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ORIGINAL RESEARCH

PROTOCOL OF COMPARATIVE EVALUATION OF MMP2 & E-CADHERIN IN SALIVA WITH MMP9 IN SERUM BY ELISA METHOD IN ORAL SQUAMOUS CELL CARCINOMA (OSCC) PATIENTS

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ABSTRACT

Background: Oral cancer is one among the foremost fatal public health problems within the Indian subcontinent and it is the second leading cancer.[1] This is attributable to the habitual and persistent use of various cost-effective tobacco products including reverse smoking. [2]

There is a need to identify biomarkers with potential for behaviour prediction of oral cancer.[3] MMP-2 and MMP-9 are a subgroup of proteinases and in oral Squamous cell Carcinoma(OSCC) patients may be useful to predict the disease free survival period by, predicting metastatic potential & recurrence potential of the disease as earlier studies have indicated the likelihood of their association with the metastasis and prognosis of OSCC .[9] Also E-cadherin disruption and shortening has been shown to play a synergistic role in the process of metastasis.[10]

Objective: In our study, we will evaluate MMP2, E-Cadherin in saliva with & MMP9 in serum in pre-operative & follow-up OSCC patients as it is expected to help in improving assessment of prognosis of the patient.

Method: The protocol of this investigation were approved by the Institutional Review Board. Unstimulated saliva & serum of pre-surgical OSCC patients and six-monthly follow-up patients will be analysed for selected parameters by ELISA method and compared with each other and with controls.

Results: The study results will give an indication of metastatic potential and recurrence probability of the OSCC patients thereby helping to plan the treatment for them to give best possible prognosis besides indicating that saliva is a vital diagnostic tool for disease mapping.

Conclusion: The likely drawbacks, if any, will be small sample size and errors in methodology and the high cost of ELISA tests besides it being a very sensitive essay method. However, since many researchers have stated the usefulness of these biomarkers based on their studies and as the combination of gelatinases in their most predictable bodyfluid assays alongwith soluble or free form E-cadherin estimation is more likely to give information on tumour behavior in terms of, metastases and recurrence. If the hypothesis of this study is proven valid then, it will be an important reason to develop a Point-of-care device for mass screening of the study parameters and will also help the surgeon in better treatment designing for the benefit of patients.

***Keywords*:** Oral Squamous cell Carcinoma (OSCC), Matrix metalloproteinases (MMPs), E-cadherin, β-catenin, Enzyme-linked Immunoassay(ELISA)

INTRODUCTION

Oral cancer is among the most fatal public health issues on the Indian subcontinent. Oral carcinoma is second most common cancer in India, despite being the eleventh most common cancer worldwide. A quarter (77,000 cases) of all cases of oral cancer worldwide occur in India alone. In India, most prevalent type of oral cancer is carcinoma of the buccal mucosa. The consistent and widespread use of smokeless tobacco products like snuff, gutkha, betelquid chewing (with or without

tobacco) is largely responsible for India's noticeably higher incidence of oral carcinoma. This habit makes the majority of Indians, especially its youth, more susceptible to oral premalignant disorders, and as habits and frequency of consumption rise, the likelihood of oral carcinoma developing in younger patients increases.[1]

In India, when gender distribution is considered, oral cancer ranks as primary type of cancer among men and women also are having a relatively high incidence rate of oral cancer with third most common cancer among female population. The phenomenally high incidence rate is attributable to the habitual and persistent use of various cost-effective tobacco products including reverse smoking. Other predisposing factors that give a synergistic synchronisation to the harmful effects of tobacco encompass a large spectrum from alcohol and socioeconomic status to poor hygiene & /or diet, repetitive infections and sometimes chronic irritation from prosthetic fittings. Persons who are habitual as well as regular and persistent tobacco & alcohol consumers with or without betel quid chewing are at a higher risk compared to individuals exposed to any one of these habits or conditions. The typical age of diagnosis is 55–60 years for men and 50–54 years for women, with maximum prevalence observed in the sixth decade of life. Males are affected twice as frequently as females. Majority of oral squamous cell carcinoma cases in Southeast Asia, as well as India, typically arise in the vestibule or buccal mucosa, as well as in the oral cavity's commissural regions.[2]

This lesion is regarded as aggressive owing to its hallmark invasion tendency of spreading into the tissues in its immediate vicinity and also a tendency to spread to cervical lymphatic chain nodes. This behaviour therefore contributes to severe morbidity, decreased survival rates, and a high rate of recurrence of oral carcinomas.

