IDENTIFICATION OF HELICOBACTER PYLORI AND ITS CONNECTION WITH ORAL CANCER IN THE ORAL CAVITY: A CROSS SECTIONAL STUDY

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ABSTRACT: Helicobacter pylori, group I carcininogen bacteria. The possible role of this organism in oral cavity has been studied in this study. We adopted Polymerase Chain Reaction to identify the organism in subjects with gingivitis, periodontitis, recurring oral aphthae, oral cancer and gastric cancer and compared with control group. The virulence of this organism in oral cavity has been detected by ELISA by identifying proteins such as CAG A and VAC A. For statistically important (p value 0.00001) in prevalence of Helicobacter pylori in the diseased groups it is compared with the control community. Helicobacter pylori positive saliva appears to be methodologically important (p value 0.00001) for comparative analysis of all virulent proteins distribution in the control community of the diseased population. 24 subjects which is showed in the existence of Helicobacter pylori, 15 had CAG A protein, 19 had VAC A protein and nine had all the virulent proteins. All the three subjects were useless but an organism’s existence was demonstrated by all of them out of which two showed positivity for all proteins. These three subjects have seen the absence of HPV 16 and 18. With nicotine habits and the presence of organism substantial findings were found in patients. For Helicobacter pylori Rima Oris can serve as a niche. The oral cavity is likely to be high due to the similarities between the oral mucosa and the gastric mucosa for the propensity of CAG A and VAC A proteins which is to be pathogenic.

Keywords Helicobacter pylori, inflammatory disorders, oral cancer, PCR, ELISA.

INTRODUCTION
Indian population constitutes about one third of total world oral cancer [1]. According to National Institute of Cancer Prevention and Research (NICPR), in India around 2.5 million people are estimated to live with the disease. In 1994, Helicobacter pylori was declared as Group I carcinogen [2]. The proteins which assess the virulence are formed by the Helicobacter pylori for CAG A and VAC A proteins. Recently it has been discovered that CAG A induces a cytotoxic reaction and have p53 mutations in oncoprotein. VAC A causes cell death and results in the production of ulcers by motivating the organism to colonise tissues. [3] The modification of host immune response was supposed to be done by the pathogenesis of the gastric tumour of this organism. The same pathogenesis may have been inherited by the oral cavity carecinoma of this organism. The one who are highly affected by
this organism are people living in unsanitary habitats, back country, thin populated areas and political as well as socially developing regions. In the oral cavity Sub-gingival plaque acts as a niche for many bacteria. For enabling the production of Helicobacter pylori these regions serve as a positive space for certain gram negative micro organisms that are micro aerophilic and anaerobic. In establishing it as a reservoir for this organism recently oral cavity is gaining more importance.

The aim of this research was to classify Helicobacter pylori in the sub gingival plaque in subjects with poor oral hygiene, Inflammatory condition such as gingivitis, periodontitis, ulcerative condition (without oral cancer)- Group A, with oral cancer- Group B, with gastric Cancer-Group C, without any lesions and without any related habits- Group D (control group) by PCR and the presence of polymerase chain reaction to detect (PCR) virulent organism by estimating cag A and vac A protein in saliva of positive subjects by Enzyme Linked Immuno Sorbent Assay (ELISA). As the oral mucosa resembles gastric mucosa the possibility of such organism to cause oral cancer is evaluated in this study.

MATERIALS AND METHODOLOGY
Case history of all the patients, which included age, gender, educational qualification, occupation, history of habits, medical history, oral hygiene status was analysed using OHI-S, intraoral and extra oral examination were taken. Based on their disease status they were divided into four groups. From each patient paired sub gingival samples containing plaque and saliva were collected. Separate Eppendorf tubes containing 0.5 ml of (PBS) 1X phosphate buffered saline solution were put in the plaque samples. Unstimulated whole saliva was collected from the patient by asking the patient to swallow the residual saliva; the saliva was allowed to accumulate for 5 minutes and expectorated in a sterile container. The saliva samples were pipetted into the Eppendorf tubes centrifuged and the supernatant was stored. The subgingival plaque samples and saliva samples were stored in -40˚c until used.

The eligibility criteria for all the subjects included age group of 30-60 years, absence of any systemic diseases and immune compromised diseases, should not have undergone antibiotic therapy for about three months, with or without tobacco habits. DNA extraction was done by commercial DNA isolation kit. To classify the organism, Helicobacter pylorii, a Real-time polymerase chain reaction in quantitative terms (q-RT-PCR) was carried out. In the present study, primer 16srRNA was selected by the method of Damla Aksit Bicak et al 2017. For annealing and extension at 60 c for 60 s, the PCR was designed to run 45 cycles with 95 c for 15 s. Enzyme Linked Immuno-Sorbent Assay (ELISA) was used to analyse the presence of virulent proteins cag A and vac A in saliva of positive patients. Positive controls were used in PCR and ELISA. The result obtained was statistically analysed using SPSS version 16.

