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ASSOCIATION OF HELICOBACTER PYLORI IN ORAL SQUAMOUS CELL CARCINOMA – A RETROSPECTIVE STUDY USING POLYMERASE CHAIN REACTION

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ABSTRACT

Background: Oral Squamous Cell Carcinoma (OSCC) is the predominant malignancy of the oral cavity, frequently associated with habits such as tobacco and alcohol usage. Helicobacter pylori (H. pylori), a known gastric pathogen, has been hypothesized to play a role in OSCC pathogenesis. **Aim:** To evaluate the association of H. pylori in OSCC using Real-time Polymerase Chain Reaction (RT-PCR) on formalin-fixed paraffin-embedded (FFPE) tissue sections. **Methods:** This retrospective study analyzed 40 FFPE tissue samples, comprising 25 OSCC cases with habits, 5 OSCC cases without habits, and 10 control samples from non-cancerous individuals. DNA was extracted using a modified Proteinase-K method, and H. pylori presence was assessed by RT-PCR using JW22 and JW23 primers. The standard strain of H. pylori was used to establish a quantitative standard curve. **Results:** H. pylori was detected in 42.5% (17/40) of samples. Among OSCC with habits, 47.8% of well-differentiated and 42.9% of moderately differentiated cases showed positivity. Control samples showed 30% positivity. No significant association was observed between the presence of H. pylori and the occurrence of OSCC or with type of habit. **Conclusion:** Although H. pylori DNA was present in several samples, the study found no significant association between H. pylori and OSCC. Further large-scale research is necessary to confirm any potential role of H. pylori in oral carcinogenesis.

INTRODUCTION

oral squamous cell carcinoma(oscc) is the most common malignant tumor of the oral cavity and represents a major global health concern. It accounts for nearly ninety percent of all oral malignancies1and shows a particularly high incidence in developing countries such as India.[1] The high prevalence of tobacco usage, alcohol consumption, and betel quid chewing has contributed significantly to the disease burden[2].

The development of OSCC is multifactorial and involves a complex interaction between environmental factors, lifestyle habits, genetic alterations, and host immune responses[3,4]. In addition to these well-established risk factors, chronic infections and inflammation have gained attention for their potential role in carcinogenesis[5].Persistentmicrobialinfectionsareknowntoinduceprolonged inflammatory responses, which may

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contribute to epithelial damage and malignant transformation.

Helicobacter pylori is a gram-negative, microaerophilic bacterium that is well recognized for its role in gastric inflammation, peptic ulcer disease, and gastric carcinoma[6,7]. Beyond the stomach, the organism has been detected in various extra-gastric sites, including the oral cavity[8]. Dental plaque, saliva, and oral mucosal tissues have been proposed as possible reservoirs for the bacterium[11].

Several virulence factors of Helicobacter pylori, such as urease, vacuolating cytotoxin (VacA), and cytotoxin-associated-gene A (CagA), enable it to survive in hostile environments and modulate host"immune responses[9,10]. These properties have led to speculation regarding its possible contribution to oral carcinogenesis. However, existing literature presents conflicting results, with some studies reporting an association between Helicobacter pylori and oral cancer, while others fail to establish a significant relationship[13].

Conventional diagnostic methods such as culture and histopathology have limitations in detecting Helicobacter pylori, particularly in formalin-fixed tissues. Molecular techniques, including real-time polymerase chain reaction,offergreatersensitivityandspecificity[14,15].Therefore, the present study was undertaken to evaluate the presence of Helicobacter pylori in oral squamous cell carcinoma using real-time PCR and to assess its possible association with oral malignancy.

MATERIALS AND METHODS

This retrospective study was conducted on archived formalin-fixed paraffin-embedded tissue samples obtained from the Department of Oral and Maxillofacial Pathology. A total of forty tissue blocks were included in the study. The study sample consisted of twenty-five cases of oral squamous cell carcinoma associated with oral habits, five cases of oral squamous cell carcinoma without habits, and ten control samples obtained from individuals undergoing routine dental procedures such as impacted tooth removal or crown lengthening.

