results which are helpful especially in resource-limited settings. It is based on the principle that H. pylori produces an enzyme urease (21). Minimum of 10D bacteria are required for a positive test result (21,22). The reagent includes 250mg urea, 400pl gentamicin and 400pl of phenol red in 15 ml distilled water and test isobtained in few minutes to 24 hours (23,24). Sensitivity and specificity are 80-100% and 97-99% respectively (21).

Polymerase Chain Reaction(PCR)

PCR amplifies the DNA of H. pylori and helps in identifying even minute amounts of bacterial load. It is as sensitive as histology. It can be done in various samples as saliva, stools, gastric biopsy, gastric juice, dental plaques, etc(25).

Fluorescence In -Vivo Hybridization (FIVH)

FIVH is recently developed technology which helps in direct visualization of bacteria in mucosa during endoscopy. As it requires a confocal laser endomicroscopy its use is limited to certain organs(26).

Stool Antigen Test (SAT)

This method of antigen testing started in 1997(27). Stool antigen testing is a convenient method of detection of H. pylori with sensitivity and specificity of 94% and 97%, which was identified in a meta-analysis done globally (28).

It can be done using:

1. Enzyme immunoassay (EIA)
2. Immunochromatography assay (ICT)

EIA based tests are more accurate when compared to ICT based tests(28). But ICT is easy to perform and requires no special equipment which is suitable for low resource settings. These tests can be monoclonal antibody- based or polyclonal antibody-based. Though initially polyclonal based tests were used many studies showed a better sensitivity and specificity with monoclonal antibodies (27,28). Advantages of SAT is it can be used for epidemiological studies and screening in mass surveys. EIA based tests are also recommended for assessing the efficacy of H. pylori eradication after treatment. Also, it is a convenient test for children (27).

Accuracy of this test depends on several factors: (27)

1) Antigen may degrade as it passes along the alimentary canal
2) In patients with atrophic gastritis, this test is less accurate
3) Antibiotic use
4) Use of proton pump inhibitors
5) Use of mucolytic like N- acetylcysteine
6) Upper gastrointestinal bleeding
7) Storage temperature
8) Transport time, etc.

Urea Breath Test (UBT)

UBT works on the principle that urease secreted by H. pylori hydrolyses urea. Urea labelled with isotopic carbon is given orally and exhaled carbon dioxide is measured for isotopic carbon (29). It is a simple and safe test. This test has a sensitivity and specificity greater than 90% (30,31).

Helicobacter Pylori Saliva Antigen Test

It is a simple, rapid non-invasive test to diagnose H. pylori. It can be done in large populations in a short period and helps in recognizing oral H. pylori infection which has a potential for gastric colonization so that eradication can be done. It is sensitive in detecting low bacterial load but not very specific(32).

Serology

Serology is the most commonly used test for H. pylori. After about 22-23 days of infection, there is IgG sero conversion which can be identified by enzyme immunoassays (33). H. pylori is a heterogenous organism which produces many immune active proteins like lipopolysaccharides, CagA, catalase, heat shock protein, VacA, UreA, Omp, GroEL, FliD protein, etc.(27,28) Enzyme immunoassays or immunochromatography tests can be used for detection of antibodies of which EIA show better results(27). As the prevalence of H. pylori and its varied stains differ from area to area, the efficacy of test depends on using

the appropriate kit which could identify the local strains rather than foreign strains (27,28). Sensitivity and specificity vary with product from 60 to 100% (31). Recently some kits are based on pooled antigens. Calibrating the titres to local population also helps it improving the sensitivity and specificity of the tests.

Advantages of this test includes: (27,28)

1) Inexpensive, accurate, rapid
2) Can be used for epidemiological studies
3) Not affected by bleeding, gastric atrophy, use of PPI or antibiotics

Disadvantages include: (27,28)

1) Cannot differentiate active and past infection
2) Immune response varies from individual to individual, nutritional status
3) Cross-reactivity with other related bacteria like Campylobacter
4) Geographical variation of strains

Ammonia Breath Test (ABT)

The ammonia produced from urea by H. pylori is excreted through kidneys and lungs. ABT quantifies the ammonia excreted through lungs (34, 35). It is rapid, simple and has a sensitivity and specificity of 71.43% and 88.9% respectively (34).

ASSOCIATION OF HELICOBACTER PYLORI WITH DIFFERENT DISORDERS

Helicobacter pylori is a heterogenous organism with high genetic variability. Its virulence factors and its ability to alter the host immune system leads to pathogenicity in a myriad of disorders. Though its role in gastric pathology is well studied and established, its association with others is far from conclusion. Some advocate that H. pylori has beneficial effects by reducing the gastric acid secretion reducing the chance of oesophageal disorders and preventing allergies. Yet its association with serious disorders should not be overlooked (17).

H. pylori has many virulence factors which include sheathed flagella, hypo inflammatory lipopolysaccharide antigen, CagA pathogenicity island, molecular mimicry, etc each playing their role to help alter the host immune responses and colonization of organism eventually leading to many disorders.

H. pylori was identified in different regions of the body by different methods like culture, polymerase chain reaction and histologic examination. These include skin, eyes, nasal and oral cavities, ear, coronary arteries, stomach, liver, gallbladder, large intestine and peritoneum (36).

Disorders with causative role well studied include dyspepsia, gastric and duodenal ulcers, gastritis, gastric malignancies which include gastric

adenocarcinoma and MALT lymphoma. Gastritis can be pangastritis, corpus- predominant gastritis or antral predominant gastritis of which pangastritis and corpus-predominant gastritis are associated with gastric malignancy (36,37). Other potential cancers associated include colorectal, pseudomyxoma peritonei, laryngeal and pharyngeal cancers and lymphomas. Extra gastric conditions which were studied and shown some correlation with H. pylori are (36, 38)

1. Iron deficiency anaemia
2. Vitamin B 12 deficiency
3. Idiopathic thrombocytopenic purpura
4. Skin disorders like rosacea, psoriasis, chronic prurigo, chronic idiopathic purpura
5. Diseases of ear, throat, nose as nasal polys, otitis media, etc
6. Diseases of pregnancy- preeclampsia, hyperemesis
7. Ocular disorders
8. Liver and gallbladder
9. Pulmonary
10. Neurodegenerative
11. lnsulin resistance, diabetes mellitus
12. Pancreatic disorders
13. Cardiovascular disorders

ASSOCIATION OF *HELICOBACTER PYLORI* WITH CANCER

Helicobacter pylori is identified as a class I carcinogen by International Agency of Research on Cancer alongside others like smoking, asbestos and radiation (36). H. pylori-associated gastric malignancies account for 25% of infection-related malignancies and 5.5% of all cancers globally (36).

Development of gastric malignancy occurs in a progressive manner from chronic non-atrophic active gastritis to atrophic gastritis to intestinal metaplasia to dysplasia to carcinoma(39). H. pylori causes carcinogenesis by different mechanisms directly or indirectly by changing the host response to chronic active infection.

(CagA strains are associated with more severe inflammation and high rate of cancers. It causes transformations in cell lines into more immature stem cells which are prone to carcinogenesis, it leads to gradual accumulation of mutations over time and tumour genesis eventually(40). CagA interacts with the SH-2 domain in host cells leading to signal transduction in tyrosine kinases via SHP-2 phosphatase. This phosphatase is identified in many tumours. Other proteins include c-MET(36).

COLORECTAL MALIGNANCY AND RISK FACTORS

Colorectal cancers are the third most commonly diagnosed malignancies accounting for the fourth leading cause of cancer-related deaths (41). Multiple genetic mutations accumulate causing a change in

normal colorectal mucosa to transform into adenomas and carcinomas. These mutations can be sporadic or germ line. Environmental and genetic factors play an important role in pathogenesis. Around 70 % of them are sporadic, 10 % are inherited syndromes and 20% are familial clustering. Cancers identified and treated at an early stage have a much better prognosis when compared to those diagnosed at a later stage, thus emphasizing the importance of screening, which may improve the survival. Some of the risk factors for colorectal cancers include smoking, alcohol, lifestyle factors, metabolic syndrome (42).

Development of cancers occurs through intrinsic or extrinsic pathways. Intrinsic includes oncogenic activation and extrinsic include infection and inflammation. As we observe from proximal to distal colon there are gradual changes pertaining to microorganisms, anatomy and biomarkers. In colon cancers interaction and balance among genetic factors, microbial environment and host immune response lead to tumorigenesis. The above factors lead to the production of certain proteins which interact with receptors involved in pathways leading to colon cancer initiation. They eventually lead to increased expression of certain chemokines, cytokines, prostaglandins and COX-2 which provide an inflammatory microenvironment for cancer development. Some of the well-described factors are interleukin 6, transforming growth factor beta, microsatellite instability, etc.(43)

Studied risk factors for colorectal cancers include the following(44,45):

1. Old age
2. Male sex
3. Diabetes mellitus
4. Hypertension
5. Metabolic syndrome
6. Elevated triglycerides and cholesterol
7. Obesity
8. Alcohol consumption

COLORECTAL CANCERS AND BACTERIA

The colon contains a large load of microbiome containing about $10^{14}$to 10^{15} bacteria and the quantity gradually increases as we go distally. Many studies showed changes in environment and lifestyle of people lead to changes in colon microbiota(43). Though they have a symbiotic role in humans, alterations in the same may promote cancer genesis directly or indirectly. Some studies also showed that they have a role in the treatment of colon cancers by changing the host immunological response to cancer and medication. First observation of association of gut flora with colorectal cancers was made in 1975 in rats. Most likely theories include dysbiotic microenvironment and some bacteria causing DNA mutations and promoting the growth of other bacteria causing carcinogenesis (46).

Different mechanisms proposed are as follows (47):

1. Inflammatory bowel disease
2. Genotoxins
3. Metabolism - eg. Metabolism of bile acids to carcinogenic deoxycholic acid in a high fatty meal.
4. Chronic inflammation
5. Modulation of immune response

Organisms observed to have an association with colorectal cancers include the following (47,48):

1. *Fusobacterium nucleatum*
2. *Colibactin-producing Escherichia coli*
3. *Enterotoxigenic Bacteroides fragilis*
4. *Enterococcus faecalis*
5. *Streptococcus gallolyticus*
6. *Porphyromonas sp.*
7. *Salmonella sp.*
8. *Prevotella sp.*
9. *Helicobacter pylori*

HELICOBACTER PYLORI AND COLORECTAL CANCERS

Helicobacter pylori infection is noted in more than 70% of the population. Its role in colorectal neoplasm was first identified in 1990's (14). Some of the studies showed a plausible role of infection in tumour

carcinogenesis in colorectal carcinomas. Though its causative role is not fully established this environmental factor can be controlled with antibiotic treatment and reduce the risk for colorectal carcinomas. In a meta-analysis conducted between 1991 and 2002 odds ratio of 1.4 was observed for correlation of colorectal cancers and H. pylori infection (49). A recent study conducted in western population identified an association of H. pylori positive gastritis and colonic cancers with respect to number, size and histopathological progression(50). Ye Yan et al studied intestinal metaplasia with H. pylori infection as a risk factor for colorectal adenomas which demonstrated a significant association(51). The same study showed that this increased risk is with H. pylori infection with intestinal metaplasia and not without intestinal metaplasia signifying chronic infection resulting in chronic sequelae of intestinal metaplasia.

Mechanism

The association between H. pylori infection and colorectal cancers is being studied, though the exact mechanism and risk of colorectal malignancy are still inconclusive. A possible mechanism is H. pylori-related chronic atrophic gastritis leading to hypergastrinemia, by a reverse feedback mechanism, which causes changes in the lower gastrointestinal mucosa (52). Also, reduction in gastric acid secretion leads to changes in intestinal microflora attributing to carcinogenesis (6). Chronic inflammatory response to infection may also contribute to cancer

pathogenesis (1). Hyperchlorhydria impairs protein digestion resulting in accumulation of certain metabolites and., bacterial overgrowth contributing to colorectal malignancy (51).

Chronic infection leads to atrophic gastritis in 8.1% patients per year accounting for the 10-fold rise in atrophic gastritis risk. A study conducted by Sonnenberg A et al (2013) showed an association between advanced colorectal cancers and Helicobacter py/o/7-induced atrophic gastritis (53). A cross-sectional study conducted by Ji Young Lee et al demonstrated that H. pylori-associated atrophic gastritis acts as an independent risk factor for advanced colorectal malignancies (15). Brim et al. studied African American population of greater than 40 years, which revealed that gastric H. pylori infection is associated with increased risk of neoplastic and non-neoplastic colonic lesions (54). So they advocated screening and eradication of H. pylori in a patient with chronic gastritis.

Lee et al. identified an elevated risk of colorectal malignancies with H. pylori infection based on serological tests (15). These tests don't differentiate between past and current infection. This discrimination is important because current infection induces chronic inflammation causing oncogenic sequelae. Evaluation of histology and immunohistochemistry for CD44 (an indicator of cancer stem cells and bone marrow-derived stem cells) showed a high prevalence of Helicobacter in colorectal adenomas and carcinoma (38). A pilot study conducted by Mary Jones et al.

demonstrated an increased prevalence of H. pylori in tubulovillous adenomas and adenocarcinomas but not in villous adenomas by histology (55). Some studies identified the organism within the neoplastic tissue. Direct identification using histopathology showed an association with colonic adenomas, indicating that current infection is an independent risk factor for colorectal neoplasia (53,56)

The carcinogenic pathways vary for proximal and distal cancers. The pathways with chromosomal instability are seen in distal colon and those with CpG island methylator phenotype and microsatellite instability are seen in the proximal colon (1,57). Stomach microflora changes induced by chronic atrophic gastritis lead to methylation changes for which proximal colon is more susceptible. Also, the altered bacterial environment causes increased secretion of secondary bile acids which increases the risk for proximal colon cancers(58,59). The urease secreted by H. pylori increases the luminal ammonia which increases colonic malignancy risk (60).