OSCC development is characteristically having multiple steps with assimilation of increasing mutations and forward mutations of certain controlling genetic pathways. As a result of, summation of mutational events that occur in epithelial stem cells of oral mucosa, with simultaneous rapid cellular proliferation of DNA-altered cells facilitating there build-up in the affected oral epithelium area and a continuous provocation by the habit related ill-effects, there is development of an infiltrative disease in the affected area.

Early diagnosis of oral cancer is solely based on clinical examination which often does not provide a clear-cut diagnostic accuracy between oral potentially malignant disorders with and without morphological changes to identify the high-risk group among them. The lesions also remain asymptomatic or mildly symptomatic for a long time. Therefore, detection of early-stage oral cancer is still a far-fetched goal. This can be explained by the lack of an adequate and sensitive assessment method of molecular changes along with the routine examination. The development of an authentic diagnostic method for early lesions of OSCC would be a significant step in achieving early diagnosis of oral cancer and would also serve to aid in procuring an early treatment, so improving the post-treatment lifespan and life quality and would help to minimise the fatalities associated with delayed diagnosis and treatment as for advanced tumours.[3]

For OSCC confirmatory diagnosis is based on histopathology, wherein diagnosis is confirmed by virtue of presence of dysplastic epithelial cells and dysplastic epithelial architectural features with variable extent of squamous differentiation invading the connective tissue. Although the majority of stromal host cells have some tumor-suppressive properties, the stromal changes that typically occur during malignancy ultimately promote growth, invasion, and metastasis, serving as tumor promoters rather than suppressors. Both normal and malignant epithelial tissues depend on the host tissue stroma for preservation.[4]

It is noteworthy that, owing to a delay in diagnosis coupled with reliance only on clinical staging of the tumours to decide the treatment plan, it is seen that, two tumours although of the same stage tend to behave in biologically different ways leading to differing prognosis. The consequence, therefore, is that only a marginal betterment were noted, in five-year survival rate of patients for more than four to five decades even though the therapeutic methods for head and neck squamous cell carcinoma were significantly improvised in same period.[5]

At present, a confirmatory diagnosis of oral carcinoma is done by tissue biopsy & its histo-pathological analysis report. Beyond the need of highly trained personnel to do such procedures and examinations, it is associated with patient distress and the risk of secondary infection, tumour spread or contamination of the lesion is also likely, as it is a surgical intervention and it involves significant cost besides an assessment period of atleast 5-6 days. One of the most relevant approaches would be to detect oral squamous cell carcinoma in its early stage thus creating an opportunity of revamping the post-treatment prognosis and decreasing morbidity in these patients. Therefore, there is a need to develop or identify a biomarker with potential for diagnosis & behaviour prediction of oral cancer.

Saliva is a biofluid that can be utilised for the detection of human diseases by virtue of its contents which tend to show changes & so, their qualitative &/or quantitative changes may prove important indicators of health state of an individual. According to some researchers, its presence in relation to the oral mucosa permits a symbiotic equation between the systemic influence of molecular markers and their expression in saliva. When found in the saliva of diseased patients, the molecules—which include proteins, metabolites, coding and

noncoding RNAs, and DNA—may be useful to analyse an individual's current state of disease. Researchers investigated the role of inflammatory cytokines as possible biomarkers of mouth cancer because saliva analysis has shown promising findings even in cases of OSCC. Some of components of saliva as well as serum, can probably, serve as efficient biomarkers for early screening, diagnosis, prognosis evaluation, observing therapy for debilitating diseases like oral carcinoma. [3]

Matrix metalloproteinases (MMPs) are proteinases that are secreted during all phases of a solid tumour, like oral carcinoma, and when activated, they affect the surrounding microenvironment, leading to dynamic tissue relationship changes. In OSCC infiltration and metastasis, the loss of integrity of the basement membrane (BM) between the epithelium and lamina propria, basement membrane around malignant cell clusters, and surrounding vascular structures is a key alteration. This alteration is facilitated by, a significant increase of proteases in the local mileu with subsequent changes in both the BM and extracellular matrix (ECM), and that in turn allows tumour cells to migrate and metastasise through the vascular and lymphatic systems. MMP-2 (gelatinase A) is acts on and disintegrates type-IV collagen, which is a major element of BM, and thereby paves way for invasion and metastasis of OSCC tumour cell clusters. This correlation of the expression of MMP-2 with tumour invasion and nodal involvement in OSCC is indicated by many researchers and that can be utilised as, MMP-2 may indicate the metastatic potential of oral SCCs. Similarly another gelatinase MMP9 when evaluated in serum is a very strong indicator of recurrence.[6]