Ethical clearance was obtained from the Institutional Ethical Committee prior to the commencement of the study. Relevant demographic and clinical details were retrieved from departmental records.

Sections of four micrometer thickness were cut from each paraffin block and collected in Tris-EDTA buffer. DNA extraction was carried out using a modified Proteinase-K digestion method. The samples were subjected to repeated washing, lysis, and incubation to obtain purified DNA, which was subsequently stored at minus twenty degrees Celsius until further analysis.

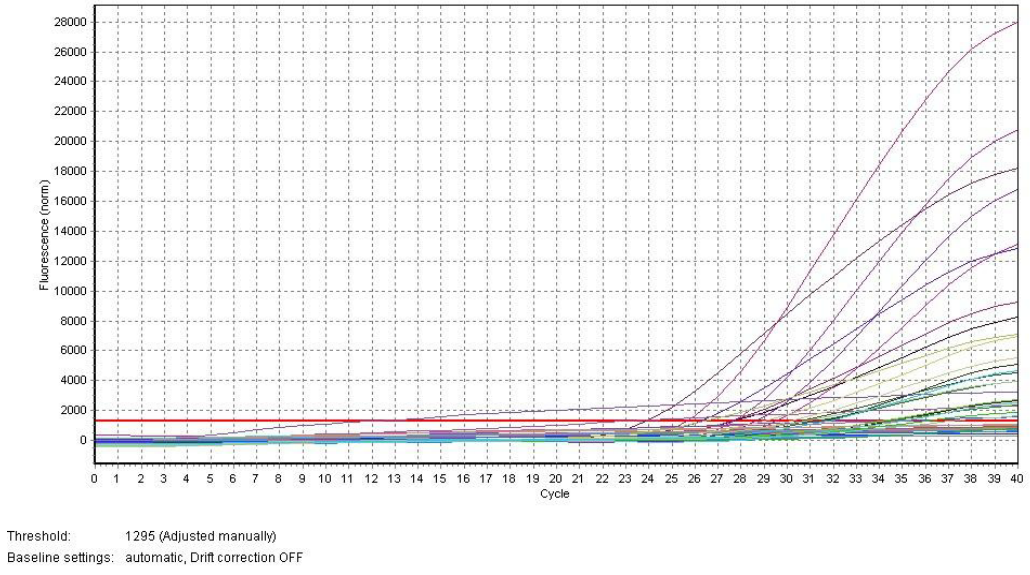
Real-time polymerase chain reaction was performed using specific primers targeting Helicobacter pylori (JW22 and JW23). Each reaction mixture contained SYBR Green master mix, primers, Template DNA, and molecular-grade water in a total volume of twenty microliters. The amplification protocol included an initial denaturation step followed by thirty-five cycles of denaturation, annealing, and extension.

A standard strain of Helicobacter pylori was used to generate a quantitative standard curve. Serial dilutions were prepared and cycle threshold values were plotted to estimate bacterial load in the test samples. Fluorescence data were analyzed using dedicated PCR software (Graph 1).

Statistical analysis was performed using appropriate non-parametric tests. The Kruskal–Wallis tes was used to compare bacterial load among groups, and Spearman's correlation coefficient was applied to assess relationships between clinical variables and bacterial quantity. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Graph 1: Fluorescence graph showing Cycle Threshold (CT) value for each sample



Interpretation: The peak value is correlated with the sample; earlier the peak (low CT value) - Higher is the quantity of *H. pylori.*

The study population comprised forty subjects, including eighteen males and twenty-two females. Among the oral squamous cell carcinoma cases with habits, a slight female predominance was observed. Control samples also showed a higher proportion of female subjects.