Helicobacter pylori and Hypergastrinemia

H. pylori infection cause increase in blood gastrin levels, both fasting and stimulated (52). The chronic inflammation causes hypergastrinemia and also increases cytokines like TNF-alpha and interleukin 1 in gut mucosa which promote carcinogenesis (3).

Gastrin in Colorectal Malignancy

Gastrin is a 17 amino-acid peptide and secreted by G cells in antral mucosa, duodenum and pancreas. It increases pancreatic enzyme activity, promotes gastric emptying and increases gastric acid secretion. By increasing gastric acid secretion, it promotes digestion and prevents overgrowth of intestinal bacteria (3). It is also a growth-promoting factor with a possible role in tumour development including colon, lung and pancreas (61-65). By acting as a growth factor, it increases angiogenesis, promotes tumour spread, activates transcription factors in colon adenoma to carcinoma transformation and impairing antiapoptotic factors. Its correlation with colorectal carcinoma is still controversial. Gastrin, when administered exogenously in cell lines, causes an increase in DNA synthesis (66, 67). Also, administration of Penta gastrin to implanted colon cancer cells in mice resulted in an increase in tumour burden and reduced survival (66, 68, 69). Increased circulatory levels of gastrin were observed in colorectal carcinomas, yet its significance in raised levels as a causative factor or gastrin from tumour functioning as autocrine growth factor is not determined (52). A progressive reduction in gastrin levels was observed in colorectal cancers from early to late stages indicating a possible role in early carcinogenesis and is less secreted by poorly differentiated tumours (3). Expression of gastrin and its receptors occurs in colonic polyps showing a role in early adenoma-carcinoma sequence (51). In patients with

Zollinger Ellison syndrome higher markers of colonic proliferation was identified (12), although some studies showed no correlation.

CagA (CYTOTOXIN-ASSOCIATED GENE A)

Helicobacter pylori are majorly sub-grouped into two based on their ability to secrete a 120-145 k Da protein called CagA. It is encoded by a gene CagA located in CagA pathogeni city island. Helicobacter pylori strains in the American population and east Asian population are CagA positive, 60% and almost 100% respectively (70). Positive strains are cause more severe inflammation and gastritis which play a major role in the promotion of gastric / carcinoma, leading to higher risk for gastric cancer. CagA interacts with tyrosine phosphatase SHP-2, which acts as an oncoprotein is a major mechanism which promotes malignancy. CagA gene induces overproduction of lnterleukin-8 which is a known growth factor for colorectal cancers in humans (41). A prospective study conducted by Paul J Limburg et al. did not identify any significant correlation between CagA seropositive H. pylori and colorectal carcinomas (71).

AIM AND OBJECTIVE

AIM AND OBJECTIVE

Aim

To evaluate the association of H. pylori infection and colorectal cancers.

Primary objectives

To determine the prevalence of Helicobacter pylori infection in patients with colorectal cancers and compare with controls.

Secondary objectives

To examine the possible correlation of overall H. pylori infection and the CagA strains with the site, histopathological differentiation, stage and metastasis of colorectal cancer.

METHODOLOGY

METHODOLOGY

The study was conducted in the Department of Surgery, GRH, Madurai from August 2018 to August 2019. An informed consent was obtained from all participants included in the study.

STUDY DESIGN

It is a single centre prospective case-control study.

STUDY PARTICIPANTS

The participants were categorized into two groups namely study and control groups. The study group included all consecutive patients of age >18 years with histologically proven colorectal malignancy in the Department of Surgery, GRH, Madurai. The control group included age and gender-matched patients undergoing groin hernia repair (males) and treatment for extra abdominal benign conditions (females) in the Department of Surgery, GRH, Madurai.

The following patients were excluded from the study:

1. Patients receiving gastric anti-secretory medications and NSAIDs on a long-term basis.
2. History of previous gastro-duodenal surgery.
3. History of Zollinger Ellison syndrome.

The effect of Non-steroidal anti-inflammatory drugs on H. pylori and vice versa was proposed and studied by many. But both, independent risk factors for gastric diseases, being synergistic or antagonistic is not

identified in any study. Though many mechanisms were proposed none of them was proved. As the interaction between both risk factors was not well understood, patients receiving these drugs on a long-term basis were excluded (72, 73).

In patients who underwent any gastroduodenal surgeries, bile reflux will affect the growth of H. pylori and also some studies showed there is the spontaneous eradication of H. pylori after surgery (56).

Patients with Zollinger Ellison syndrome were excluded as they have hypergastrinemia which acts as a confounding factor for colorectal malignancies (67-69).

SAMPLING

Patients were included by convenience sampling. A sample size of 32 in each group is calculated based on the requirement to detect the difference in prevalence in two groups if any (10); the level of significance being 5% and power of the study set to 90%, using Open Epi (Fleiss with continuity correction) software expecting a dropout rate of 10% sample size of 36 was set for each group.

STUDY PROCEDURE

Informed consent was taken from the patients satisfying the inclusion criteria. Five ml of fasting blood sample and stool samples were collected from all the patients and subjected to CagA ELISA and H. pylori stool antigen testing respectively. The following parameters were noted

and correlated with the H. pylori status.

1. Patient characteristics
2. Stage of colorectal malignancy
3. Histological type of neoplasia
4. Presence of metastasis if any
5. lntra-operative details (if the patient undergoes surgical management)

The results in the two groups were analysed for statistical significance.

Definition of Helicobacter pylori Status

The patient was considered to have H. pylori infection if either CagA ELISA test or H. pylori stool antigen test or both the tests were positive. The patient was considered negative for H. pylori infection if both the tests were negative.

Procedure of CagA ELISA Test

Five ml of fasting blood sample was collected and immediately transported to Microbiology department. The sample was allowed to settle for clot formation and then centrifuged for serum separation. Separated serum was stored at -20° till use. The ELISA test was done using H. pylori CagA ELISA kit procured from Bioassay Technology Laboratory.

This kit was based on a qualitative reverse phase enzyme immunoassay technique. Antibodies in the sample bind to the antigen on

the plate. Unbound antibody is washed away during washing. A Horseradish Peroxidase conjugated detection antibody is added and incubated. Unbound HRP was washed away. The substrate is then added, and colour develops. This reaction is stopped using a stop solution and intensity of colour checked at 450 nm. Then the optic density is measured and compared with positive and negative controls. The test was done in following steps:

1. Preparation of reagents, samples, controls and set a blank well with no solution. Wash fluid was prepared at 1 in 30 dilutions.
2. Add 50 pi of positive and negative controls in each well.
3. Dilute 10 pi sample with 40 pi diluent solution.
4. Incubate for 30 min at 37°C
5. Wash for 5 times
6. Add HRP to each well and incubate for 30 minutes at 37°C
7. Wash for 5 times
8. Add substrate A and substrate B and incubate for 10 min at 37°C
9. Add 50 pi of stop solution
10. Read the OD value within 15 minutes at 450 nm.

Quality control

OD blank < 1

OD positive >1

OD negative<1

Cut off value=negative control value + 0.15

If OD sample < 1: negative

If OD sample >1: positive

Procedure of Stool Antigen Testing

The stool sample was collected from all the patients in sterile containers and transported to the Microbiology laboratory and immediately frozen at -20°C until use. Stool antigen testing was done using the On-Site H. pylori rapid test.

This test is a sandwich lateral flow chromatographic immunoassay. The test strip contains a burgundy coloured conjugate pad containing monoclonal anti-H. pylori antibody conjugated with colloidal gold and a nitrocellulose membrane strip with test and control line. The test line is pre-coated with another monoclonal anti-H. pylori antibody and control line is pre-coated with goat anti-mouse IgG antibody. When the specimen disperses into the cassette it migrates by capillary action. If the antigen is present it binds to the anti-H. pylori conjugates and the immunocomplex is captured by the pre- coated antibody on T line. It was done in following steps:

1. The collected and stored samples were thawed and brought to room temperature
2. The stool sample was mixed with extraction buffer and a homogeneous liquid suspension was made.

3. Two drops of solution were dispensed in the well of cassette
4. Result's were read after 15 min and not later than 20 minutes.

The test was considered valid only if control line develops. They are considered invalid if there is no development of control line.

The positive result was indicated by the formation of both C and T lines.

The negative result was indicated by the formation of only C line.

DATA COLLECTION

Data was collected from both the study group and control group using a pre-approved data collection Proforma.

It included independent variables such as:

1. Age
2. Gender
3. History of smoking
4. Prevalence of H. pylori infection

The outcome variables recorded were:

1. Histopathological type
2. Stage of colorectal malignancy
3. Presence/absence of lymph node metastasis.
4. Metastasis
5. Differentiation of the tumour

STATISTICAL ANALYSIS

The data from the proformas were tabulated on Microsoft Excel spreadsheet and analyzed using SPSS version 25. A probability value of less than 0.05 was considered as significant. Continuous variables like age of the patient were expressed as mean and the difference was tested using student's t-test. Gender was expressed as proportions. Categorical variables such as the history of smoking, histopathological type, stage of the tumour and metastasis were tested using the Chi-square test and Fisher's exact test.

RESULTS

RESULTS

A total of 90 participants were included in the study, of which 47 were in the study group and 43 were in the control group.

Table I shows the comparison of age distribution in study and control groups. In the study group, 36.2% were in age group 51-60 and 19.1% were in 61-70 age group. In the control group, 34.9% were in 51-60 group and 27.9% in 61-70 age group. Mean age group in the study group, was 55.02 with a standard deviation of 14 and in the control group it was 54 with a standard deviation of 12.8. On comparing the mean age in two groups p-value was 0.719, showing no significant variation of age distribution between both groups. Figure I shows age distribution in the study group (n=47). Figure II shows age distribution in control group (n=43).

Table II shows gender distribution in study and control groups. There were 30 (63.8%) males and 17 (36.2%) females in study group and 23 (53.5%) males and 20 (46.5%) females in control group. The male to female ratio in the study group was 1.15 to land 1.76 to 1 in the control group.

Table III shows the comparison of smoking status in study and control groups. 25.5 % of study participants and 28 % of control participants were smokers. There was no significant variation statistically with a p-value of 0.799.

Table IV shows the prevalence of stool antigen positivity, CagA seropositivity for H. pylori and overall H. pylori positivity in study and control groups (n=90). In study group 18 (38.3%) had stool antigen positive, 18 (38.3%) had CagA for H. pylori positive and 31 (66%) had overall H. pylori positive. In control group 18(42%) had stool antigen positive, 9 (21%) had CagA for H. pylori positive and 23 (53.5%) had overall H. pylori positive.

Table V shows the prevalence of H. pylori in study and control groups. The rate of H. pylori infection in the study group was 66 % (31/47) and in the control group was 53.5% (23/43). On analysis test statistically, there was no significance with a p-value of 0.228. Figure III shows the prevalence of H. pylori in study and control groups.

Table VI shows the prevalence of H. pylori infection according to age distribution in study and control groups. In the study group, 38.7% of H. pylori positive population was in 51-60 age group whereas 34.8 % of infection rate was found in controls of the same age group.

Table VII shows the comparison of site-specific association of H. pylori infection and colorectal cancers in the study group (n=47). In patients with right colon cancers, 5 (71.4%) were positive for H. pylori infection and 2 (28.5%) were negative for the same. In patients with left colorectal cancers, 26 (65%) patients were positive and 14 (35%) were negative for H. pylori infection. On comparing both groups the p-value was

1.000 which was not significant. Figure IV shows the comparison of overall H. pylori prevalence and CagA prevalence for H. pylori with the site of colorectal cancers in the study group (n=47).

Table VIII shows the comparison of histopathological differentiation of the tumour with H. pylori infection in the study group (n=47). A total of 29 patients had well-differentiated adenocarcinoma among which 18 (62%) were positive for H. pylori infection. Histopathology of 18 patients was moderately- differentiated adenocarcinoma with 13 (72.2%) of them being positive for the infection. On statistical analysis, the p-value was 0.475, which was not significant.

Table IX shows the comparison of stage-specific association of H. pylori infection and colorectal cancers in the study group (n=47). Of 23 patients with high stage cancers, 15 (65.2%) were positive for H. pylori infection and among 24 patients with low stage cancers, 16 (66.7%) were positive for H. pylori infection. The prevalence was similar with no statistical significance as p-value was 0.917.

Table X shows the comparison of metastasis with H. pylori infection in the study group (n=47). A total of 7 people had metastasis with 4 (57%) positive for H. pylori infection. In patients with no metastasis, 27 (67.5%) had H. pylori infection. No statistical significance was noted between the two groups, p- value = 0.676. Figure IV shows the comparison of H. pylori infection in relation to the site, histopatholcgical differentiation, stage and

metastasis of colorectal cancers in the study group (n=47).

As CagA strains of H. pylori is known to be more virulent in carcinogenesis of colorectal cancers, a separate analysis of CagA seroprevalence in H. pylori-positive patients was done.

Table XI shows the prevalence of CagA seropositivity for H. pylori in study and control groups. The rate of H. pylori infection with CagA strains in the study group was 38.3 % (18/47) and in control group was 21% (9/43). On analysis, there was statistically no significance with a p-value of 0.073. Figure

Table XII shows the prevalence of CagA seropositivity for H. pylori according to age distribution in study and control groups. In the study group, 44.4 % CagA positive population is in 51-60 age group, whereas 33.3 % of infection rate was found in controls of the same age group and 44.4% in 61- 70year age group.