Postoperative tumour free tenure indicates prognosis and is inversely proportional to the prognosis & quality of life both. Therefore, it is vital to analyse periodically, those biomarkers which stipulate the chances of recurrence of OSCC as, any change in them can be immediately taken note of and further investigations can be done ultimately reducing postoperative recurrence episodes. Duration from first surgery to pathologically confirmed recurrence is referred to as Recurrence Time and it ranges from 2 to 96 months. The general criteria of low recurrence include: T1-T2 stage with pN0 tumours, well differentiated tumours, flap repair, presence of a negative tumour resection margin, and lesions with no extracapsular invasion. In the study by Liu et al. Vimentin up-regulation and E-cadherin and β-catenin down-regulation had been seen to be related with recurrence and survival of OSCC patients. [7,8]

MMP-9 enzyme has the function of depletion of extracellular matrix and so, is a role player in cancer pathogenesis. In various researches on MMP 9 in OSCC, significant differences were noted in MMP-9 levels of OSCC patients' group and their respective control groups. The results of this comparison analysis for serum or plasma levels of MMP-9 show a statistically essential difference between the two groups described above; as a result, this parameter may be helpful for observing treatment response and predicting disease recurrence. A class of proteinases called MMP-2 and MMP-9 may be helpful in predicting the disease-free survival period for OSCC patients by predicting the likelihood of the disease's metastasis and recurrence.[9]

Earlier studies have shown that, the greater the quantity of Matrix metalloproteinases the more is the likelihood of its association with the metastasis to regional lymph nodes and this in turn would lead to a poor patient prognosis.[10]

E-cadherin is a Ca2+-dependent glycoprotein that functions as an intercellular adhesion molecule in between epithelial cells and also regulates the epithelial cell-to-cell adhesion. When tumour cells have to migrate and invade, the E-cadherin junctions tend to be compromised and so, there is a relationship between the quantity of this marker and invasion tendency of tumour cells. Also, the catenin-cadherin epithelial cell junction formed by β-catenin and E-cadherin, is instrumental in development of epithelial structure as well as subsequent maintainance of cell–cell adhesion. However, in case of 'switching on' of canonical WNT signalling pathway, there is a β-catenin dissociation from cadherin-catenin protein complex of cell junction, with subsequent translocation and accumulation of β-catenin in nucleus. In nucleus, β-catenin interacts with TCF/LEF to induce downstream gene expression that also promotes tumour proliferation with E-cadherin remaining in a soluble form. In this way, invasion, migration, and cell proliferation tend to be affected. This biochemical pathway therefore, explains the reason for loss of E-cadherin mediated cell junctions and their imporatnace in oral squamous cell carcinoma wherein a two-fold effect occurs consequent to disrupted cell junctions leading to metastatic dissemination and also to activation of several EMT transcription factors.[5]

Researchers have noted that ecto-domain shedding of E-cadherin could be an essential step in mileu of altered epithelial cells boosting the invasive activity of these cells which in turn is associated with tumour progression. Additionally, the shortening of E-cadherin by MMPs also as a result leads to altered cell junctions and less number of adherent epithelial cells. This highlights therefore that, in the same way as the gelatinases activate and enhance the metastasis and tissue invasion, E-cadherin disruption and shortening also perform a synergistic function in the process of metastasis. This has been shown in the study by Vajaria et.al, which indicated that elevated expression of MMPs causes loss of cell–cell adhesion by increased truncation of E-cadherin protein and decrease in E-cadherin levels by disruption of cell–cell adhesion.[10]

In our study, we will evaluate the quantity of MMP2, E-Cadherin in saliva with & MMP9 in serum in pre-operative & follow-up OSCC patients as it is expected to indicate the tendency of metastasis and recurrence and thereby help in improving assessment of prognosis of the patient.

In order to evaluate the status of at risk group for their proneness to fatalities, there should be investigative methods that can be used to achieve a good amount of reactivity index to serve as an important parameter, with the least possible false negative results.

This strategy, being non-invasive, might prove to be a significant method to assess the probability of non-overt metastases and extent of metastases along-with the likely treatment outcome during post-treatment follow-up in patients with oral cancer.

