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Protocol of Comparative evaluation of MMP 2 and E-cadherin in saliva with MMP 9 in serum by ELISA method in Oral Squamous cell Carcinoma (OSCC) patients

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Abstract

Oral cancer is one among the foremost fatal public health problems within the Indian subcontinent and it is the second leading cancer.[1] 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 synchronization to the harmful effects of tobacco are encompassing a large spectrum from alcohol and socioeconomic status to poor hygiene & /or diet, repetitive infections and sometimes chronic irritation from prosthetic fittings.[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 indicate the tendency of metastasis and recurrence and thereby help in improving assessment of prognosis of the patient.

Method: The protocol of this study has been 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.

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

Introduction

Oral cancer is one among the foremost fatal public health problems within the Indian subcontinent. Although oral cavity carcinoma is the eleventh leading cancer globally, in India, unfortunately, it is the second leading cancer. India alone accounts for a quarter (77,000 cases) of total number of oral cancer cases across the world. Among oral cancers, carcinoma of the buccal mucosa is the commonest oral cavity cancer in India. The notably greater incidence of oral carcinoma in India is in a big way due to a consistent and common consumption of the smokeless tobacco products snuff, gutkha and betel quid chewing (with or without tobacco), which makes a large majority of Indian population, particularly its youth develop a proneness to oral premalignant disorders and with continuation of habits and often an increase in frequency of consumption, leading to increased likelihood of oral carcinoma occurrence in younger patients. (1)

In India, oral cancer ranks as the 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 are encompassing 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 male are affected twice more often than the females and the average age at the time of diagnosis is 55-60 years in males and 50-54 years in females with highest prevalence noted during the sixth decade of life. In Southeast Asia and India, most cases of oral squamous cell carcinoma tend to occur in the buccal mucosa or vestibule as well as in the commissural areas of the oral cavity. (2)

It is considered an aggressive lesion 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 of it therefore contributes to severe morbidity, decreased survival rates, and high rate of recurrence of oral carcinomas.

OSCC development is characteristically having multiple steps with assimilation of increasing mutations and forwarded mutations of certain controlling genetic pathways. As a result of, summation of mutational events that occur in the epithelial stem cells of oral mucosa, with the 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 will also serve to aid in procuring an early treatment, so improving the post-treatment lifespan and life quality and would help to minimize 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. The host tissue stroma plays a crucial role for the maintenance of both normal epithelial tissues and its malignant counterparts, and although most stromal host cells possess certain tumour-suppressing capabilities, the stromal changes which tend to occur during malignancy in due course of time promote growth, invasion, and metastasis and thus serve as tumour promoters and not as suppressors. (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 has been noted, in the 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 have been significantly improvised in the 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 at least 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 utilized 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. Its presence in relationship with the oral mucosa facilitates a symbiotic equation between the expression of molecular markers in saliva and their systemic impact, according to some researchers. The molecules range from metabolites, proteins to coding and noncoding RNAs, and DNA which when detected in saliva of diseased patients may prove valuable in terms of analysis of disease status of an individual. Even in oral squamous cell carcinoma, the role of saliva when analysed, has shown encouraging results and hence, the role of inflammatory cytokines has been investigated as potential biomarkers of oral cancer by researchers. Some of the components of saliva and serum, can probably, serve as efficient biomarkers for early screening, diagnosis, prognosis evaluation, and monitoring of 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 component 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 oral squamous cell carcinoma 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. The duration from the 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 downregulation were seen to be associated with recurrence and survival of OSCC patients. (7,8)

MMP-9 enzyme has the function of degradation 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 the OSCC patients' group and their respective control groups. In this comparative analysis the results for Serum or plasma levels of MMP-9 give a statistically significant difference in the above-mentioned two groups and hence, this parameter may be useful for monitoring treatment response and predicting recurrence of the disease. MMP-2 and MMP-9 are a subgroup of proteinases and in OSCC patients may be useful to predict the disease-free survival period by, predicting metastatic potential & recurrence potential of the disease. (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 and subsequent maintenance of cell–cell adhesion. However, in case of 'switching on' of canonical WNT signalling pathway,

there is a β-catenin dissociation from the cadherin-catenin protein complex of cell junction, with subsequent translocation and accumulation of β-catenin in the nucleus. In the 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 (Figure-1). In this way, cell proliferation, migration, and invasion tend to be affected. This biochemical pathway therefore, explains the reason for loss of E-cadherin mediated cell junctions and their significance 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 important step in the 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 tissue invasion and metastasis, E-cadherin disruption and shortening also plays a synergistic role 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)