Table XIII shows the comparison of site-specific association of CagA seroprevalence for H. pylori and colorectal cancers in the study group (n=47). In patients with right colon cancers, 3 (43%) were positive for CagA and 4 (57%) were negative for the same. In patients with left colorectal cancers, 15 (37.5%) patients were positive and 25 (62.5%) were negative for CagA. On comparing both groups the p-value was 1.000 which was not significant.

Table XIV shows the comparison of site-specific prevalence of

CagA seropositivity for H. pylori among H. pylori positive patients in the study group (n=31). Of 5 patients who tested positive for H. pylori in right colon cancers, 3 (60%) were positive for CagA and in left colon and rectal cancers, 15 (57.6%) patients tested positive for CagA among H. pylori positive patients. There was no statistical significance with a p-value of 1.000.

Table XV shows the comparison of histopathological differentiation of the tumour with CagA seroprevalence for H. pylori in the study group (n=47). A total of 29 patients had well-differentiated adenocarcinoma, among which 14 (48%) were positive for CagA serology. Histopathology of 18 patients was moderately-differentiated adenocarcinoma with 4 (22%) of them being positive for the CagA serology. On statistical analysis, the p-value was 0.074 which was not significant.

Table XVI shows the comparison of histopathological differentiation with the prevalence of CagA seropositivity for H. pylori among H. pylori patients in the study group (n=31). Among 18 patients with well-differentiated adenocarcinoma and H. pylori infection, 14 (77.8%) were positive for CagA antibody. In patients with moderately-differentiated adenocarcinoma and H. pylori infection, 4 (30.7%) were CagA positive. On analysis, there was a significant p-value of 0.009.

Table XVII shows the comparison of stage-specific association of CagA seroprevalence for H. pylori and colorectal cancers in the study

group (n=47). Of 23 patients with high stage cancers, 9 (39%) were positive for CagA seroprevalence for H. pylori and among 24 patients with low stage cancers, 9 (37.5%) were positive for CagA seroprevalence for H. pylori. Though prevalence was similar, there was no statistical significance as the p-value was 0.908.

Table XVIII shows the comparison of stage-specific prevalence of CagA seropositivity for H. pylori among H. pylori positive patients in the study group (n=31). Out of 15 patients of high stage cancers who were infected with H. pylori, 9(60%) were positive for CagA and in patients with a low stage of cancers (n=16) having H. pylori positive test, 9 (56%) were CagA positive. Calculated p-value was 0.833, which was not significant.

Table XIX shows the comparison of metastasis with CagA seroprevalence for H. pylori in the study group (n=47). A total of 7 people had metastasis with 2 (28.5%) positive for CagA serology. In patients with no metastasis, 16 (40%) had CagA positivity. No statistical significance was noted between the two groups, the p-value was 0.692.

Table XX shows the comparison of metastasis with CagA seroprevalence for H. pylori among H. pylori positive patients in the study group (n=31). Among the 4 patients who had H. pylori infection and metastasis, 2 (50%) were positive for CagA antibodies. In 27 patients without metastasis and with H. pylori positive test, 16(59.2%) patients tested for CagA serology. Calculated p-value was 1.000 which was not

significant. Figure VI shows the comparison of CagA seroprevalence for H. pylori in relation to the site, histopathological differentiation, stage and metastasis of colorectal cancers in the study group (n=47). Figure VII shows the comparison of CagA seroprevalence for H. pylori in relation to the site, histopathological differentiation, stage and metastasis of colorectal cancers in overall H. pylori positive patients of the study group (n=31).

TABLES

Table I: Comparison of age distribution in study and control groups.

Age group (years)	Study group No. (%)	Control group No. (%)
18-30	2 (4.3)	2 (4.7)
31-40	5(10.6)	4 (9.3)
41-50	9(19.1)	8(18.6)
51-60	17(36.2)	15(34.9)
61-70	9(19.1)	12 (27.9)
71-80	4 (8.5)	2 (4.7)
>80	1 (2.1)	0(0)
Total	47(100)	43 (100)
Mean age ± SD	55.02 ±14	54 ±12.8
	p-value* - 0.719	

*Unpaired T-test

SD- Standard deviation

Table II: Gender distribution in study and control groups.

Gender	No.	Study group No. (%)	Control group No. (%)	p-value*
Male	53	30 (63.8)	23 (53.5)	0.319
Female	37	17 (36.2)	20 (46.5)	

*Chi-square test

Male and female ratio: Study group-1.15:1, Control group- 1.76:1

Table III: Gender distribution in study and control groups.

Smoking status	No.	Study group No. (%)	Control group	p- value*
Smokers	24	12 (25.5)	12 (28)	0.799
Non-Smokers	66	35 (74.5)	31 (72)	

*Chi-square test

Table IV: Prevalence of stool antigen positivity, CagA seropositivity for H. pylori and overall H. pylori positivity in study and control groups.

Group	No.	Stool Antigen positive No. (%)	CagA for H. pylori positive No. (%)	Overall H. pylori positive No. (%)
Study	47	18 (38.3)	18(38.3)	31(66)
Control	43	18 (42)	9(21)	23 (53.5)

Table V: Prevalence of H.pylori in study and control groups.

Group	No.	H. pylori status		p- value*
		Positive No. (%)	Negative No. (%)	
Study group	47	31(66)	16(34)	
Control group	43	23 (53.5)	20 (46.5)	0.228

Odds ratio (OR) -1.69; 95% Confidence Interval (Cl) - 0.72 - 3.94

*Chi-square test

Table VI: Prevalence of H.pylori infection according to age distribution in study and control groups.

Age group (years)	H. pylori status	
	Study group No. (%)	Control group No. (%)
18-30	0(0)	2 (8.7)
31-40	3 (9.7)	1 (4.3)
41-50	7 (22.6)	3(13)
51-60	12(38.7)	8 (34.8)
61-70	6(19.4)	8 (34.8)
71-80	2 (6.4)	1 (4-3)
>80	1 (3.2)	0(0)
Total	31 (100)	23(100)

Table VII: Comparison of site-specific association of H.pylori infection and colorectal cancers in the study group (n=47)

Site	No.	H. pylori status		p- value*
		Positive No. (%)	Negative No. (%)	
Right colon	7	5(71.4)	2 (28.5)	1.000
Left colon and rectum	40	26 (65)	14(35)	

*Chi-square test/Fisher's exact test

Table VIII: Comparison of H.pylori infection in relation to histopathalogical differentiation in the study group (n=47)

Differentiation	No.	H. pylori status		p- value*
		Positive No. (%)	Negative No. (%)	
Well-differentiated adenocarcinoma	29	18(62)	11(38)	
Moderately-differentiated adenocarcinoma	18	13(72.2)	5(27.8)	0.475

*Chi-square test/Fisher's exact test

Table IX: Comparison of stage-specific association of H.pylori infection and colorectal cancers in the study group (n=47)

Stage	No.	H. pylori status		p- value*
		Positive No. (%)	Negative No. (%)	
High stage**	23	15(65.2)	8(34.8)	0.917
Low stage***	24	16(66.7)	8(33.3)	

*Chi-square test

**High stage – Stage III/IV

***Low stage – Stage I/II

Table X: Comparison of H.pylori infection in relation to metastasis in the study group (n=47)

Metastasis	No.	H. pylori status		p- value*
		Positive No. (%)	Negative No. (%)	
Present	7	4(57)	3(43)	0.676
Absent	40	27 (67.5)	13(32.5)	

*Chi-square test/Fisher's exact test

Table XI: Prevalence of CagA seropositivity for H.pylori in study and control groups.

Group	No.	CagA seroprevalence for H. pylori		p- value*
		Positive No. (%)	Negative No. (%)	
Study group	47	18(38.3)	29 (61.7)	0.073
Control group	43	9(21)	34(79)	

OR - 2.35: 95%-0.92-6.00

*Chi-square test

Table XII: Prevalence of CagA seropositivity for H.pylori according to age distribution in study and control groups.

Age group (years)	CagA seroprevalence for H. pylori	
	Study group	Control group
	No. (%)	No. (%)
18-30	0(0)	0(0)
31-40	2(11.1)	1 (11.1)
41-50	5 (27.8)	1 (11.1)
51-60	8 (44.4)	3 (33.3)
61-70	2 (11.1)	4 (44.4)
71-80	0(0)	0(0)
>80	1 (5.6)	0(0)
Total	18(100)	9(100)

Table XIII: Comparison of site-specific association of CagA seroprevalence for H.pylori and colorectal cancers in the study group (n=47).

Site	No.	CagA seroprevalence for H. pylori		p- value*
		Positive No. (%)	Negative No. (%)	
Right colon	7	3(43)	4(57)	1.000
Left colon and rectum	40	15(37.5)	25 (62.5)	

*Chi-square test/Fisher's exact test

Table XIV: Comparison of site-specific prevalence of CagA seroprevalence for H.pylori among overall H.pylori positive patients in the study group (n=31).

Site	No.	CagA seroprevalence for H. pylori		p- value*
		Positive No. (%)	Negative No. (%)	
Right colon	5	3(60)	2(40)	1.000
Left colon and rectum	26	15(57.6)	11 (42.4)	

*Chi-square test/Fisher's exact test

Table XV: Comparison of CagA seroprevalence for H. pylori in relation to histopathological differentiation in the study group (n=47).

Differentiation	No.	CagA seroprevalence for H. pylori		p- value*
		Positive No. (%)	Negative No. (%)	
Well-differentiated adenocarcinoma	29	14(48)	15(52)	0.074
Moderately-differentiated adenocarcinoma	18	4(22)	14(78)	

*Chi-square test/Fisher's exact test

Table XVI: Comparison of prevalence of CagA seropositivity for H. pylori in relation to histopathological differentiation among overall H. pylori positive patients in the study group (n=31).

Differentiation	No.	CagA seroprevalence for H. pylori		p- value*
		Positive No. (%)	Negative No. (%)	
Well-differentiated adenocarcinoma	18	14(77.8)	4 (22.2)	0.009
Moderately-differentiated adenocarcinoma	13	4 (30.7)	9(69.3)	

Well differentiated - OR - 5.44; 95% Cl - 1.35 - 21.89

Moderately differentiated - OR - 0.69; 95% Cl - 0.16 - 2.93

*Chi-square test/Fisher's exact test

Table XVII: Comparison of stage-specific association of CagA seroprevalence for H.pylori and colorectal in the study group (n=47).

Stage	No.	CagA seroprevalence for H. pylori		p- value*
		Positive No. (%)	Negative No. (%)	
High stage**	23	9(39)	14(61)	0.908
Low stage***	24	9 (37.5)	15(62.5)	

*Chi-square test

**High stage- Stage lll/IV

***Low stage- Stage l/ll

Table XVIII: Comparison of stage-specific association of CagA seroprevalence for H.pylori among H.pylori positive patients in the study group (n=31).

Stage	No.	CagA seroprevalence for H. pylori		p- value*
		Positive No. (%)	Negative No. (%)	
High stage**	15	9 (60)	6(40)	0.833
Low stage***	16	9(56)	7(44)	

*Chi-square test

**High stage- Stage lll/IV

***Low stage- Stage l/ll

Table XIX: Comparison of CagA seroprevalence for H.pylori in relation to metastasis in the study group (n=47).

Metastasis	No.	CagA seroprevalence for H. pylori		p- value*
		Positive No. (%)	Negative No. (%)	
Present	7	2 (28.5)	5(71.5)	0.692
Absent	40	16(40)	24 (60)	

*Chi-square test/Fisher's exact test

Table XX: Comparison of CagA seroprevalence for H.pylori in relation to metastasis among overall H.pylori positive patients in the study group (n=31).