Aim of this investigation is to evaluate and compare the effectiveness of three biomarkers mentioned in assessing the metastatic and recurrence potential of OSCC.

Study Design

Main outcome of this investigation will be based on comparison of Quantitative assessment values of saliva levels of E-cadherin, MMP2 & serum levels of MMP9 between Controls and OSCC Patients preoperatively in order to prove the usefulness of the quantitative values as an indicator to improve prediction of metastases in OSCC patients & thereby an establishment of a method for the early predictor of probable prognosis of the individual patient. Also, quantitative assessment of saliva levels of E-cadherin, MMP2 & serum levels of MMP9 when compared between Pre-op OSCC, Controls and Post-op OSCC patients might serve as a measure in improving the prediction of recurrence in OSCC patients.

The latest research has shown a promising relationship of correlation in quantitative changes of MMP 2 & E-Cadherin in saliva with MMP 9 levels in serum with tumour progression as well as recurrence. A quantitative biochemical assessment & analysis is thus likely to have a clinically important effect if, it serves as a helpful indicator of the chances of metastases & recurrence. In order to detect like a clinical effectiveness at a particular level of 5% and a statistical power of 80%, 30 patients in each of the three groups (controls, pre-operative OSCC patients and Post-operative OSCC patients at six monthly follow-up) will be required.

Aims of study

1. To acquire by quantitative assessment the levels of E-cadherin & MMP2 in saliva & MMP9 in serum of Controls.

2. To evaluate by quantitative assessment the levels of E-cadherin & MMP2 in saliva & MMP9 in serum of OSCC Patients preoperatively for prediction of metastases

3. To evaluate by quantitative assessment the levels of E-cadherin & MMP2 in saliva & MMP9 in serum of OSCC patients postoperatively for prediction of recurrence.

Objectives of study:

1. To evaluate the E- cadherin & MMP 2 levels in Saliva of Controls & OSCC patients pre-operatively & post-operatively

2. To evaluate the MMP 9 levels in serum of Controls & OSCC patients pre-operatively and post-operatively

4. To compare the E-cadherin & MMP2 levels of Controls with OSCC patients pre- & post-operatively

5. To compare the MMP9 levels of Controls with OSCC patients pre- & post-operatively.

6. To compare E-Cadherin, MMP2 & MMP9 levels in pre –operative and post-operative of OSCC

7. To analyze pre-operative readings of OSCC patients for probability of metastases in the patients

8. To analyze post-operative readings of OSCC patients for probability of recurrence in the patients

Hypotheses of the study:

1. Quantitative assessment of saliva levels of E-cadherin, MMP2 & serum levels of MMP9 when compared between Controls and OSCC Patients preoperatively may be useful to improve prediction of metastases in OSCC patients.
2. Quantitative assessment of saliva levels of E-cadherin, MMP2 & serum levels of MMP9 when compared between Pre-op OSCC and Post-op OSCC Patients may be useful to improve prediction of recurrence in OSCC patients.

Recruitment & Methodology

The protocol of this study has been approved by the Institutional Review Board of University of Datta Meghe Institute of Medical Sciences and an ethical approval has been obtained. This study involves Oral squamous cell carcinoma pre-surgical and post-surgical patients. Informed written consent will be obtained from each participating patient. To be eligible to join this study, the patients who have histopatholigically confirmed Primary OSCC who have not received any prior treatment & are to be surgically treated will be

selected. In follow-up of the same patients who underwent surgical treatment before 6 months the saliva & serum will be re-analyzed for the same parameters as done prior to surgery. Patients not willing to participate, patients with history of other tumors, patients indicated non-surgical treatment and those with recurrent lesions will be excluded from the study. A case history sheet will be used to identify participants who fulfil the above-mentioned study criteria.

According to previous studies, decreased expression of E-cadherin is associated with increased invasiveness and a poor differentiation & prognosis of OSCC. It also gives an indication of the probability of MMP-9-mediated degradation of E-cadherin mediated cell attachment complexes. However, as most studies for E-Cadherin are done to detect the membrane bound molecule by IHC, such studies have lead to false-positive detection of E-cadherin, owing to, detection of either intra or extra-membranous component of the biomolecule only.[11]

