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ASSOCIATION OF HELICOBACTER PYLORI INFECTION IN COLORECTAL CANCER-A PROSPECTIVE STUDY

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ABSTRACT :

Background : 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. . 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.

Methods: The study was conducted in the Department of Surgery sree mookambikai institute if medical sciences from August 2023 to August 2025. An informed consent was obtained from all participants included in the study.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,sree mookambikai institute.

Results: 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.

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.

Keywords: Colorectal cancers, Helicobacter pylori

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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 thedevelopment 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.

AIM AND OBJECTIVES OF THE STUDY:

- To evaluate the association of H. pylori infection and colorectal cancers.
- To determine the prevalence of Helicobacter pylori infection in patients with colorectal cancers

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and compare with controls.

- 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.

MATERIALS AND METHODS:

The study was conducted in the Department of Surgery sree mookambikai institute if medical sciences from August 2023 to August 2025. An informed consent was obtained from all participants included in the study.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,sree mookambikai institute. The control group included age and gender-matched patients undergoing groin hernia repair (males) and treatment for extra abdominal benign conditions (females) The following patients were excluded from the study:Patients receiving gastric anti-secretory medications and NSAIDs on a long-term basis.History of previous gastro-duodenal surgery.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).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.

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.

The results in the two groups were analysed for statistical significance.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.Five ml of fasting blood sample was collected and immediately transported to Microbiology department. The

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

Two drops of solution were dispensed in the well of cassette,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.

Statistical analysis was done using the statistical package for social sciences (SPSS).Different statistical methods were used as appropriate. Mean ± SD was determined for quantitative data and frequency for categorical variables. The independent t- test was performed on all continuous variables. The normal distribution data was checked before any t-test. The Chi-Square test was used to analyze group difference for categorical variables. A p- value < 0.05 was considered significant

RESULTS:

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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)

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)	

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Control group	43	23 (53.5)	20 (46.5)	0.228

COMPARISON OF H.PYLORI INFECTION IN RELATION TO HISTOPATHALOGICAL DIFFERENTIATION IN THE STUDY GROUP (N=47)

		H. pylori status		
Differentiation	**No.**	**Positive No. (%)**	**Negative No. (%)**	**p- value***
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

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

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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.

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

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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).

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 andhigh 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

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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 beconsidered. 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.

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

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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.

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.

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