Metastasis	No.	CagA seroprevalence for H. pylori		p- value*
		Positive No. (%)	Negative No. (%)	
Present	4	2(50)	2(50)	1.000
Absent	27	16(59.2)	11 (40.8)	

*Chi-square test/Fisher's exact test

FIGURES

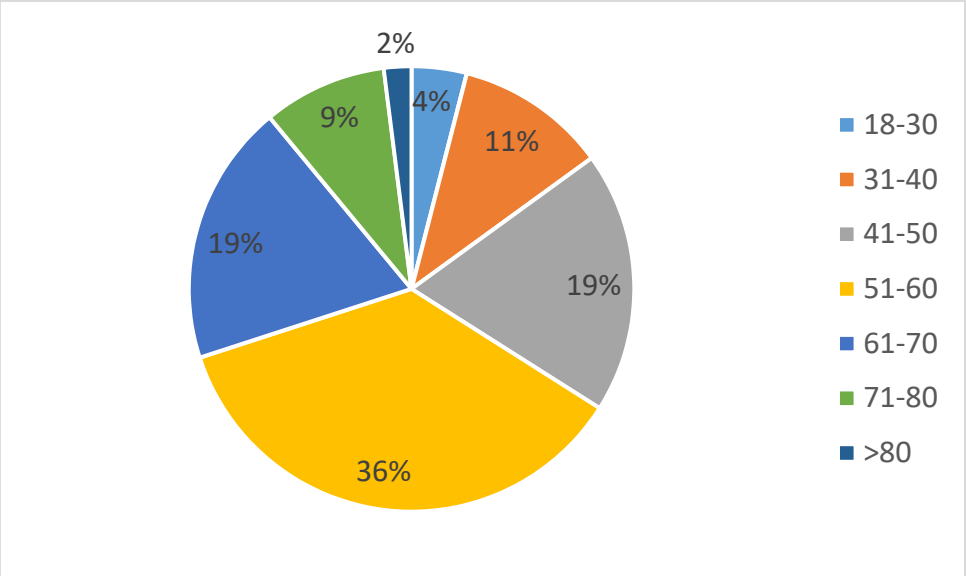

Figure I: Age distribution (in years) in the study group (n=47)

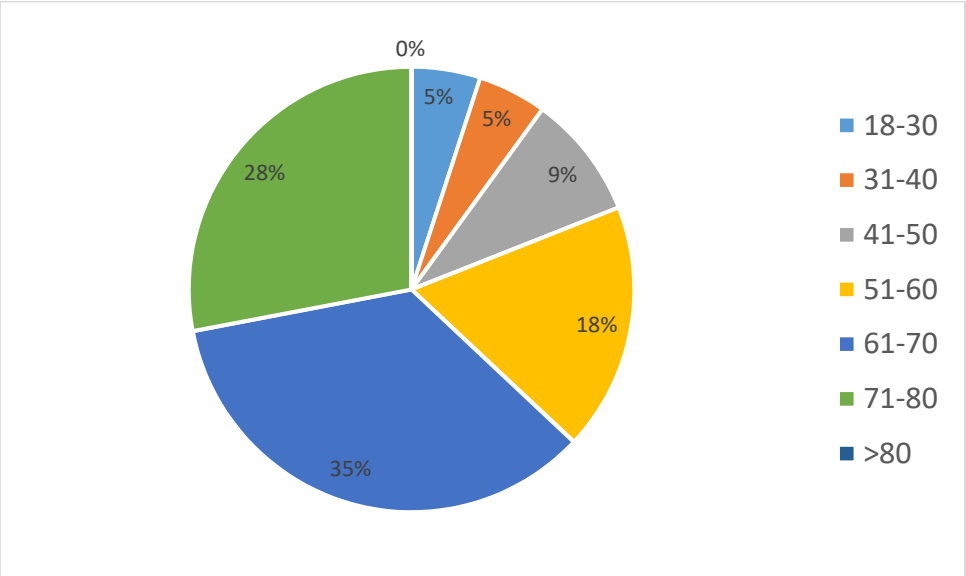

Figure II: Age distribution (in years) in the Control group (n=43)

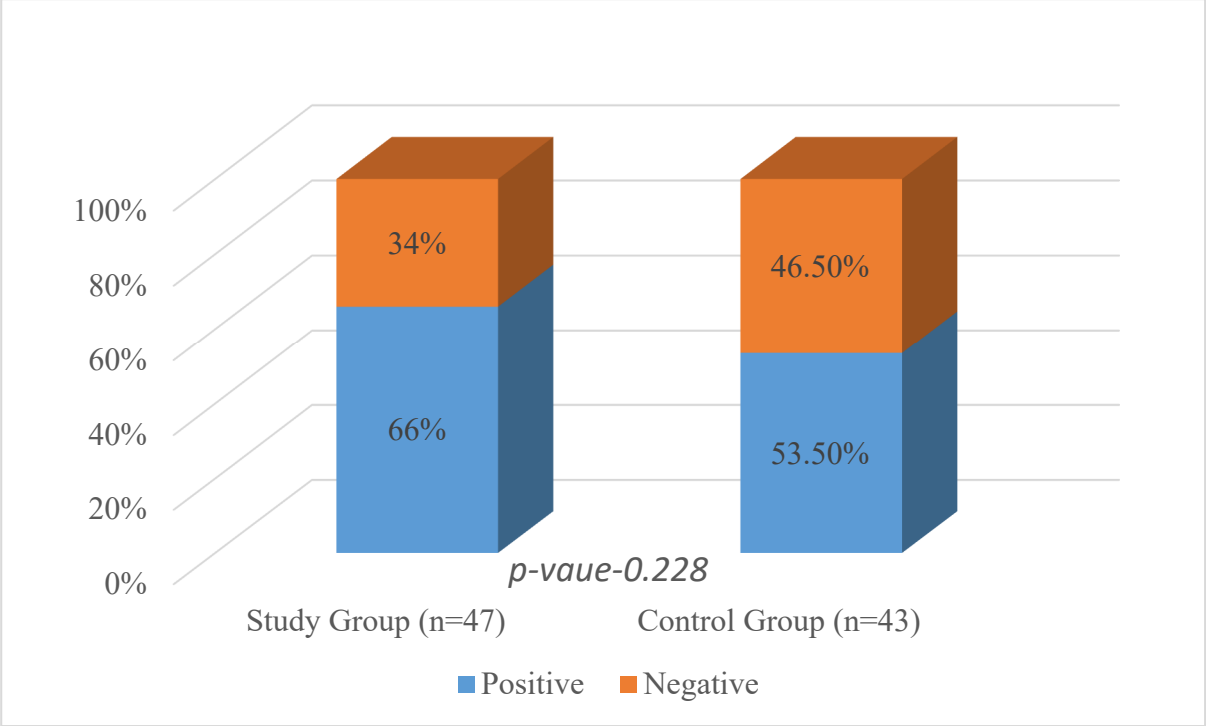

Figure III: Prevaence of H.pylori infection in study and control groups.

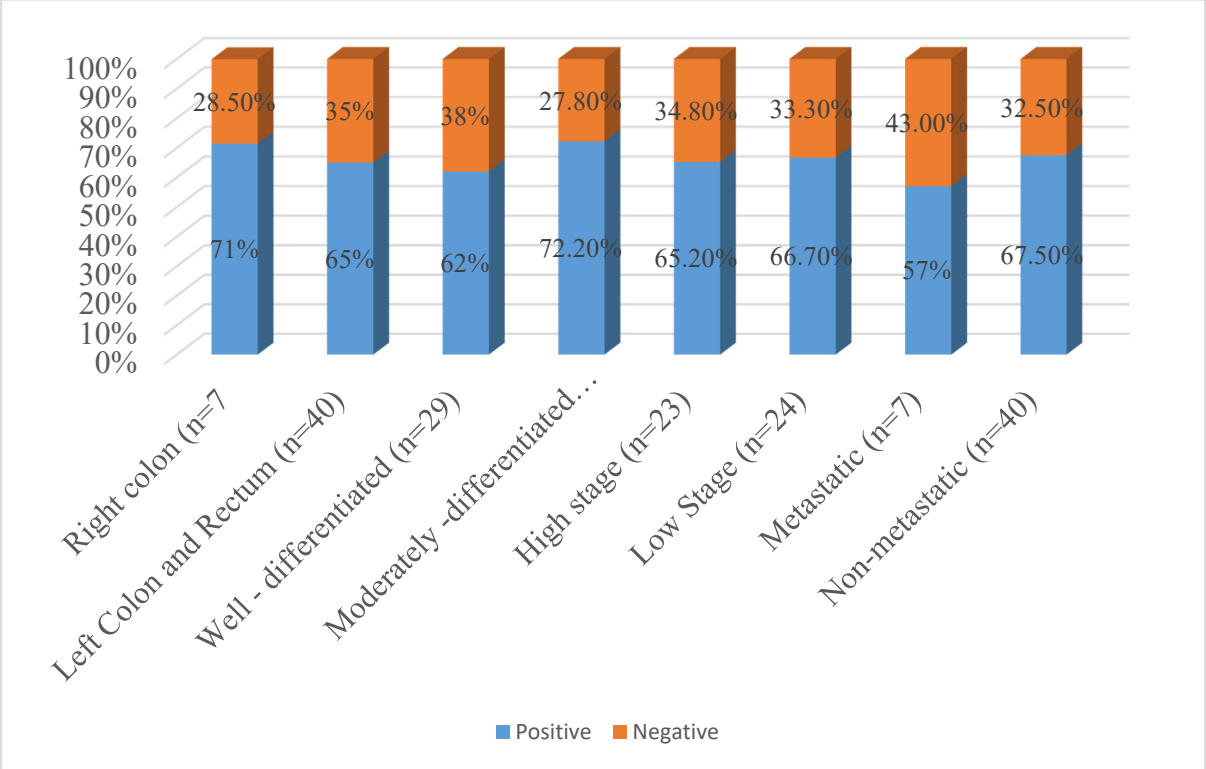

Figure IV: Comparison of H.pylori infection in relation to the site, histopathological differentiation, stage and metastasis of colorectal cancers in the study group (n=47)

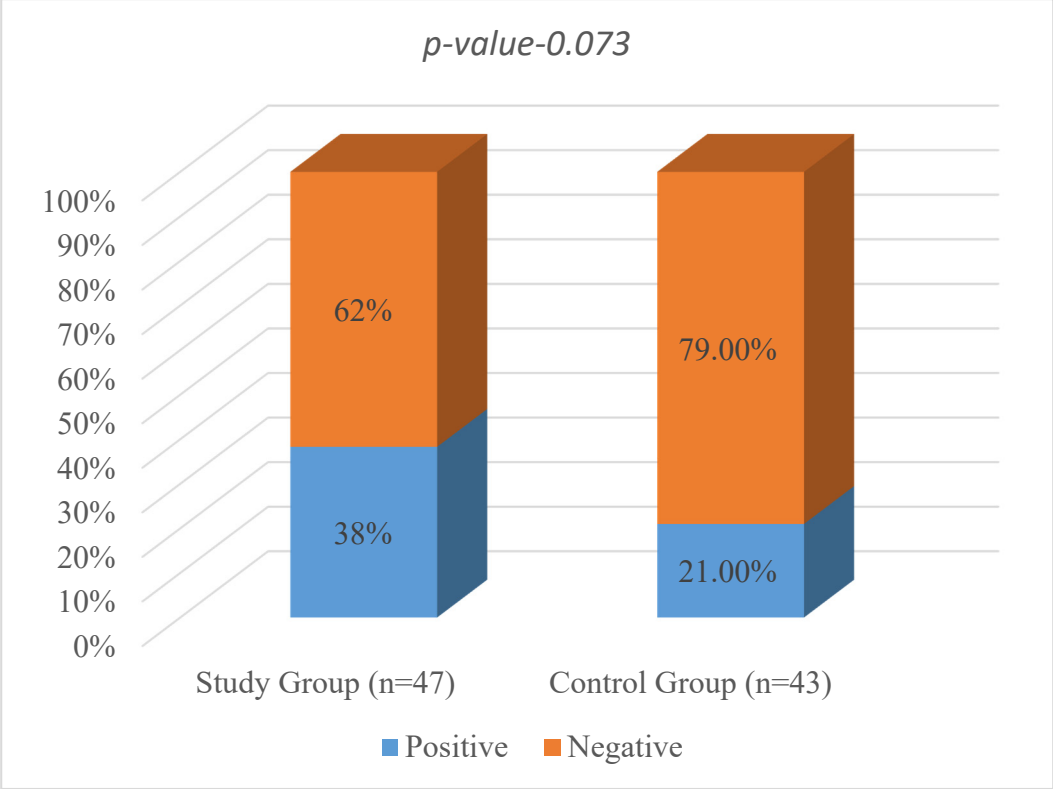

Figure V: Prevalence of CagA seroprevalence of H-pylori in study and control groups.

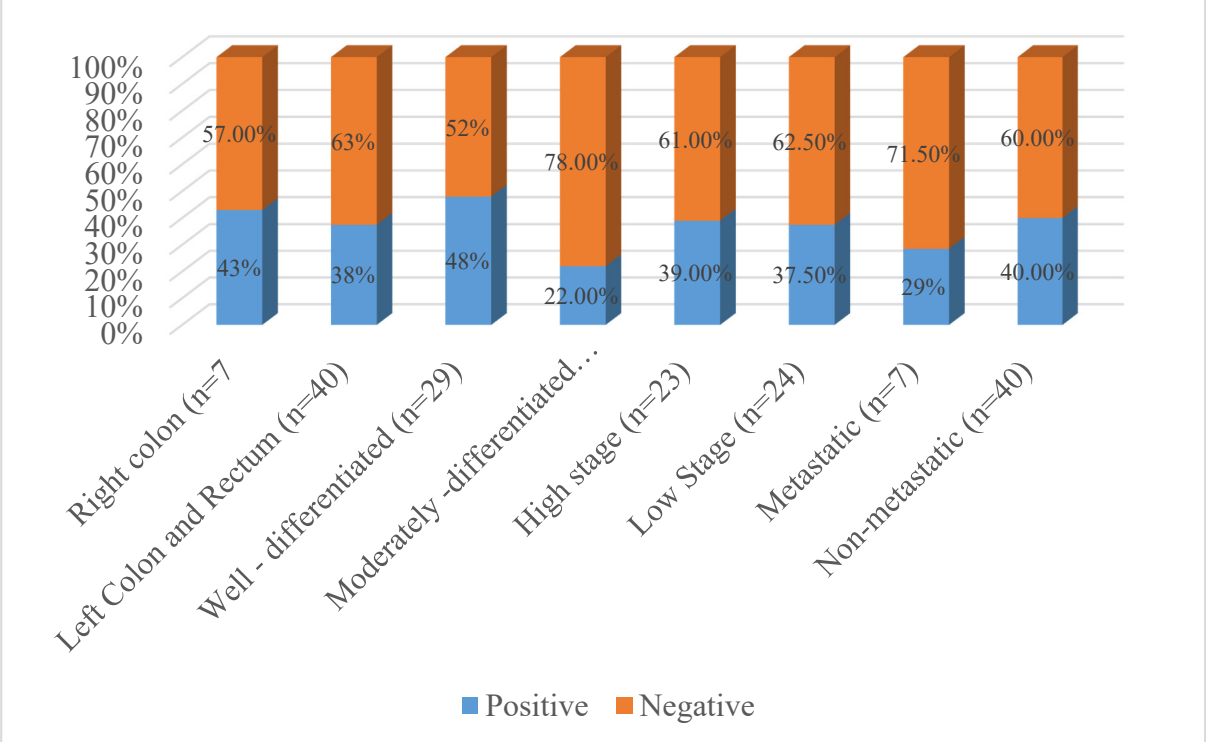

Figure VI: Comparison of CagA seroprevalence for h.pyori in relation to the site, histopathological differentiation, stage and metastasis of colorectal caners in the study group (n=47).