RESULTS
Identification of Helicobacter Pylori in Different Groups
Identification of Helicobacter pylori in each group was done. 90% of organism was found in group C gastric carcinoma, 80% in group B oral cancer, 76.6% in group A inflammatory condition. An overall of 82.2% of prevalence of Helicobacter pylori was found in disease group (Table 1). This shows that there is increased evidence of organism in subjects with poor oral hygiene and with inflammatory diseases such as gingivitis and periodontitis.
Table 1: Number of subjects showing presence of Helicobacter pylori in each group

| Subjects | Presence of Helicobacter Pylori (n=30) | Percentage of Presence of Organism in Each Group |
|----------|----------------------------------------|-----------------------------------------------|
| GROUP A  | 23                                     | 76.6%                                         |
| GROUP B  | 24                                     | 80%                                           |
| GROUP C  | 27                                     | 90%                                           |
| GROUP D  | 1                                      | 3.3%                                          |

Statistical comparison of Helicobacter pylori in study group vs the control group

Helicobacter pylori in inflammatory, oral cancer and gastric cancer groups were found to be statistically significant on comparison with control group with a p value of 0.00001 (Table 2). Among study groups the organism was found to be 30.7%, 32.0% and 36.0% in group A, B, C respectively.

Table 2: Test of significance for test group vs control group

| Test of Significance | Value  | df | Asymp. Sig. (2-sided) |
|---------------------|--------|----|-----------------------|
| Pearson Chi-Square  | 60.978a| 3  | .000                  |
| Likelihood Ratio    | 67.881 | 3  | .000                  |
| Linear-by-Linear Association | 27.989 | 1   | .000                  |
| N of Valid Cases    | 120    |    |                       |

OHI and Presence of organism

As the Oral Hygiene Index (OHI) gets poorer, there is increase in evidence of the organism. Statistical significance was observed between poor OHI and presence of Helicobacter pylori.

Fig 1. Graphical representation of OHI in all groups

Presence of Helicobacter pylori& virulent protein (CAG A and VAC A)

Presence of virulent proteins (cag A and vac A) in each group was calculated and tabulated. Overall, 56.0% of both virulent proteins were found to be in diseased groups. Comparative analysis of the presence of both the virulent proteins in the saliva of the Helicobacter pylori positive individuals in diseased group with control group shows statistically significant p value as 0.00001 (table 4).
Table 3: Virulent proteins in each group

| Group   | Presence of *Helicobacter pylori* | Presence of CAG A in positive individuals | Presence of VAC A in positive individuals | Presence of both (CAG A and VAC A) in positive individuals |
|---------|-----------------------------------|-------------------------------------------|------------------------------------------|---------------------------------------------------|
| GROUP A | 23                                | 15                                        | 18                                       | 13                                                |
| GROUP B | 24                                | 15                                        | 19                                       | 9                                                 |
| GROUP C | 27                                | 23                                        | 25                                       | 21                                                |
| GROUP D | 1                                 | 0                                         | 0                                        | 0                                                 |

Table 4: Test of significance for presence of virulent proteins

|                          | Value     | df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|--------------------------|-----------|----|-----------------------|----------------------|----------------------|
| Pearson Chi-Square       | 38.769a   | 1  | .000                  |                      |                      |
| Continuity Correctionb   | 36.347    | 1  | .000                  |                      |                      |
| Likelihood Ratio         | 52.498    | 1  | .000                  |                      |                      |
| Fisher's Exact Test      |           |    | .000                  | .000                 |                      |
| Linear-by-Linear Association | 38.446   | 1  | .000                  |                      |                      |
| N of Valid Cases         | 120       |    |                       |                      |                      |

Oral Cancer with *Helicobacter pylori* with cag A & vac A
In group B with oral cancer n=30, 24 subjects were detected with *Helicobacter pylori*, of which 15 had cag A protein, 19 had vac A protein and 9 had both the virulent proteins and 91.6% of individuals had gastritis/gastric ulcer (Fig 2).

Oral cancer without habits
Three subjects were without habit but all of them showed presence of organism out of which two of them showed positivity for both the proteins. Statistical analysis did not show a significant p value. (Table 5).
Table 5: Test of significance for subjects without habits

| Test                      | Value  | Df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---------------------------|--------|----|-----------------------|----------------------|----------------------|
| Pearson Chi-Square        | 2.134a | 1  | .144                  |                      |                      |
| Continuity Correctionb    | .635   | 1  | .426                  |                      |                      |
| Likelihood Ratio          | 1.930  | 1  | .165                  |                      | .207                 |
| Fisher's Exact Test       |        |    |                       | .207                 | .207                 |
| Linear-by-Linear Association | 2.063 | 1  | .151                  |                      |                      |
| N of Valid Casesb         | 30     |    |                       |                      |                      |

Habits Vs Helicobacter Pylori
On comparing presence of Helicobacter pylori and habits, 46.1% of subjects showed positivity for virulent proteins with history of habits. While 15.9% of subjects showed presence of both the virulent proteins in absence of habits. Statistical analysis shows that more virulent organism was present in subjects with habits than without habits with a significance of p value of 0.001.