Histopathological evaluation revealed that well-differentiated oral squamous cell carcinoma constituted the majority of cases, followed by moderately differentiated carcinoma. The most commonly involved anatomical site was the buccal mucosa, followed by the lateral border of the tongue and vestibule.

Helicobacter pylori DNA was detected in seventeen out of forty samples, accounting for a positivity rate of forty-two point five percent. Among oral squamous cell carcinoma cases associated with habits, a higher detection rate was observed when compared to cases without habits and control samples.

Quantitative analysis showed a higher mean bacterial load in oral squamous cell carcinoma cases with habits compared to other groups; however, statistical analysis using the Kruskal–Wallis test did not reveal a significant difference among the groups. Spearman's correlation analysis demonstrated no significant correlation between age, duration of oral habits, and bacterial load.

Overall, the results indicate the presence of Helicobacter pylori in both malignant and non-malignant oral tissues, without a statistically significant association with oral squamous cell carcinoma.

DISCUSSION

Oral squamous cell carcinoma(OSCC) is acomplex disease influenced by multiple factors such as tobacco consumption, alcohol intake, betel nut chewing, genetic susceptibility, and environmental exposures. In addition to these established risk factors, chronic infections and persistent inflammation have been increasingly explored for their possible contribution to carcinogenesis[5,17-19]. Helicobacter pylori, a gram-negative bacterium well known for its role in gastric pathology, has recently gained attention for its potential involvement in extragastric malignancies, including oral cancer[6,7,20]. The present retrospective study evaluated the presence of Helicobacter pylori DNA in OSCC tissues using real-time polymerase chain reaction. Although Helicobacter pylori was detected in both OSCC and control samples statistical analysis did not reveal a significant association between bacterial presence and OSCC. This finding indicates that detection of Helicobacter pylori in oral tissues does not necessarily imply a direct role in malignant transformation[13,25].

A comparatively higher bacterial load was observed in OSCC cases associated with oral habits when compared to OSCC cases without habits and control samples;however,this difference was not statistically significant. This suggests that oral habits may alter the local oral environment, facilitating microbial persistence without establishing a causal relationship with carcinoma development.

The identification of Helicobacter pylori in a considerable proportion of samples supports the concept that the oral cavity can act as a reservoir for the organism[11,21,23]. The ability of Helicobacterpylori to survive in the oral environment has been attributed to its virulence factors, which promote colonization and inflammatory responses[8,9,24]. Chronic inflammation induced by such infections may contribute to epithelial damage; however, the absence of a statistically significant association in the present study suggests that this mechanism alone may not be sufficient to initiate oral carcinogenesis[5,17].

Discrepancies regarding the published studies regarding the role of Helicobacter pylori in OSCC may be due to variations in study design, sample size, demographic factors, and diagnostic techniques[22,24]. The use of real-time PCR in the present study allowed sensitive detection of bacterial DNA from formalin-fixed paraffin-embedded tissues[15,16]. Despite this methodological advantage, the limited sample size remains a notable limitation.

Based on the findings of this study, Helicobacter pylori does not appear to function as a primary ccausative agent in OSCC. Instead, its presence may reflect secondary colonization influenced by oral habits and local tissue conditions. Further research involving larger sample sizes and molecular analysis is required.

CONCLUSION

The present study demonstrated the presence of Helicobacter pylori DNA in both oral squamous cell carcinoma and non-cancerous oral tissue sample susing real-time polymerase chain reaction. However, no statistically significant association was observed between Helicobacter pylori detection and the occurrence of OSCC, nor with the presence or duration of oral habits. These findings suggest that although the oral cavity may serve as a potential reservoir for primary etiological factor in oral squamous cell carcinoma remains unproven. Present secondary colonization influenced by local oral conditions rather than a direct contributor to malignant transformation.

Further large-scale studies incorporating larger sample sizes and detailed microbial analysis are required to clarify the possible contributory role of Helicobacter pylori in oral carcinogenesis.

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