Figure VIII: Positive test for *H. pylori* stool antigen by Immunochromatography Assay (On-Site *H. pylori* rapid test); positive (arrow)

Figure IX: Negative test for *H. pylori* stool antigen by Immunochromatography assay (On-Site *H. pylori* rapid test).

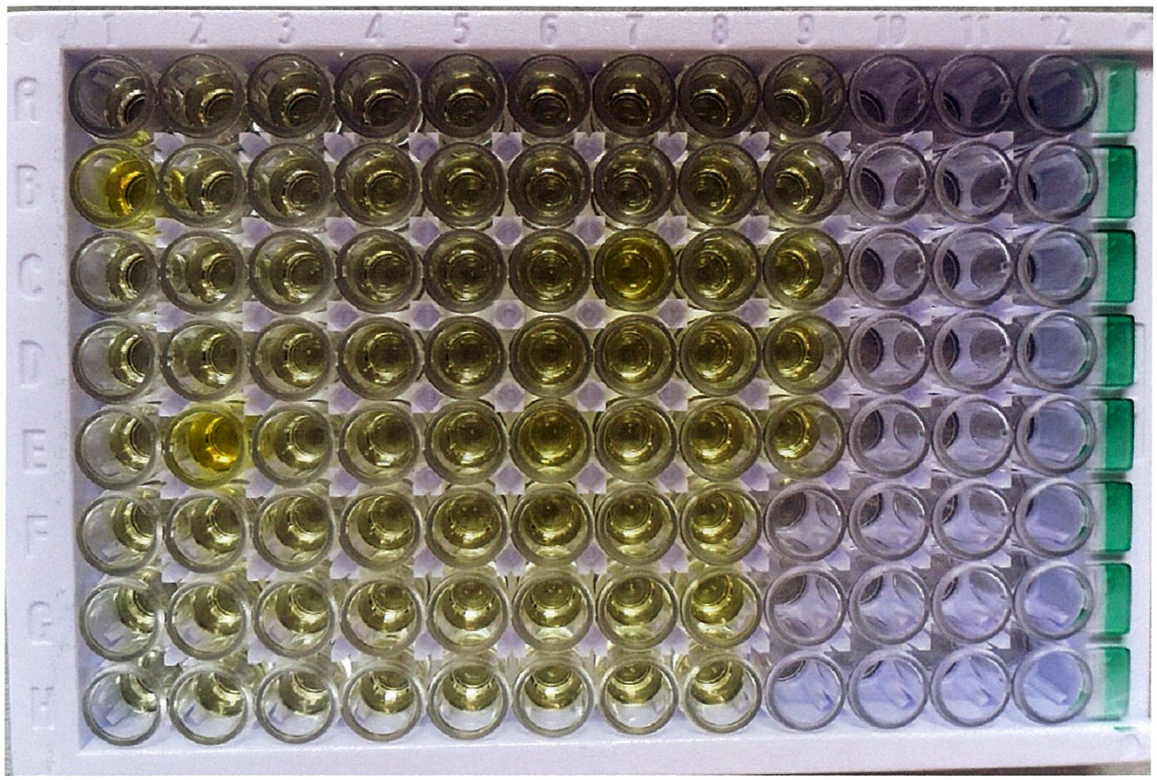

Figure X: *H. pylori* CagA ELISA kit in process (Bioassay Technology Laboratory). ELISA-Enzyme-Linked Immunosorbent Assay

DISCUSSION

DISCUSSION

H. pylori is a ubiquitous organism with a high prevalence in general population despite the geographical variations. Many recent studies were done to identify the epidemiological association of this organism with different gastric and extra-gastric diseases(74). Though some have adequate evidence to define its causative role in gastric cancer, MALToma, etc., others are still under evaluation. The clinical outcome from H. pylori infection depends on various host response factors, different strains of bacteria and environmental factors. With the recent evidence showing possible correlation with colorectal cancers, there is growing interest in studying their correlation worldwide. Colorectal cancers are one of the top five leading causes of cancer- associated mortality globally. The multifactorial aetiology for these cancers is well known and evaluated. However, further research to identify other possible risk factors which could elaborate our knowledge on the aetiology and aid in the management of colorectal cancers is needed. Many case- control, cross-sectional studies and meta-analysis were done widely in pursuit of knowing the relationship between H. pylori infection and colorectal cancers(41). Yet their association is far from any conclusion with conflicting evidence and many of the studies are not without limitations.

The present study was carried out to study the association between H. pylori infection and colorectal cancers, with special reference to CagA

strains and also its association with respect to the site, histopathological differentiation, stage and metastasis of malignancy. In the present study, age distribution, gender distribution and smoking status were similar in both groups. A higher trend of the prevalence of H. pylori positivity was identified in the study (colorectal cancer) group when compared to controls (66% vs 53.5%), however, the analysis showed no significant difference. Similarly, CagA seroprevalence for H. pylori was high in the study group (38.3% vs 21%) when compared to the control group, however, the difference was not significant. In the present study, no association was found between colorectal cancer and H. pylori infection. Also, no correlation of colorectal cancer was found with CagA strains of H. pylori. Further analysis of the study group with respect to the site, differentiation, stage and metastasis between H. pylori infection and specifically with CagA strains did not reveal positive correlation. In patients with positive H. pylori infection in the study group, a significant correlation of CagA strains of H. pylori was found with histopathological differentiation. Here a higher prevalence of these strains was observed in well-differentiated adenocarcinomas (p-value-0.009).

Even though abundant studies were available over different cohorts, they have their own limitations in providing strong and reliable evidence for the correlation. Some of those limitations include small sample size, selection bias from hospital-based sampling and inability to correct for

confounding factors. Studies done retrospectively are less reliable when compared to prospective studies. In retrospective studies, the duration of risk exposure and the lag period from risk exposure to onset of disease cannot be studied. Some studies have shown a positive correlation between the two (7-9,11,75-77) whereas others showed no association (10,71,78-84). Two studies which were based on urea breath test did not show any correlation (82,85). A Japanese study where three non-serological tests as rapid urease test, histology and urea breath test showed a positive correlation(75). Most of the studies were case-control studies and only two prospective studies were done but they were limited by their small sample size(71,84). There is a dearth of studies in Asian population particularly in India where there is a need for reliable large population-based analysis of H. pylori infection and its associations with different diseases.

The present study showed an overall H. pylori prevalence of 60%, which is comparable to the prevalence in general population (15). A prevalence of 66% in study group and 53.5% in control group was noted in the present study which is relatively higher in comparison to a large population-based case-control study conducted in Germany by Zhang et al, which showed a seroprevalence of H. pylori as 46.1% and 40.1% in cases (n=1712) and controls (n=1669). The previous report demonstrated a positive association of H. pylori with colorectal cancers (odds ratio of 1.30; p-value of 0.001) (86). A meta-analysis conducted by Zumkeller et al from

1991-2002 had a prevalence of 67%(666/997) in cases and 60%(881/1476) in controls with an odds ratio of 1.4(2). Strofilas et al conducted a prospective case- control study in Greece which showed a prevalence of 71% (66/93) in colorectal cancers and 65% in control group with no statistical significance(12). A similar association was found in the present study as well, although not significant. A meta-analysis done by Wu et al revealed a pooled OR of 1.39 and 1.42 in Western and Eastern studies(13). Limburg et al studied H. pylori with colorectal cancer risk which showed an H. pylori seroprevalence of 72% in cases and 78% in controls with an odds ratio of 0.83(71).

The present study demonstrated a CagA seroprevalence for H. pylori of 38.3% in the study and 21% in the control groups. Among the H. pylori- infected colorectal cancer patients CagA prevalence was 58%. In the control group, CagA seroprevalence was 39% among overall H. pylori-infected patients. These results show a higher prevalence in the study group and less prevalence in the control group in comparison to a study, which showed a CagA seroprevalence of 34% and 29.9% in cases and controls respectively(86), however, there was no significant difference in CagA seroprevalence in both groups. Also, CagA prevalence increased with age in both groups in this study. A study conducted by Strofilas et al demonstrated a CagA positivity of 56% in cases and 38.4% in control group with no statistical significance (12). Wu et al meta-analysis on H.

pylori and colorectal cancers showed a pooled OR of 1.37 for CagA positivity(13). The prevalence of CagA antibodies noted in a study conducted by Limburg et al was 59% in control group and 62% in cases with an odds ratio of 1.21, but with no statistical significance).

On site-specific analysis, the present study revealed that 5(71.4%) out of 7 patients with right-sided colon cancers and 26(65%) out of 40 patients with left colon and rectal cancers were positive for H. pylori. Although a higher trend was noted, the number of patients with right colon cancers was low to draw conclusions. When compared the published reports by Zhang et al which showed a prevalence of 43.9%(243/553) in right colon cancers and 47% (553/1176) in left colon and rectum cancers, the prevalence in the present study was high. The adjusted odds ratio for H. pylori in left colorectal cancers was 1.32(86). Only two studies have evaluated the site-specific association of H. pylori with colorectal cancers(71,80). As there is increasing evidence for a difference in aetiologies and behaviour of malignancy according to the site, this type of analysis is required(42,86).

In the present study, considering CagA positive strains correlation with the site of cancer, a 43% prevalence in right colon cancers and 37.5% prevalence in left colon and rectal cancers was observed. The prevalence of CagA in right colon was 60.1% (146/243) when compared to 64.2% (355/553) in left colorectal cancers, in a study where adjusted odds ratio

was 1.22 (95% CI-1.05 - 1.57) in left colorectal cancers and OR was 1.00 (95% CI-0.77 - 1.29) in right colon cancers(86). The present study showed a lower prevalence in comparison with the above study in both groups.

With respect to differentiation of histopathology H. pylori prevalence of 62% in well differentiated and 72.2% in moderately differentiated carcinomas was noted. A study conducted by Kapetanakis et al revealed a high prevalence of H. pylori infection in colorectal cancers with mild dysplasia (mild dysplasia-89% and moderate/severe dysplasia-83%) (87).

In the present study, 65.2% of high stage cancers and 66.7% of low stage cancers had H. pylori infection. A higher prevalence was observed when compared to the prevalence of 47.7% (443/929) in low stage and 44.4% (346/780) in high stage cancers, which were noted in a study with an adjusted odds ratio of 1.34 in low stage and 1.16 in high stage cancers (86). But there was no significant difference noted in the present study. Analysis for the stage was emphasized as there was evidence for association with colorectal adenomas (7,9,75). Some studies observed that gastrin causes mucosal proliferation in the colon by activating certain receptors which were found to have a role in advanced malignancy and adenoma-carcinoma sequence (8,66,88).

The present study showed the prevalence of CagA in the low and high stage as 39% and 37.5%, with no significant variation. The observed

results were less when compared to a case-control study, which showed a CagA seroprevalence of 65% (288/443) in low stage and 60.1% (208/346) in high stage cancers with an adjusted odds ratio of 1.48 and 1.16 respectively(86).

The results from the present study provide data regarding the prevalence of H. pylori infection in colorectal cancers, which can be used as a basis for further studies. As the prevalence varies in different cohorts, the present study which was carried out in a single centre, it can provide data for this region which can be compared with other regions.

Colorectal cancers have a multifactorial aetiology which needs to be studied elaborately to define the causative role of each factor. H. pylori infection is easy to diagnose and it can be eradicated with a combination of antibiotics in a short duration effectively. As H. pylori is highly prevalent in general population especially in developing country like ours, any evidence of its association with colorectal cancers would direct for its eradication in these patients, particularly in the high-risk population. H. pylori eradication is a cost- effective and acceptable method of primary prevention. This method was found to decrease the incidence of gastric malignancies(89,90). Some researchers have proposed that if any correlation is recognised, in patients with gastric cancers infected with H. pylori, surveillance by colonoscopy for colorectal cancers may be considered. However, attempts to eradicate this organism should be limited

to high-risk patients considering the high prevalence in general population because achieving complete eradication is financially demanding and difficult. Also, the long-term outcomes after eradication are not known.

In the present study, we studied overall H. pylori prevalence and also more virulent CagA strains in our centre. Two tests were used to increase the sensitivity of identifying H. pylori infection. H. pylori prevalence and CagA seroprevalence for H. pylori were compared with respect to stage, differentiation, site and metastasis in the study group. Very few studies in the past few decades included the above analysis and the present study gives a comprehensive analysis of the above. The tests used in our study are non- invasive, simple, acceptable, which can be adopted over large populations. Special emphasis was given to CagA strains as they were known to cause a greater inflammatory response, higher elevation in serum gastrin levels and are associated with increased risk of gastric carcinomas. Their role in colorectal cancers is inconclusive (2,8,10,71,80). Shmuely et al identified a 10- fold rise in risk with CagA strains.

The present study has its own limitations. The study was done in a single centre among hospital patients, which sometimes due to the limited number of patients can become a disadvantage. Although age and smoking were analysed, metabolic syndrome and other factors were not included in the study which act as confounding factors. This study being a case-control study has an inherent drawback of inability to identify a causative role in

disease pathology.