Table1. RESEARCH STUDY DESIGN

	PHASE I	PHASE II	PHASE III
Objectives	1.Patient case history was taken after informed written consent 2. Normal Healthy adults without any habit history or systemic or oral disease will be selected,case H/O will be taken after informed written consent 3.Recording clinical data in Excel sheet	1.Saliva and Serum of OSCC Patients will be collected, centrifuged and refrigerated for future use with sample labelling 2.Saliva and serum of Controls will be collected following study protocol and stored after centrifugation for future analysis 3.Estimation will be done of MMP 2 & soluble E-cadherin in collected saliva samples and MMP 9 in serum samples by ELISA method and obtained values were recorded	1.Patients will be re-called for follow-up at 6-months,12 months and 18-months Post surgery and saliva and serum samples will be recollected, centrifuged, labelled and refrigerated for future analysis 2. Estimation of MMP 2 & soluble E-cadherin in collected saliva samples and MMP 9 in serum samples by ELISA method & obtained values were recorded
Type of Study	Observational	Prospective non-interventional analytical study	Follow-up & Molecular estimation & analysis in saliva and serum
Sample Size	n=60(30/each group)	n=60(30/each group)	n=30

Table2. Timeline of Phasic evaluation

Criteria	Enrollment					
TIMEPOINT[b]	$-t_i$ to 0	0	(At Diagnosis)-t_1	(6 months Post-R_X)-t_2	(12Months Post-R_X)-t_3	(18 Months Post-R_X)-t_4
ENROLLMENT:	20 MONTHS					
Eligibility screen	As Per Inclusion & Exclusion Criteria					
Informed consent	YES					
[List other procedures]	NONE					
Randomization	YES	X				
ASSESSMENTS: [List baseline variables and tests]	1. General & Lesional examination of Patients 2. MMP 2 & sE-cadherin quantification in Saliva and MMP 9 quantification in Serum IN Healthy controls		Collect Patient data, saliva & serum. Centrifuge & Label them for storage until all samples are collected	Collect Patient data, saliva & serum. Centrifuge & Label them for storage until all samples are collected	Collect Patient data, saliva & serum. Centrifuge & Label them for storage until all samples are collected	Collect Patient data, saliva & serum. Centrifuge & Label them for storage until all samples are collected
	MMP 2 & sE-cadherin quantification in Saliva and MMP 9 quantification in Serum at the time of Patient enrollment for Treatment	X	MMP 2 & sE-cadherin quantification in Saliva and MMP9 quantification in Serum at the time of diagnosis. Analyse & interpret controls & Pre-op. data	MMP 2 & sE-cadherin quantification in Saliva and MMP9 quantification in Serum at 6 month followup Analyse & interpret Quantified data of saliva & serum at 6 months	MMP 2 & sE-cadherin quantification in Saliva and MMP9 quantification in Serum at 12 month followup Analyse & interpret Quantified data of saliva & serum at 12 months	MMP 2 & sE-cadherin quantification in Saliva and MMP9 quantification in Serum at 18 month followup Analyse & interpret Quantified data of saliva & serum at 18 months Compare & interpret all results by statistical analysis
[List outcome variables and tests]		AT 6,12 & 18 MONTHS POST-R_X MMP 2 & sE-cadherin quantification in Saliva and MMP 9 quantification in Serum	MMP 2 & sE-cadherin quantification in Saliva and MMP 9 quantification in Serum analysed & quantified by ELISA	MMP 2 & sE-cadherin quantification in Saliva and MMP 9 quantification in Serum analysed & quantified by ELISA	MMP 2 & sE-cadherin quantification in Saliva and MMP 9 quantification in Serum analysed & quantified by ELISA	MMP 2 & sE-cadherin quantification in Saliva and MMP 9 quantification in Serum analysed & quantified by ELISA Statistical analysis
[List other data variables and tests]	NIL	Age, sex and site of lesion		X		X

Though the results of various studies assessing OSCC have not been integrated, the value of MMP-2 and MMP-9 on cancer diagnosis, progression, and metastasis have varied. Nevertheless, all of the studies show that MMPs play a role in evaluating whether oral SCC is advanced or early stage, and that they may be used as an early prognosis predictor. Instead of serum levels of MMP2 of HNSCC, saliva levels of MMP 2 would be evaluated in my study since, serum levels were not significantly higher than that of healthy and treated patients in the previous studies.