Fig:1- Five Extracellular Dimers of E-Cadherin with Ca^{2+} & intra-cellular core forms complex with intra-cellular β-Catenin. Dissociation of the cadherin-catenin protein complex causes β-Catenin to translocate into the nucleus causing Tumor Suppresor cascade dysfunction.

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 approach, 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.

The aim of this study is to evaluate and compare the effectiveness of three biomarkers mentioned in assessing the metastatic and recurrence potential of Oral squamous cell carcinoma.

Study Design

The main outcome of this study 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 significant effect if, it serves as a helpful indicator of the chances of metastases & recurrence. In order to detect such a clinical effectiveness at a significance 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)

Studies evaluating OSCC have reported different results regarding the value of MMP-2 and MMP-9 on the cancer diagnosis, progression and metastasis with no integration between findings of different studies, but all studies are indicative of role of MMPs in the evaluation of the advanced or early stage of oral SCC and possibility of their use as early predictor of their prognosis. 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. Since previous studies have also found high levels of MMP-9 marker in the patients' serum, we expect the serum levels of MMP-9 to prove to be a reliable marker for diagnosis and analysis of the response to treatment in OSCC patients, particularly, with respect to lymph node invasion. It is noteworthy that researchers have found that, MMP9 showed an increase in diagnostic performance when tested in combination with MMP-2. (12,13)

Based upon the results from Nosratzehi T et al and using an Alpha(α) level of 0.05 as well as Beta(β) level of 0.20 i.e. power= 80%. The minimum estimated sample size is 26 subjects per group. Sample size calculation was performed using G*Power Version 3.1.9.2. 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 baseline data collection according to the inclusion & exclusion criteria, participants will be assigned in each group.

Statistical methods:

Data will be analyzed by SPSS (version 16) software. Descriptive analysis will be done by using tables and charts, while the explanatory analysis will be conducted using the 2 appropriate mean comparison test (based on the normality or non-normality of the data, the t-test or Man Whitney test was used, respectively) and the Spearman correlation test. The significance level will be set at ≤ 0.05 for all tests. The assessment of the relationship between the markers and metastases, grade, recurrence, and age (continuous variables) will be done with the independent t-test, while the relationship of the markers with the stage of the disease and correlation between the serum and salivary levels of each protein will be assessed with the Pearson test. (12,15)

If, there is large in-born variability of parameters, particularly in saliva, median values will be calculated and analyzed using non-parametric statistical tests, as is acceptable for small sample size groups (less than thirty individuals in a group) and in accordance with common practice. Distributions of categorical variables were compared and analyzed by Fisher–Irwin exact test, where appropriate. The medians between subgroups of patients were compared by Kruskal–Wallis (non-parametric multiple comparison test). Also, for categorical variables, frequencies, percentages and distribution can be calculated and for continuous variables, ranges and medians can be calculated. (13)

Sample collection &Assessment

Saliva samples 5 ml of un-stimulated 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 the supernatants will be stored at −20 °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 −20 °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 standardization 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.

A total of 3 mL of blood will be drawn and the serum separated and stored at -20°C. Serum levels of MMP2 and MMP-9 will be measured using commercially available ELISA kits. Methods will be as described in the manufacturer's protocol. Assay range for MMP-2 was 10-3000 ng/ml and for MMP-9 was 30-90000 ng/L. The sensitivity limit of the assay for MMP-2 was 5.64 ng/ml and for MMP9 was 15.12 ng/L.

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)

The readings of Controls will be taken as guideline readings & compared with readings of post-op. OSCC patients after 6 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.