DISCUSSION
Many scholarly works have been done to identify Helicobacter pylori in oral cavity. The purpose of this study was to identify the Helicobacter pylori organism in the oral cavity and to quantify the toxic proteins produced by the organism (cag A and vac A and their interaction with oral carcinoma in the positive subjects’ saliva. Oral-oral route and oral faecal route of transmission, has been accepted as the common route of transmission of this organism [4]. Oral cavity is believed to be the possible extra gastric reservoir for this organism [5]. Helicobacter pylori tend to flourish in microaerophilic region such as sub gingival plaque and endodontically infected root canals [6,7]. If Helicobacter pylori can persist in oral cavity, the possibility of the organism to cause gastritis or reinfection is high [8], as well, the possibility of the organism to cause inflammation of the gingivae thus compromising periodontal health also seems to be high.
In this study we were able to identify the organism in the oral cavity of diseased group and positive correlation between presence of the organism and poor periodontal health was identified [9], added, those patients with inflammatory conditions such as gingivitis, periodontitis also showed positivity for virulent protein CAG A secreted by this organism, which has been accepted as a potent inducer of inflammation in gastritis as well proved to cause mutation of p53[10]. But dental treatments such as scaling will kill these microorganisms and their fate during antibiotic use is uncertain for other diseases. Many experiments have shown unfavourable reports in the detection of these organisms [11] in the oral cavity, the cause could be due to the inadequate collection of the samples, the volume of sample obtained, its storing and handling, the bad environment, the vulnerability to technique and the accuracy of the test, the selection of subjects.
Although there are many studies relating Helicobacter pylori and oral diseases [12], the possibility of this organism to cause oral diseases such as gingivitis, periodontitis, recurrent aphthous ulcer or its role in oral cancer is still an enigma. Several experiments have found that various species are found in the oral cavity and gastric cavity, debates are running to show that organism in oral cavity are just a part of regurgitation rather than, they harbour in oral cavity [13], yet studies also showed both strains to be the same, substantiating that mouth could harbour Helicobacter pylori naturally[14].
One of the aetiologies of oral cancer is chronic inflammation, several authors researched the role of Helicobacter pylori in oral cancer [15,16], still the organism’s role can neither be accepted nor be denied completely. We tried to identify the oncoproteins cag A and vac A in saliva of those *Helicobacter pylori* positive oral cancer patients, by ELISA. Many theories have been put forth to show mutation of p53 and pRB by cag A and vac A[17], which results in chronic inflammation of gastric mucosa and ends up in gastric cancer. Thereby identification of these proteins in oral cavity seems to be a major breakthrough. The possibility of these oncoproteins to cause chronic inflammation in oral mucosa similar to gastric mucosa seems to be high.

Smoking by itself has been accepted as a sole aetiology of oral cancer. Role of *Helicobacter pylori* as a cofactor in causing oral cancer has not been researched widely. Studies have shown that smoking could increase the growth and virulence of this organism in gastric cavity [18], similarly, a clear correlation between smoking and Helicobacter pylori was found in this study.

Changing trend in demographic details of the oral cancer patients was also observed in this study which has been established by many other studies [19]. In addition, three female participants with a negative history of cigarette habits, two of them in the fourth decade, one well-differentiated tongue squamous cell carcinoma and two well-differentiated buccal mucosal squamous cell carcinoma with gingivo-buccal sulcus involvement, exhibited positivity for Helicobacter pylori and its oncoprotein cag A. While we were able to see very small numbers of patients without the use of nicotine, a case-control trial could pave the way.

**CONCLUSION**

To sum up, this analysis is a starting point for understanding of Helicobacter pylori and its oral cavity oncoprotein. The results suggest that the oral cavity of this organism can be a natural extra gastric reservoir and that saliva bathes the whole oral cavity, the discovery of CAG A and VAC A toxic proteins in saliva is revolutionary. The sample size should be expanded to indicate a clear correlation. For the same subjects who showed the positivity of the organism in the oral cavity, also in the gastric cavity, it should be verified that this analysis would have brought more individuality.

**ETHICAL REQUIREMENTS ENFORCEMENT**

**Difference of Opinion:** No conflict of interest exists in them is being said by the Authors.

**Funding:** No support was collected from an external body.

**Ethical Approval:** Both procedures conducted in this study involving human subjects were in compliance with the academic testing committee's ethical guidelines and with the Helsinki declaration of 1964 and its corresponding revisions or similar ethical standards.

**Informed consent:** From all individual participants informed consent was obtained.

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