The evidence on the relationship between H. pylori infection and colorectal cancers is not as strong as that identified in relation to gastric conditions. The results are inconsistent and far from any conclusion. In this study even though a trending high prevalence was noted, no significant correlation was found. Further evaluation requires large-scale studies over a large geographical area over an adequate time period with rigorous methodology considering all confounding factors for colorectal cancers. Considering the plausible role of H. pylori in colorectal cancers preventive measures should be taken and all attempts should be made to elucidate the correlative pathology in colorectal cancers. In view of the high general prevalence of H. pylori, further prospective interventional studies with targeted treatment for high-risk patients with H. pylori infection are warranted. Further research may include risk factors as gastrin, atrophic gastritis, level of CagA antibodies and other antibodies to major virulence factors which help in identification of the mechanism of carcinogenesis and also risk stratification of patients for the decision on the time of intervention.

SUMMARY
AND
CONCLUSION

SUMMARY AND CONCLUSION

SUMMARY

The study was a prospective, single centre case-control study conducted from August 2018 to August 2019, in Department of Surgery, GRH, Madurai. It was done to evaluate the association of H. pylori and colorectal cancers by determining its prevalence in patients with colorectal cancers and comparing it with that of controls. We also examined for correlation of overall H. pylori infection and CagA strains with the site, histopathological differentiation, stage and metastasis of colorectal cancers. A total of 47 patients in study group and 43 patients in control group were taken for study after careful consideration of inclusion and exclusion criteria. These patients were tested for stool H. pylori antigen by ICT and serum CagA by ELISA and compared in both groups.

In the present study, age groups, gender distribution and smoking status were comparable between the two groups. The mean age in the study group was 55.02 ± 14 years and in the control group was 54 ±12.8 years. The male to female ratio was 1.15:1 in the study group and 1.76:1 in the control group. In study group 18 (38.3%) had stool antigen positive, 18 (38.3%) had CagA for H. pylori positive and 31 (66%) had overall H. pylori positive. In control group 18(42%) had stool antigen positive, 9 (21%) had CagA for H. pylori positive and 23 (53.5%) had overall H.

pylori positive.

The overall prevalence of H. pylori was 60% with 66% (31/47) of study patients and 53.5%(23/43) of control patients being positive. There was no significant difference between the two groups on analysis. On site-specific analysis, 71.4% (5/7) of study and 65% (26/40) of control group patients wereH. pylori infection positive. By comparing the histopathological differentiation, 62% (18/29) of well-differentiated adenocarcinoma and 72.2% (13/18) of moderately-differentiated adenocarcinoma patients had H. pylori infection. On stage-specific analysis, 65.2% (15/23) of high stage cancers and 66.7% (16/24) of low stage cancers were infected with H. pylori. With regard to metastasis, the prevalence of infection was 57% (4/7) in the metastatic group and 67.5% (27/40) in the non-metastatic group. The comparison of H. pylori infection with above parameter showed no significant variation.

In the present study the CagA seroprevalence in the study group was 38.3% (18/47) and in the control group was 21% (9/43), which was not significant on comparison. With respect to the site, a seroprevalence of 43% (3/7) in right colon cancers and 37.5% (15/40) in left colon and rectal cancers were noted. In patients with well-differentiated adenocarcinoma CagA seroprevalence was 48% (14/29) and in those with moderately-differentiated adenocarcinoma 22% (4/18) had CagA seroprevalence. On comparing stage- specific association with CagA strains of H. pylori

infection, 39% (9/23) of high stage tumours and 37.5% (9/24) of low stage tumours were CagA seropositive. In metastatic patients, a seroprevalence of 28.5% (2/7) and in non-metastatic patients seroprevalence of 40% (16/40) was observed. On comparing the above parameters for association with CagA strains of H. pylori, the correlation was not significant. However, on further analysis of CagA strains among the H. pylori positive patients in the study group with different parameters as the site, histopathological differentiation, stage and metastasis, a significant p-value of 0.009 was noted with histopathological differentiation. Of 31 patients in this group, 77.8% (14/18) with well- differentiated adenocarcinomas and 30.7% (4/13) with moderately- differentiated adenocarcinomas were CagA seropositive.

The present study showed that there was no association between H. pylori infection and colorectal cancers and also there was no association of site, histopathological differentiation, stage and presence of metastasis in the tumour with this infection. Special reference to CagA strains also showed no correlation with colorectal cancers. Except for identified risk with CagA strains of H. pylori in relation to well-differentiated adenocarcinoma among all H. pylori-infected patients, there was no association of CagA strains with the site, histopathological differentiation, stage and metastasis of disease.

CONCLUSION

Though our study did not show any correlation of H. pylori infection with colorectal cancers, it would add a small amount of evidence to the large pool of further research required to objectify the correlation between the two. A continuing effort to find the same with better-designed studies is warranted.

BIBLIOGRAPHY

BIBLIOGRAPHY

1) Inoue I, Kato J, Tamai H, Iguchi M, Maekita T, Yoshimura N, et al. Helicobacter pylori-related chronic gastritis as a risk factor for colonic neoplasms. World J Gastroenterol. 2014;20:1485-92.

2) Zumkeller N, Brenner H, Zwahlen M, Rothenbacher D. Helicobacter pylori infection and colorectal cancer risk: a meta-analysis. Helicobacter. 2006;11:75-80.

3) D'Onghia V, Leoncini R, Carli R, Santoro A, Giglioni S, Sorbellini F, et al. Circulating gastrin and ghrelin levels in patients with colorectal cancer: correlation with tumour stage, Helicobacter pylori infection and BMI. Biomed Pharmacother Biomedicine Pharmacother. 2007;61:137-41.

4) Ryberg B, Axelson J, Hakanson R, Sundler F, Mattsson H. Trophic effects of continuous infusion of [Leu15]-gastrin-17 in the rat. Gastroenterology. 1990;98:33-8.

5) Renga M, Brandi G, Paganelli GM, Calabrese C, Papa S, Tosti A, et al. Rectal cell proliferation and colon cancer risk in patients with hypergastrinemia. Gut. 1997;41:330-2.

6) Kanno T, Matsuki T, Oka M, Utsunomiya H, Inada K, Magari H, et al. Gastric acid reduction leads to an alteration in lower intestinal microflora. Biochem Biophys Res Commun. 2009;38:666-70.

7) Meucci G, Tatarella M, Vecchi M, Ranzi ML, Biguzzi E, Beccari G, et al. High prevalence of Helicobacter pylori infection in patients with colonic adenomas and carcinomas. J Clin Gastroenterol. 1997;25:605-7.

8) Hartwich A, Konturek SJ, Pierzchalski P, Zuchowicz M, Labza H, Konturek PC, et al. Helicobacter pylori infection, gastrin, cyclooxygenase- 2, and apoptosis in colorectal cancer. Int J Colorectal Dis. 2001;16:202-10.

9) Breuer-Katschinski B, Nemes K, Marr A, Rump B, Leiendecker B, Breuer N, et al. Helicobacter pylori and the risk of colonic adenomas. Colorectal Adenoma Study Group. Digestion. 1999;60:210-5.

10) Shmuely H, Melzer E, Braverman M, Domniz N, Yahav J. Helicobacter pylori infection is associated with advanced colorectal neoplasia. Scand J Gastroenterol. 2014;49:35-42.

11) Inoue I, Mukoubayashi C, Yoshimura N, Niwa T, Deguchi H, Watanabe M, et al. Elevated risk of colorectal adenoma with Helicobacter pylori- related chronic gastritis: a population-based case-control study. Int J Cancer. 2011;129:2704-11.

12) Strofilas A, Lagoudianakis EE, Seretis C, Pappas A, Koronakis N, Keramidaris D, et al. Association of Helicobacter pylori infection and colon cancer. J Clin Med Res. 2012;4:172-6.

13) Wu Q, Yang Z-P, Xu P, Gao L-C, Fan D-M. Association between Helicobacter pylori infection and the risk of colorectal neoplasia: a systematic review and meta-analysis. Colorectal Dis Off J Assoc Coloproctology G B Irel. 2013;15:e352-364.

14) WHO. IARC monographs on the evaluation of carcinogenic risks to humans: schistosomiases, liver flukes and Helicobacter pylori, vol 6, Lyon, France: IARC, 1994, 177-240.

15) Lee JY, Park HW, Choi JY, Lee J-S, Koo JE, Chung EJ, et al. Helicobacter pylori Infection with Atrophic Gastritis Is an Independent Risk Factor for Advanced Colonic Neoplasm. Gut Liver. 2016;10:902-9.

16) Peleteiro B, Bastos A, Ferro A, Lunet N. Prevalence of Helicobacter pylori Infection Worldwide: A Systematic Review of Studies with National Coverage. Dig Dis Sci. 2014;59:1698-709.

17) Ahmed N. 23 years of the discovery of Helicobacter pylori: is the debate over? Ann Clin Microbiol Antimicrob. 2005;4:17.

18) Lee JY, Kim N. Diagnosis of Helicobacter pylori by invasive test: histology. Ann Transl Med. 2015;3:10.

19) Perez-Perez Gl. Accurate diagnosis of Helicobacter pylori. Culture, including transport. Gastroenterol Clin North Am. 2000;29:879-84.

20) Hirschl AM, Makristathis A. Methods to detect Heiicobacter pylori: from culture to molecular biology. Helicobacter. 2007;12:6-11.

21) Uotani T, Graham DY. Diagnosis of Helicobacter pylori using the rapid urease test. Ann Transl Med. 2015;3:9.

22) Allahverdiyev AM, Bagirova M, Caliskan R, Tokman HB, Aliyeva H, Unal G, et al. Isolation and diagnosis of Helicobacter pylori by a new method: microcapillary culture. World J Gastroenterol. 2015;21:2622-8.

23) Boey J, Wong J, Ong GB. Bacteria and septic complications in patients with perforated duodenal ulcers. Am J Surg. 1982;143:635-9.

24) Tomtitchong P, Siribumrungwong B, Vilaichone R-K, Kasetsuwan P, Matsukura N, Chaiyakunapruk N. Systematic review and meta-analysis:

Helicobacter pylori eradication therapy after simple closure of perforated duodenal ulcer. Helicobacter. 2012;17:148-52.

25) Rimbara E, Sasatsu M, Graham DY. PCR detection of Helicobacter pylori in clinical samples. Methods Mol Biol Clifton NJ. 2013;943:279-87.

26) Fontenete S, Leite M, Cappoen D, Santos R, Ginneken CV, Figueiredo C, et al. Fluorescence In Vivo Hybridization (FIVH) for Detection of Helicobacter pylori Infection in a C57BL/6 Mouse Model. PloS One. 2016;11:e0148353.

27) Patel SK. Diagnosis of Helicobacter pylori: What should be the gold standard? World J Gastroenterol. 2014;20:12847.

28) Wang Y-K, Kuo F-C, Liu C-J, Wu M-C, Shih H-Y, Wang SS, et al. Diagnosis of Helicobacter pylori infection: Current options and developments. World J Gastroenterol WJG. 2015;21:11221-35.

29) Chey WD. Accurate diagnosis of Helicobacter pylori. 14C-urea breath test. Gastroenterol Clin North Am. 2000;29:895-902.

30) Gisbert JP, Pajares JM. Review article: 13C-urea breath test in the diagnosis of Helicobacter pylori infection - a critical review. Aliment Pharmacol Ther. 2004;20:1001-17.

31) Garza-Gonzalez E, Perez-Perez Gl, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol. 2014;20:1438-49.

32) Yang B-L, Yeh C, Kwong W-G, Lee S-D. A novel one-step Helicobacter pylori saliva antigen test. J Chin Med Assoc JCMA. 2015;78:96-100.

33) Navarro F, Coll P, Sainz S, Mirelis B, Cardenosa N, Alonso C, et al. [Evaluation of two commercial kits for detection of Helicobacter pylori specific antibodies in patients undergoing gastroscopy. A seroprevalence study in an asymptomatic population]. Enferm Infecc Microbiol Clin. 1992;10:190-4.

34) Daino DF, Soifer L, Pedesta J, Rome J. [Pilot test for Helicobacter pylori detection with ammonia breath test]. Acta Gastroenterol Latinoam. 2015;45:12-7.

35) Kearney DJ, Hubbard T, Putnam D. Breath ammonia measurement in Helicobacter pylori infection. Dig Dis Sci. 2002;47:2523-30.

36) Testerman TL. Beyond the stomach: An updated view of Helicobacter pylori pathogenesis, diagnosis, and treatment. World J Gastroenterol. 2014;20:12781.

37) Watari J. Helicobacter pylori associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. World J Gastroenterol. 2014;20:5461.

38) Franceschi F, Zuccala G, Roccarina D, Gasbarrini A. Clinical effects of Helicobacter pylori outside the stomach. Nat Rev Gastroenterol Hepatol. 2014;11:234-42.

39) Correa P, Houghton J. Carcinogenesis of Helicobacter pylori. Gastroenterology. 2007;133:659-72.

40) Amieva M, Peek RM. Pathobiology of Helicobacter py/o/7-lnduced Gastric Cancer. Gastroenterology. 2016;150:64-78.

41) Papastergiou V, Karatapanis S, Georgopoulos SD. Helicobacter pylori and colorectal neoplasia: Is there a causal link? World J Gastroenterol. 2016;22:649-58.

42) Nam JH, Hong CW, Kim BC, Shin A, Ryu KH, Park BJ, et al. Helicobacter pylori infection is an independent risk factor for colonic adenomatous neoplasms. Cancer Causes Control CCC. 2017;28:107-15.