We expect that serum levels of MMP-9 will prove to be a reliable marker for diagnosis and study of the response to treatment in OSCC patients, especially with regard to lymph node invasion, as prior studies have also discovered significant levels of the MMP-9 marker in the patients' serum. Particularly, the researchers discovered that when MMP9 and MMP-2 were tested combined, MMP9 showed increased diagnostic performance.[12,13]

Using an Alpha (α) level of 0.05 and a Beta (β) level of 0.20, or power = 80%, based on the findings of Nosratzehi T et al. For each group, a minimum estimated sample size of 26 participants is required. To calculate the sample size, G*Power Version 3.1.9.2 was used. Since there are three groups, on rounding off minimum sample size comes to 30 per group. The three groups will be of: 30 controls, 30 pre-operative OSCC patients, 30 post-operative six monthly follow-up patients. Therefore the overall sample size is determined to be 90 (30* 3=90).

After the collection of baseline data in accordance with the inclusion and exclusion criteria, participants will be allocated to each group.
Statistical methods:

Statistical analyses will be performed using SPSS software. Normality of data will be checked with Shapiro-Wilk test and followed by that, the data descriptive statistics, frequency analysis and percentage analysis will be used for categorical variables and the mean & S.D will be used for continuous variables to compare sE-cadherin, MMP-2 & 9 levels across the three groups. To find the significant difference between the bivariate samples in Paired groups the Paired sample t-test will be used & for Independent groups the Independent sample t-test will be used. For the multivariate analysis the one way ANOVA with Tukey's Post-Hoc test will be used and for repeated measures the Repeated measures of ANOVA will be used with Bonferroni correction to control the type I error on multiple comparison .To assess the relationship between the variables Pearson's Correlation will be used. To find the significance in qualitative categorical data Chi-Square test will be used similarly if the expected cell frequency is less than 5 in 2×2 tables then the Fisher's Exact will be used. In all the above statistical tools the probability value .05 will be considered as significant level.

Sample collection &Assessment

PHASE I **Table 1**

Saliva samples 5 ml of unstimulated saliva will be collected between 8:30–11:30 a.m. in a pre-marked sterile collection tube. Samples will be centrifuged at 3000 rpm for 10 min at room temperature immediately and supernatants will be stored at −80 °C until use in sterile, stoppered tubes.

Blood samples will be collected at the same visit by venipuncture after the saliva collection. It will be centrifuged at 3000 rpm for 15 minutes within half an hour of collection and the serum will be obtained, transferred to 5 ml sterile, stoppered tubes & stored at −80 °C until use.

Assessment of analyte levels in saliva & serum will be performed using Human protease ELISA kits for MMP 2, MMP9, E-Cadherin with 96-well plate containing precoated wells and standardisation wells according to manufacturer’s instructions. The readings will reflect the standardization readings in the first column followed by sample readings in subsequent columns in ng/ml.

Three millilitres of blood will be extracted, the serum will be separated, and it will be kept at -80°C. ELISA kits that are available commercially will be utilized to measure the serum levels of MMP2 and MMP-9. The procedures will follow those outlined in manufacturer's protocol. MMP-2 and MMP-9

have assay ranges of 10–3000 ng/ml and 30-90000 ng/L, respectively. (Table 1)

The assay's
Expected results: sensitivity limits for MMP-2 and MMP9 were 5.64 ng/ml and 15.12 ng/L, respectively.

PHASE II Table 1&2

The readings of Controls will be taken as guideline readings & compared with readings of pre-op. OSCC patients to study the molecular basis of metastases. (10)

PHASE III Table 1&2

The readings of Controls will be taken as guideline readings & compared with readings of post-op. OSCC patients after 6 months, 12 months and 18 months of surgery to study the molecular basis of recurrence.

The comparison of pre-operative and post-operative levels of parameters will be helpful in noting the extent of change, if any, in the quantitative values of the parameters and thereby allow to assert the study objectives. (Table 1)

Interpretation & Prognostic significance

Biomolecular quantification of MMP2 & E-cadherin in saliva and MMP 9 in serum of OSCC patients at time of diagnosis and six months after treatment may be effective in aiding:

- To evaluate, whether, the tumor has high or low metastatic potential.
- To predict, recurrence of OSCC
- The diagnostic accuracy is likely to improve with a confidence interval of above 80%.
- Our results will also support the idea that salivary diagnostics has a potential to be used in clinical practice for early diagnosis, to know metastatic potential and reccurrence likelihood and most importantly to allow large-scale screening in high incidence groups.
- It can thus, help in designing treatment plan & determining prognosis.

Analyzing the range of parametric quantities in the histopathological diagnosis of oral carcinomas that are well-, moderately-, and poorly-differentiated may also be helpful.

