Helicobacter Pylori as a risk indicator for Oral Squamous Cell Carcinoma – A PCR-Based Study

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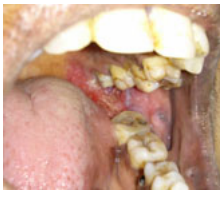
Introduction

- Oral cancer is the 3rd most common cause of death in India
- Established etiological factors - tobacco in the form of smoking and chewing, gutkha, areca nut, poor oral hygiene
- Increased cases of oral cancer reported even in the absence of such habits
- These act as co-factors to tobacco and areca nut – leading to chemical carcinogenesis
- These factors include microbial (bacterial, viral and fungal), psychosomatic and chronic irritations
- Various infectious agents including HPV, Candida and Helicobacter pylori have been investigated



H. pylori cagPAI
↓ *Encodes*
Type four secretion system
↓ *Facilitates*
Adhesion of VacA, CagA, PGN to epithelial cells
↓ *Leading to*
Incorporation in host DNA
↓
Multiple intracellular pathways are activated
↓ *Including*
1. Increased inflammatory cytokine production
2. Immune cell infiltration
3. Affecting host cell apoptosis
4. Proliferation and differentiation
↓ *Leading to*
Epithelial cell oncogenic transformation

Review of Literature

First to recover viable H. pylori from patient's saliva positive for gastric H. pylori	H. pylori - cofactor in aphthous ulceration recurrence	Detected H. pylori by culture and PCR in dental plaque & saliva	Betel chewing predisposing to colonisation with H. Pylori in digestive tract	Presence of H. pylori in plaque as the result of gastroesophageal reflux	Possible association of H. pylori with an increased risk of oral cancer
Ferguson D. A. et al, **1993**	Kilmartin C. M, **2002**	Dowsett S.A. et a, **2003**	Fernando N. et al, **2009**	Panahi O. et al, **2011**	Dayama A. et al, **2011**

Lacunae of studies No studies could correlate the association between H. pylori and oral cancer with age, sex and site

Statement of Problem

Clinicopathological profiling of cases based on age, sex and site is needed to correlate the association of H. pylori with oral cancer

Material & Methods

Sampling Methodology

- GCF sample collected from 48 lesional side adjacent to oral cancer in TE buffer for PCR analysis
- Primers used - JW22 and JW23 (Chromous Biotech, Bangalore)
- Technique used for DNA isolation – Modified Proteinase K Method
- 2µl of template DNA added to each tube and subjected to PCR
- PCR program carried out in thermocycler & included an initial denaturation step at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, & final extension at 72°C for 1 minute

Detection of amplified products

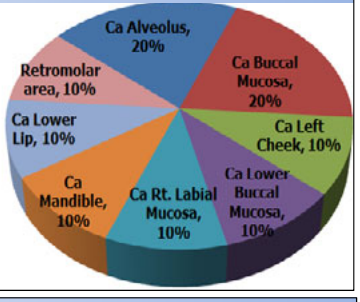
- Products obtained were analysed on 2% agarose gel electrophoresis performed in Tris-acetate-EDTA buffer.
- Gel was stained with 0.5 µg/ml ethidium bromide and visualised on an ultraviolet transilluminator.
- Expected product after amplification - 300 base pairs in length

Discussion points

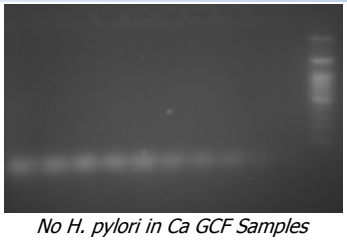
Though GCF is an ecological niche for H. pylori, tobacco chewing may alter this niche leading to absence of organism from the same

Test Results & Trends

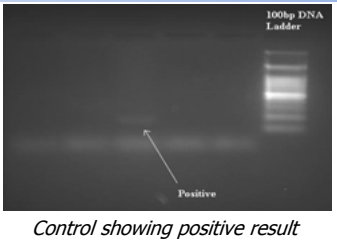
- Average age of patients developing squamous cell carcinoma was 51.6 yrs and included 70% males and 30% females
- 90% of patients developing OSCC show Lymph node involvement



Interim Conclusion



No H. pylori in Ca GCF Samples



Control showing positive result

- No H. pylori detected in GCF samples in our preliminary study
- Pilot study – difficult to draw conclusions

Future scope

- Same sample may need higher molecular analysis
- Tissue samples may be studied to find the colonisation of H. pylori rather than in GCF samples
- Need to isolate tobacco chewing and non tobacco chewing patients who develop oral cancer

References

- Anand Dayama et al. Helicobacter pylori and Oral Cancer: Possible Association in a Preliminary Case Control Study. Asian Pacific J Cancer Prev, 12, 1333-1336
- Neluka Fernando et al. Presence of Helicobacter pylori in betel chewers and non betel chewers with and without oral cancers. BMC Oral Health 2009, 9:23
- Gupta A. A., Kheur S. M., Kheur M., Saner S.R. Helicobacter pylori – possible role as biomarker for oral cancer. Dental Hypothesis 2014; 5(1): 3 - 6.

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