Expected results

Bio-molecular quantification of MMP2 & E-cadherin in saliva and MMP 9 in serum of oral squamous cell carcinoma patients at the 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 Oral squamous cell carcinoma
- 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 recurrence likelihood and most importantly to allow large-scale screening in high incidence groups.
- It can thus, help in designing treatment plan & determining prognosis.

It may also help to analyse the range of parametric quantity in the histo-pathological diagnosis of well-differentiated, moderately differentiated and poorly-differentiated oral carcinomas.

The 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 along with 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 the sites of tissue damage, inflammation & invading front of metastasizing tumours. (15, 17)

Decades after their discovery, it has been proved that E - cadherin also plays an important role in morphogenesis including cell recognition, boundary formation, coordinated cell movements, tissue polarity and also maintains the structure and functions of cells. Hence defective expression of E - cadherin is linked directly with disruption of normal tissue architecture, metastatic cancer, etc.

Many authors have studied the role of E-cadherin in oral premalignant disorders and oral squamous cell carcinoma by IHC. Some authors concluded that there are variations in the expression of E - cad but its usage as a prognostic marker is questionable. Others found alterations in the expression of E – cadherin to be contributors to the malignant transformation of dysplasia and aggressiveness of the caner. (16)

MMP-9 plays a role in inflammation, wound healing, tissue remodeling, movement of matrix-bonded growth factors, and cytokine's processing. Also, an elevation of their levels is seen in tumour endothelium and in urine of cancer patients. In studies of Wang et al., Cheng et al., and Ranuncolo et al., the MMP-9 concentration was reported to be significantly higher in patients with HNSCC compared to the control group. Also, in the study by Dalirsani et al., although the salivary levels of MMP-9 were higher in case subjects, the difference was not significant but serum levels of MMP 9 were higher and with statistically significant difference between levels in cases and controls (healthy individuals) as well as treated patients. Previous studies have also indicated that, MMP 9 is a better marker for evaluation of metastasis and malignant changes and for assessing the clinical features, the prognosis predicting factor, and the accurate grading of tumours. (15, 18, 19, 20)

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 body fluid assays along with 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.

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Legend Figure

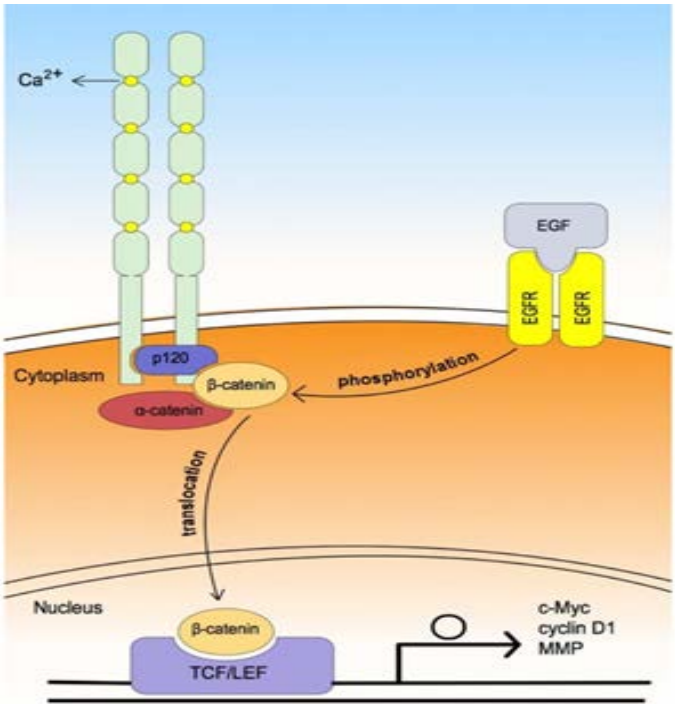

Fig.1: Five Extracellular Dimers of E-Cadherin with Ca^{2+} & intra-cellular core forms complex with intra-cellular β-Catenin. Dissociation of the cadherin-catenin protein complex causes β-Catenin to translocate into the nucleus causing Tumor Suppresor cascade dysfunction.