Initial investigation will be done in a patient with a primary lesion of oral carcinoma and the subsequent readings will be evaluated six –months post- surgery as already mentioned in the study design.

DISCUSSION

It has been understood for a long time that, early detection and diagnosis of oral carcinoma can lead to a greater survival rate and play an important role in successful clinical treatment. Early diagnosis, however, is often not achieved, as, the lesions tend to be painless and asymptomatic until in advanced stages in most cases. To aid in the purpose of diagnosis and to get an indication of lesional behaviour, saliva can be used as the body fluid of analysis of oral health status of a patient because, saliva is in the immediate vicinity of the lesion hence, biomolecules in saliva are more likely to reflect the lesion behaviour and besides it avoids the risk of contamination or secondary infection. About 100 or more potential salivary biomarkers have been identified by various researchers so far, and, behaviour analysis of OSCC lesions with the analysis of salivary biomarkers might serve to avert undue complications of metastases and also prevent recurrence and thereby might help to improve overall patient prognosis.

In the present study, we will analyse MMP2 in saliva alongwith E-cadherin. MMP2 is a zinc-dependent proteinase that, tends to degrade collagen –IV, a major component of the basal lamina with other types basal lamina associated collagens and elastin as well as fibronectin. MMP-2 (gelatinase A) is expressed at high levels during the growth. Its expression has been reported to be high in stroma surrounding thesites of tissue damage, inflammation & invading front of metastasizing tumours. [15, 17]

E-cadherin has been proven to have a significant role in morphogenesis, including cell identification, border development, coordinated cell movements, tissue polarity, and maintaining the structure and functions

of cells, decades after its discovery. Therefore, abnormal E-cadherin expression is directly associated with abnormal tissue architecture, metastatic malignancy, etc.

E-cadherin's function in oral premalignant diseases and oral squamous cell carcinoma has been investigated by many researchers using IHC. Some researchers came to the conclusion that although E-cad expression varies, its use as a predictive marker is questionable. Others discovered that changes in E-cadherin expression contributed to the aggressiveness of the caner and the malignant development of dysplasia.[16]

MMP-9 is involved in cytokine processing, matrix-bonded growth factor movement, tissue remodeling, inflammation, and wound healing. Additionally, cancer patients' urine and tumor endothelium exhibit elevated levels of these substances. According to research by Wang et al., Cheng et al., and Ranuncolo et al., patients with HNSCC had a noticeably greater MMP-9 concentration than the control group. Additionally, in Dalirsani et al.'s study, serum levels of MMP-9 were higher and showed a statistically significant difference between levels in cases and controls (healthy individuals) as well as treated patients, even though salivary levels were higher in case subjects but the difference was not significant. Additionally, prior research has shown that MMP 9 is a more accurate marker for evaluating malignant changes and metastases, as well as for evaluating clinical characteristics, prognostic factors, and tumor grading. [15, 18, 19, 20]

CONCLUSION

The likely drawbacks, if any, will be small sample size and errors in methodology and the high cost of ELISA tests besides it being a very sensitive essay method. However, since many researchers have stated the usefulness of these biomarkers based on their studies and as the combination of gelatinases in their most predictable bodyfluid assays alongwith soluble or free form E-cadherin estimation is more likely to give information on tumour behavior in terms of, metastases and recurrence.

If the hypothesis of this study is proven valid then, it will be an important reason to develop a Point-of-care device for mass screening of the study parameters and will also help the surgeon in better treatment designing for the benefit of patients.

DECLARATIONS

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Competing Interests

The authors have no competing interests to declare.

Ethical Approval

The study was approved by the appropriate ethics committee and conducted according to relevant guidelines and regulations.

Informed Consent Not applicable.

REFERENCES

1. Bobdey S, Sathwara J, Jain A, Saoba S, Balasubramaniam G. Squamous cell carcinoma of buccal mucosa: An analysis of prognostic factors. South Asian J Cancer 2018;7(1):49-54
2. Salian V, Dinakar C, Shetty P, Ajila V. Etiological Trends in Oral Squamous Cell Carcinoma: A Retrospective Institutional Study. Cancer Transl Med 2016;2(2):33-6
3. Frederico Omar Gleber-Netto, Maha Yakob, Feng Li et al. Salivary Biomarkers for Detection of Oral Squamous Cell Carcinoma in a Taiwanese Population. Clin Cancer Res. 2016; 22 (13) 3340-3347
4. Gupta S, Kamboj M, Narwal A. Knowing the unknown in oral squamous cell carcinoma: An observational study. J Can Res Ther.2020;16(3):494-9
5. Nooshin Mohtasham et al. Expression of E-cadherin and matrix metalloproteinase-9 in oral squamous cell carcinoma and histologically negative surgical margins and association with clinicopathological parameters. Rom J Morphol Embryol 2014, 55(1):117–121