43) Merlano MC, Granetto C, Fea E, Ricci V, Garrone O. Heterogeneity of colon cancer: from bench to bedside. ESMO Open. 2017;2:e000218.

44) Lee SE, Jo HB, Kwack WG, Jeong YJ, Yoon Y-J, Kang HW. Characteristics of and risk factors for colorectal neoplasms in young adults in a screening population. World J Gastroenterol. 2016;22:2981.

45) Jung YS, Ryu S, Chang Y, Yun KE, Park JH, Kim HJ, et al. Risk factors for colorectal neoplasia in persons aged 30 to 39 years and 40 to 49 years. Gastrointest Endosc. 2015;81:637-645.e7.

46) Gagniere J. Gut microbiota imbalance and colorectal cancer. World J Gastroenterol. 2016;22:501.

47) Stary L, Mezerova K, Skalicky P, Zboril P, Raclavsky V. Are we any closer to screening for colorectal cancer using microbial markers?A critical review. Biomed Pap [Internet] 2017[cited 2018 Jan 4]; Available from: http://biomed.papers.upol.cz/doi/10.5507/bp.2017.051 .html

48) Viljoen KS, Dakshinamurthy A, Goldberg P, Blackburn JM.

Quantitative profiling of colorectal cancer-associated bacteria reveals associations between Fusobacterium spp., enterotoxigenic Bacteroides fragilis (ETBF) and clinicopathological features of colorectal cancer. PloS One. 2015;10:e0119462.

49) Crabtree JE, Taylor JD, Wyatt Jl, Heatley RV, Shallcross TM, Tompkins DS, et al. Mucosal IgA recognition of Helicobacter pylori 120 kDa protein, peptic ulceration, and gastric pathology. Lancet Lond Engl. 1991;338:332-5.

50) Kuipers EJ, Perez-Perez Gl, Meuwissen SG, Blaser MJ. Helicobacter pylori and atrophic gastritis: importance of the CagA status. J Natl Cancer Inst. 1995;87:1777-80.

51) Yan Y, Chen Y-N, Zhao Q, Chen C, Lin C-J, Jin Y, et al. Helicobacter pylori infection with intestinal metaplasia: An independent risk factor for colorectal adenomas. World J Gastroenterol. 2017;23:1443-9.

52) Ciccotosto GD, McLeish A, Hardy KJ, Shulkes A. Expression, processing, and secretion of gastrin in patients with colorectal carcinoma. Gastroenterology. 1995; 109:1142-53.

53) Sonnenberg A, Genta RM. Helicobacter pylori is a risk factor for colonic neoplasms. Am J Gastroenterol. 2013;108:208-15.

54) Brim H, Zahaf M, Laiyemo AO, Nouraie M, Perez-Perez Gl, Smoot DT, et al. Gastric Helicobacter pylori infection associates with an increased risk of colorectal polyps in African Americans. BMC

Cancer. 2014;14:296,

55) Jones M, Helliwell P, Pritchard C, Tharakan J, Mathew J. Helicobacter pylori in colorectal neoplasms: is there an aetiological relationship? World J Surg Oncol. 12;5:51.

56) Lin Y-S, Chen M-J, Shih S-C, Bair M-J, Fang C-J, Wang H-Y. Management of Helicobacter pylori infection after gastric surgery. World J Gastroenterol. 2014;20:5274-82.

57) lacopetta B. Are there two sides to colorectal cancer? Int J Cancer. 2002;101:403-8.

58) Giovannucci E, Colditz GA, Stampfer MJ. A meta-analysis of cholecystectomy and risk of colorectal cancer. Gastroenterology. 1993;105:130-41.

59) Hodge DR, Xiao W, Clausen PA, Heidecker G, Szyf M, Farrar WL. Interleukin-6 regulation of the human DNA methyltransferase (HDNMT) gene in human erythroleukemia cells. J Biol Chem. 2001;276:39508-11.

60) Clinton SK, Bostwick DG, Olson LM, Mangian HJ, Visek WJ. Effects of ammonium acetate and sodium cholate on N-methyi-N'-nitro-N-nitrosoguanidine-induced colon carcinogenesis of rats. Cancer Res. 1988;48:3035-9.

61) Rehfeld JF, Bardram L, Hilsted L. Gastrin in human bronchogenic carcinomas: constant expression but variable processing of

progastrin. Cancer Res. 1989;49:2840-3.

62) Bardram L. Gastrin in non-neoplastic pancreatic tissue from patients with and without gastrinomas. Scand J Gastroenterol. 1990;25:935-43.

63) Nemeth J, Taylor B, Pauwels S, Varro A, Dockray GJ. Identification of progastrin derived peptides in colorectal carcinoma extracts. Gut. 1993;34:90-5.

64) Van Solinge WW, Nielsen FC, Friis-Hansen L, Falkmer UG, Rehfeld JF. Expression but incomplete maturation of progastrin in colorectal carcinomas. Gastroenterology. 1993;104:1099-107.

65) Kochman ML, DelValle J, Dickinson CJ, Boland CR. Post-translational processing of gastrin in neoplastic human colonic tissues. Biochem Biophys Res Commun. 1992;189:1165-9.

66) Smith JP, Solomon TE. Effects of gastrin, proglumide, and somatostatin on growth of human colon cancer. Gastroenterology. 1988;95:1541-8.

67) Kusyk CJ, McNiel NO, Johnson LR. Stimulation of growth of a colon cancer cell line by gastrin. Am J Physiol. 1986;251: G597-601.

68) Alonso M, Galera MJ, Reyes G, Calabuig R, Vinals A, Rius X. Effects of pentagastrin and of the somatostatin analogue (SMS 201-995) on growth of CT26 in vivo adenocarcinoma of the colon. Surg Gynecol Obstet. 1992;175:441-4.

69) Winsett OE, Townsend CM, Glass EJ, Thompson JC. Gastrin stimulates growth of colon cancer. Surgery. 1986;99:302-7.

70) Hatakeyama M, Higashi H. Helicobacter pylon CagA: a new paradigm for bacterial carcinogenesis. Cancer Sci. 2005;96:835-43.

71) Limburg PJ, Stolzenberg-Solomon RZ, Colbert LH, Perez-Perez Gl, Blaser MJ, Taylor PR, et al. Helicobacter pylori seropositivity and colorectal cancer risk: a prospective study of male smokers. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2002;11:1095-9.

72) Ji K-Y, Hu F-L. Interaction or relationship between Helicobacter pylori and non-steroidal anti-inflammatory drugs in upper gastrointestinal diseases. World J Gastroenterol. 2006;12:3789-92.

73) Sostres C, Gargallo CJ, Lanas A. Interaction between Helicobacter pylori infection, nonsteroidal anti-inflammatory drugs and/or low-dose aspirin use: old question new insights. World J Gastroenterol. 2014;20:9439-50.

74) Fujimori S, Kishida T, Kobayashi T, Sekita Y, Seo T, Nagata K, et al. Helicobacter pylori infection increases the risk of colorectal adenoma and adenocarcinoma, especially in women. J Gastroenterol. 2005;40:887-93.

75) Abbass K, Gul W, Beck G, Marked R, Akram S. Association of Helicobacter pylori infection with the development of colorectal

polyps and colorectal carcinoma. South Med J. 2011;104:473-6.

76) Talley NJ, Zinsmeister AR, Weaver A, DiMagno EP, Carpenter HA, Perez-Perez Gl, et al. Gastric adenocarcinoma and Helicobacter pylori infection. J Natl Cancer Inst. 1991;83:1734-9.

77) Mizuno S, Morita Y, Inui T, Asakawa A, Ueno N, Ando T, et al. Helicobacter pylori infection is associated with colon adenomatous polyps detected by high-resolution colonoscopy. Int J Cancer. 2005;117:1058-9.

78) Fireman Z, Trost L, Kopelman Y, Segal A, Sternberg A. Helicobacter pylori: seroprevalence and colorectal cancer. Isr Med Assoc J IMAJ. 2000;2:6-9.

79) Georgopoulos SD, Polymeros D, Triantafyllou K, Spiliadi C, Mentis A, Karamanolis DG, et al. Hypergastrinemia is associated with increased risk of distal colon adenomas. Digestion. 2006;74:42-6.

80) Machida-Montani A, Sasazuki S, Inoue M, Natsukawa S, Shaura K, Koizumi Y, et al. Atrophic gastritis, Helicobacter pylori, and colorectal cancer risk: a case-control study. Helicobacter. 2007;12:328-32.

81) Moss SF, Neugut Al, Garbowski GC, Wang S, Treat MR, Forde KA. Helicobacter pylori seroprevalence and colorectal neoplasia: evidence against an association. J Natl Cancer Inst. 1995;87:762-3.

82) Penman ID, el-Omar E, Ardill JE, McGregor JR, Galloway DJ, O'Dwyer PJ, et al. Plasma gastrin concentrations are normal in patients with colorectal neoplasia and unaltered following tumour resection. Gastroenterology. 1994; 106:1263-70.

83) Siddheshwar RK, Muhammad KB, Gray JC, Kelly SB. Seroprevalence of Helicobacter pylori in patients with colorectal polyps and colorectal carcinoma. Am J Gastroenterol. 2001;96:84-8.

84) Thorburn CM, Friedman GD, Dickinson CJ, Vogelman JH, Orentreich N, Parsonnet J. Gastrin and colorectal cancer: a prospective study. Gastroenterology. 1998; 115:275-80.

85) Liou J-M, Lin J-W, Huang S-P, Lin J-T, Wu M-S. Helicobacter pylori infection is not associated with increased risk of colorectal polyps in Taiwanese. Int J Cancer. 2006;119:1999-2000.

86) Zhang Y, Hoffmeister M, Week MN, Chang-Claude J, Brenner H. Helicobacter pylori infection and colorectal cancer risk: evidence from a large population-based case-control study in Germany. Am J Epidemiol. 2012;175:441-50.

87) Kountouras J, Kapetanakis N, Polyzos SA, Katsinelos P, Gavalas E, Tzivras D, et al. Active Helicobacter pylori Infection Is a Risk Factor for Colorectal Mucosa: Early and Advanced Colonic Neoplasm Sequence. Gut Liver. 2017;11:733-4.

88) Konturek SJ, Konturek PC, Hartwich A, Hahn EG. Helicobacter pylori infection and gastrin and cyclooxygenase expression in gastric and colorectal malignancies. Regul Pept. 2000;93:13-9.

89) Take S, Mizuno M, Ishiki K, Nagahara Y, Yoshida T, Yokota K, et al. The effect of eradicating Helicobacter pylori on the development of gastric cancer in patients with peptic ulcer disease. Am J Gastroenterol. 2005;100:1037-42.

90) Hansson LE, Nyren O, Hsing AW, Bergstrom R, Josefsson S, Chow WH, et al. The risk of stomach cancer in patients with gastric or duodenal ulcer disease. N Engl J Med. 1996;335:242-9.

ANNEXURES

CONSENT FORM

Title of the project:

Association of Helicobacter pylori infection and colorectal cancer

Participant's name: Address:

The details of the study have been provided to me in writing and explained to me in my own language. I confirm that I have understood the above study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for the scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the above study.

Signature of the participate: _____ Date: _____

Signature of the witness: _____ Date: _____

Name and address of the witness:

Signature of the investigator: _____ Date: _____

DATA COLLECTION PROFORMA

S.No.: Group - study/control

1. Name

2. Age

3. Hospital no:

4. Gender

5. Smoking status

6. Address

7. Telephone. No.

8. Clinical symptoms at presentation:

   pain abdomen/ bleeding per rectum/ altered bowel habits/ others

9. Signs:

   abdominal tenderness/ palpable lump/ others

10. Per rectal examination finding:

11. Metastasis if any:

12. Colonoscopy finding:

13. Imaging:

14. Diagnosis:

15. Treatment details: NACT / SURGERY/ Adjuvant Chemotherapy/RT

16. Intraoperative details (if applicable):

17. Post-operative biopsy report:

    a. Histopathological type:

b. Stage:

c. Lymph node metastasis:

18. H. pylori Cag A ELISA report:

19. H. pylori stool antigen report:

20. Overall H. pylori status:

KEY FOR MASTER CHART:

- Group:
  - 0- control
  - 1-study
- Gender:
  - 0- male
  - 1-female
- Smoking status:
  - 0- non-smokers
  - 1-smokers
- Side of colon:
  - 0- right colon
  - 1- left colon and rectum
- Histopathology:
  - 0- adenocarcinoma
- Differentiation:
  - 0- well-differentiated
  - 1-moderately differentiated
- Metastasis:
  - 0- metastasis absent
  - 1 - metastasis present

- Category of stage:
  - 0- low stage (stage l/ll)
  - 1-high stage (stage lll/IV)
- Stool antigen test:
  - 0- negative
  - 1-positive
- CagA test:
  - 0- negative
  - 1-positive
- Overall H. pylori infection
  - 0- negative
  - 1- positive