6. Stanciu A, Zamfir-Chiru-Anton A, Stanciu M, Popescu C, Gheorghe D. Serum level of matrix metalloproteinase-9 in patients with head and neck squamous cell carcinoma. Clin Lab [Internet]. 2016;62(08/2016). Available from: http://dx.doi.org/10.7754/clin.lab.2016.160139
7. Wang B, Zhang S, Yue K, Wang X-D. The recurrence and survival of oral squamous cell carcinoma: a report of 275 cases. Chin J Cancer. 2013;32(11):614–8.
8. Liu LK, Jiang XY, Zhou XX, et al. Upregulation of vimentin and aberrant expression of E-cadherin/beta-catenin complex in oral squamous cell carcinomas: correlation with the clinicopathological features and patient outcome. Mod Pathol, 2010,23(2):213-224.
9. Patel BP, Shah SV, Shukla SN, Shah PM, Patel PS. Clinical significance of MMP-2 and MMP-9 in patients with oral cancer. Head Neck. 2007;29(6):564–72.
10. Patel P, Vajaria B, Patel K, Begum R, Patel J, Shah F. Significance of phosphorylated epidermal growth factor receptor, matrix metalloproteinases, and E-cadherin in oral cancer. Tumor Microenviron. 2018;1(1):16.
11. Bai Y, Sha J, Kanno T. The role of carcinogenesis-related biomarkers in the Wnt pathway and their effects on epithelial-mesenchymal transition (EMT) in oral squamous cell carcinoma. Cancers (Basel). 2020;12(3):555.
12. Lotfi A, Mohammadi G, Tavassoli A, Mousaviagdas M, Chavoshi H, Saniee L. Serum levels of MMP9 and MMP2 in patients with oral squamous cell carcinoma. Asian Pac J Cancer Prev. 2015;16(4):1327–30. https://doi.org/10.7314/apjcp.2015.16.4.1327
13. Spencier Pol,Anja Brouwer. MMPs in Head & Neck Cancer. *World Journal of Surgical, Medical and Radiation Oncology*.2015;*4*: 18-27. http://creativecommons.org/licenses/by/3.0
14. Shpitzer T, Bahar G, Feinmessr R. Acomprehensive salivary analysis for oral cancer diagnosis. J Cancer Res Clin Oncol .2007; 133:613–617
15. Dalirsani Z, Pakfetrat A et al.Comparison of Matrix Metalloproteinases 2 and 9 levels in Saliva and Serum of patients with Head and Neck Squamous cell carcinoma and Healthy subjects. Int J Cancer Manag.2019; 12(5):1-6
16. I Ilangani Satish,Kannan Ashokan, Krithika CL et al. Expression of E- Cadherin and Levels of Dysplasia in Oral Leukoplakia - A Prospective Cohort Study.; Asian Pac J Cancer Prev. 2020; 21 (2): 405-410
17. Peisker A, Rashke G F et al. Salivary MMP9 in the detection of oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2017;22 (3): e270-e275
18. Wang WL, Chang WL, Yeh YC, Lee CT, Chang CY, Lin JT, et al. Concomitantly elevated serum matrix metalloproteinases 3 and 9 can predict survival of synchronous squamous cell carcinoma of the upper aerodigestive tract. Mol Carcinog. 2013; 52(6):438–45. doi: 10.1002/mc.21874.
19. Cheng D, Kong H, Li Y. Prognostic value of interleukin-8 and MMP-9 in nasopharyngeal carcinoma. Eur Arch Otorhinolaryngol. 2014; 271(3):503–9. doi: 10.1007/s00405-013-2580-3.
20. Ranuncolo SM, Matos E, Loria D, Vilensky M, Rojo R, Bal de Kier Joffe E, et al. Circulating 92-kilodalton matrix metalloproteinase (MMP9) activity is enhanced in the euglobulin plasma fraction of head and neck squamous cell carcinoma. Cancer. 2002;94(5):1483–91. doi: 10.1002/cncr.10356