S.No.	Group	Age	Gender	Smoking Status	Diagnosis	Side of colon	Histopathology	Differentiation	Management	T Stage	Nodes isolated	Nodes positive	N Stage	Metastasis	M Stage	Overall Stage	Category of Stage	Stool antigen test	Serum CagA Teest	Overall H.pylori Status
1	1	58	0	0	Carcinoma rectum	1	0	0	Abdominoprineal resection	2	14	3	1b	0	0	IIIA	1	0	1	1
2	1	70	0	1	Carcinoma sigmoid	1	0	1	Sigmoid colectomy	2	37	3	1b	0	0	IIIA	1	1	0	1
3	1	58	1	0	Carcinoma rectum	1	0	0	Abdominperineal resection	2	3	0	0	0	0	I	0	0	1	1
4	1	52	0	0	Carcinoma sigmoid	1	0	0	Sigmoid colectomy	3	24	0	0	0	0	IIA	0	0	1	1
5	1	64	0	0	Carcinoma caecum	0	0	1	Total protocolectomy	3	35	3	1b	0	0	IIIB	1	1	0	1
6	1	55	1	0	Carcinoma rectum	1	0	1	Abdominoperineal resection	3	16	2	1b	0	0	IIIB	1	1	1	1
7	1	53	1	0	Carcinoma colon-sigmoid and descending colon	1	0	0	Let hemocolectomy	3	10	10	2b	1	1a	IVA	1	0	0	0
8	1	60	0	0	Carcinoma rectosigmoid junction	1	0	0	Abdominoperineal resection	2	18	0	0	0	0	I	0	0	0	0
9	1	50	1	0	Carcinoma rectosigmoid	1	0	1	Left hemicolectoy	3	20	0	0	0	0	IIA	0	1	0	1
10	1	55	1	0	Carnoma rectosigmoid junction	1	0	0	Left hemocolectomy	2	9	0	0	0	0	I	0	0	1	1
11	1	65	0	1	Carcinoma rectum	1	0	1	0	0	0	0	0	1	1a	IVA	1	0	0	0
12	1	38	1	0	Carcinoma rectum	1	0	0	Low anterior resection	2	9	1	1a	0	0	IIIA	1	0	0	0
13	1	60	1	0	Carcinoma rectum	1	0	1	Low anterior resection	2	8	1	1a	0	0	IIIA	1	1	0	1
14	1	34	0	0	Carcinoma rectum	1	0	0	0	0	0	0	0	1	1a	IVA	1	1	1	1
15	1	62	0	1	Carcinoma rectum	1	0	0	Anterior resection	2	0	0	0	0	0	I	0	0	0	0
16	1	80	0	0	Carcinoma transverse colon-hepatic flexure	0	0	1	Limited resection and anastomosis	3	10	0	0	0	0	IIA	0	0	0	0
17	1	35	0	0	Carcinoma ascending colon	0	0	1	Right hemicolectomy	3	10	0	0	0	0	IIA	0	0	0	0
18	1	65	0	1	Carcinoma rectum	1	0	0	Abdominoperineal resection	3	13	0	0	0	0	IIA	0	1	0	1
19	1	59	0	0	Carcinoma rectum	1	0	0	Low anterior resection	3	12	2	1b	0	0	IIIB	1	1	0	1
20	1	56	1	0	Carcinoma rectosigmoid junction	1	0	0	Anterior resection	2	7	1	1a	0	0	IIIA	1	0	0	0
21	1	56	0	1	Carcinoma rectum	1	0	1	Abominoperineal resection	3	6	0	0	0	0	IIA	0	0	0	0
22	1	60	0	0	Carcinoma rectum	1	0	0	Anterior resection	2	11	0	0	0	0	I	0	0	1	1
23	1	59	1	0	Carcinoma rectosigmoid junction	1	0	0	Anterior resection	2	16	2	1b	0	0	IIIA	1	0	1	1
24	1	22	1	0	Carnoma rectosigmoid	1	0	1	Anterior resection	2	10	4	2a	0	0	IIIB	1	0	0	0
25	1	75	0	1	Carcinoma rectum	1	0	1	Abominoperineal resection	3	12	1	1a	1	1a	IVA	1	1	0	1
26	1	51	0	0	Carcinoma rectum	1	0	1	Abominoperineal resection	3	10	0	0	0	0	IIA	0	0	1	1
27	1	18	0	0	Carcinoma sigmoid	1	0	0	Limited resection and anastomosis	4	0	0	0	1	1b	IVB	1	0	0	0
28	1	66	1	0	Carcinoma rectosigmoid junction	1	0	0	Anterior resection	3	15	3	1b	0	0	IIIB	1	0	0	0
29	1	72	0	1	Carcinoma rectum	1	0	0	Abominoperineal resection	2	1	0	0	0	0	I	0	0	0	0
30	1	65	0	0	Carcinoma hapatic flexure	0	0	0	Right hemiicolectomy	2	20	0	0	0	0	I	0	0	1	1
31	1	33	0	0	Carcinoma caecum	0	0	1	0	0	0	0	0	1	1b	IVB	1	0	1	1
32	1	55	1	0	Carcinoma rectum	1	0	0	Low anterior resectino	3	12	0	0	0	0	IIIB	0	1	0	1
33	1	44	1	0	Carcinoma sigmoid	1	0	0	Sigmoid colectomy	4	4	0	0	0	0	I	0	0	0	0
34	1	70	1	0	Carcinoma rectosigmoid junction	1	0	0	Anterior resection	2	6	0	0	0	0	IIA	0	1	0	1
35	1	37	1	0	Carcinoma rectosigmoid junction	1	0	1	Anterior resection	3	11	0	0	0	0	IIB	0	1	0	1
36	1	60	0	1	Carcinoma rectosigmoid	1	0	0	Anterior resection	2	0	0	0	0	0	I	0	0	0	0
37	1	50	0	0	Carcinoma rectum	1	0	0	Abominoperineal resection	2	8	2	1b	0	0	IIIA	1	0	0	0
38	1	50	0	1	Carcinoma rectum	1	0	0	Abominoperineal resection	3	6	0	0	0	0	IIA	0	1	1	1
39	1	51	0	0	Carcinoma splenic flexure	1	0	1	Left extended hemocolectoy	3	3	0	0	0	0	IIA	0	1	0	1
40	1	82	0	1	Carcinoma splenic flexure	0	0	0	Right extended hemicolectomy	2	21	5	2a	0	0	IIIB	1	0	1	1
41	1	47	0	1	Carcinoma sigmoid	1	0	1	Left extended hemocolctomy	3	0	0	0	0	0	IIA	0	0	1	1
42	1	42	0	0	Carcinoma ascending colon	1	0	0	Left extended hemocolctomy	3	9	2	1b	0	0	IIIB	1	1	1	1
43	1	67	1	0	Carcinoma sigmoid	1	0	0	Sigmoid colectomy	3	12	2	1b	0	0	IIIB	1	0	1	1
44	1	48	1	0	Carcinoma ascending colon	0	0	1	Right hemicolectomy	2	16	0	0	1	1b	IVB	1	1	0	1
45	1	50	0	0	Carcinoma rectosigmoid	1	0	0	Anterior resection	3	12	0	0	0	0	IIA	0	1	1	1
46	1	47	0	0	Carcinoma rectum	1	0	0	Abominoperineal resection	2	10	1	1a	0	0	IIIA	1	0	1	1
47	1	80	0	1	Carcinoma rectum	1	0	1	Abominoperineal resection	3	4	0	0	0	0	IIA	0	1	0	1

S.No.	Group	Age	Gender	Smoking Status	Diagnosis	Stool antigen test	Serum CagA test	Overall H.pylari status
48	0	60	1	0	Incisional hernia	0	1	1
49	0	52	1	0	Umbilical Hernia	1	0	1
50	0	69	0	0	Right Inguinal hernia	1	0	1
51	0	54	0	0	Bilateral inguinal hernia	0	0	0
52	0	49	0	1	Right Inguinal hernia	0	0	0
53	0	66	0	0	Recurrent left inguinal hernia	1	0	1
54	0	55	0	1	Bilateral inguinal hernia	0	1	1
55	0	50	1	0	Incisional hernia	1	1	1
56	0	53	1	0	Right inguinal hernia	0	0	0
57	0	64	0	1	Right inguinal hernia	0	0	0
58	0	37	0	0	Left inguinal hernia	1	1	1
59	0	64	0	1	Right inguinal hernia	1	0	1
60	0	66	1	0	Incisional hernia	1	1	1
61	0	57	0	0	Left inguinal hernia	1	0	1
62	0	53	1	0	Paraumbilical hernia	0	1	1
63	0	65	1	0	Incisional hernia	0	0	0
64	0	64	0	1	Right inguinal hernia	0	1	1
65	0	57	0	1	Right inguinal hernia	1	0	1
66	0	70	0	1	Right inguinal hernia	0	1	1
67	0	72	0	0	Bilateral inguinal hernia	0	0	0
68	0	57	1	0	Incisional hernia	1	0	1
69	0	59	1	0	Incisional hernia	0	0	0
70	0	52	1	0	Incisional hernia	0	0	0
71	0	23	0	1	Right inguinal hernia	1	0	1
72	0	60	0	0	Bilateral inguinal hernia	1	0	1
73	0	50	0	0	Right inguinal hernia	1	0	1
74	0	54	1	0	Incisional hernia	0	0	0
75	0	49	1	0	Umbilical hernia	0	0	0
76	0	64	1	0	Incisional hernia	1	0	1
77	0	50	1	0	Paraumbilical hernia	0	0	0
78	0	52	1	0	Incisional hernia	0	0	0
79	0	63	1	0	Incisional hernia	0	0	0
80	0	70	1	0	Right inguinal hernia	1	1	1
81	0	18	0	0	Right inguinal hernia	1	0	1
82	0	61	1	1	Incisional hernia	0	0	0
83	0	49	1	0	Incisional hernia	0	0	0
84	0	32	1	0	Incisional hernia	0	0	0
85	0	58	0	0	Left inguinal hernia	0	0	0
86	0	36	0	1	Left inguinal hernia	0	0	0
87	0	41	0	1	Bilateral inguinal hernia	1	0	1
88	0	36	0	1	Right inguinal hernia	0	0	0
89	0	76	0	0	Right inguinal hernia	1	0	1
90	0	38	0	0	Left inguinal hernia	0	0	0

ETHICAL COMMITTEE CERTIFICATE

MADURAI MEDICAL COLLEGE
MADURAI, TAMILNADU, INDIA -625 020
(Affiliated to The Tamilnadu Dr.MGR Medical University, Chennai, Tamil Nadu)

Prof Dr V Nagaraajan MD MNAMS DM (Neuro) DSc.,(Neurosciences) DSc (Hons)
Professor
Emeritus in Neurosciences, Tamil Nadu Govt Dr MGR Medical University
Chairman, IEC

Dr.K.Raadhika, MD.,
Member Secretary,
Asso.Professor of Pharmacology, Madurai Medical College, Madurai.

Members

1. Dr.C.Anitha Mohan, MD, Asso.Professor of Physiology & Vice Principal, Madurai Medical College

2. Dr.P.Raja, MCh., Urology, Medical Superintendent Govt. Rajaji Hospital, Madurai

3.Dr.R.Balajinathan MD., (General Medicine) Professor & HOD of Medicine, Madurai Medical & Govt. Rajaji Hospital, College, Madurai.

4.Dr.P.Amutha, MS., (General Surgery) Professor & H.O.D Madurai Medical College & Govt. Rajaji Hospital, Madurai.

5.Dr.N.Sharmila thilagavathi, MD., Professor of Pathology, Madurai Medical College, Madurai

6.Mrs.Mercy Immaculate Rubalatha, M.A., B.Ed., Social worker, Gandhi Nagar, Madurai

7.Thiru.Pala.Ramasamy, B.A.,B.L., Advocate, Palam Station Road, Sellur.

8.Thiru.P.K.M.Chelliah, B.A., Businessman,21, Jawahar Street, Gandhi Nagar, Madurai.

ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.T.S.Pushparaj
Designation	:	PG in MS., General Surgery
Course of Study	:	2017- 2020
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	A prospective study on Association of Helicobacter Pylori infection in colorectal cancer
Ethical Committee as on	:	08.04.2019

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary Chairman Dean/Convenor

Prof Dr V Nagaraajan
M.D., MNAMS, D.M., Dsc.,(Neuro), Dsc.(Hon)
CHAIRMAN
IEC - Madurai Medical College
Madurai

DEAN
Madurai Medical College
Madurai-20

மதுரை மருத்துவக் கல்லூரி
11 APR 2019
மதுரை - 625020

PLAGIARISM VERIFICATION CERTIFICATE

URKUND

Urkund Analysis Result

Analysed Document: Dr Pushparaj Madurai Medical College- Association of H Pylori Infection and Colorectal Cancer.docx (D57244320)
Submitted: 18/10/2019 14:56:00
Submitted By: drtsp690@gmail.com
Significance: 3 %

Sources included in the report:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6687094/
https://academic.oup.com/aje/article/175/5/441/175411
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5593337/
https://www.hindawi.com/journals/grp/2018/8319481/
https://www.karger.com/Article/FullText/7661?id=pmid:2914535
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1885433/
https://www.researchgate.net/publication/292575115_Elevated_risk_of_colorectal_adenoma_with_Helicobacter_pylori_related_chronic_gastritis_a_population-based_case-control_study
b52dd3f3-5007-43fb-a1b9-820608dd0b7e
c63ed6b4-f1ce-4815-af55-32d7de7499b4
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4716066/
https://www.researchgate.net/publication/290445276_Helicobacter_pylori_and_colorectal_neoplasia_Is_there_a_causal_link
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3925856/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6322532/

Instances where selected sources appear:

19

CERTIFICATE II

This is to certify that this dissertation work titled , entitled "**Prospective study on Association of Helicobacter pylori infection in Colorectal Cancer** " submitted by **Dr.PUSHPARAJ T.S** with Registration number 221711121 for the award of MASTER DEGREE in the branch of GENERAL SURGERY has been personally verified by me in urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows 3 percentage of plagiarism in the dissertation.

Guide and supervisor
sign with seal