

# SERUM LEVELS OF CA 125 IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA (OSCC) AND SUB MUCOSAL FIBROSIS (OSF) AND ITS EXPRESSION IN OSCC TISSUE SAMPLES

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## ABSTRACT

*The incidence of oral cancer is associated with habit of betel nut chewing. In Pakistan this problem is extremely common especially in cosmopolitan cities especially Karachi. The development of premalignant lesion is the first step in initiation of disease process. The proper and early diagnosis of the disease can lead to control and timely treatment of the disease. In the present study CA125 levels in blood were compared in oral squamous cell carcinoma (OSCC), oral sub mucosal fibrosis (OSF) patients and control (healthy) groups. The expression of CA125 as a marker in OSCC and a premalignant, OSF condition was also analyzed through slides stained with immunohistochemical stain to have an observation of the extent of CA125 expression. Hematological analysis of three groups including two case groups, (OSCC and oral OSF) and one control group was done for CA125 levels. Immunohistochemical staining of slides of different stages of OSCC was performed with the help of specific stain.*

*The hematological analysis of the diseases groups showed difference of values within the threshold level as compare to controls but the ratio of staining was found high in advanced stages of carcinoma as compare to initial stages. The CA125 expression on specimen tissue surface is weak positive. Serum levels of CA125 in participants (cases and controls) of three groups' i.e. OSCC, OSF and control group were found below threshold levels.*

**Keywords:** OSSC, CA125, Immunohistochemistry, OSF, Chemiluminescence assay

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## INTRODUCTION

Oral malignant and premalignant condition in the oral cavity is initiated due to irritating content such as tobacco products ultimately resulting in alteration of genetic structure of the cell.<sup>2</sup> The lack of early diagnosis is responsible for high mortality rate in oral cancer. 75% of patients in Pakistan have been reporting in OPD when they are already at t3/t4 stage<sup>3</sup>. Easy asses to the oral cavity can enable timely preformed evaluation of malignant and suspicious lesions. The

early identification of the lesion and good public awareness can reduce the risk of disease occurrence.<sup>6</sup> Habits linked to carcinogens like tobacco and betel quid is the leading cause of carcinoma prevalence in developing countries.<sup>7</sup> There are many challenges associated with head and neck carcinoma patients and overall survival rate was observed 40-50% in five years.<sup>24</sup> predominantly involved site has been found to be buccal mucosa in specifically male OSCC patients specifically in south Asia.<sup>19</sup>

Glycoprotein CA125 is mucinous in nature with about 11000 amino acids<sup>18</sup>. CA125 is a tumor marker that is expressed in different conditions it is found both on tissue surface and in blood stream. The normal level of CA125 in blood is less than 35units/ml and any value equivalent or raised is considered high.<sup>4</sup> CA125 levels were raised in malignant conditions.<sup>5</sup> Saliva along with serum is also a mean for CA125 levels assessment. The premalignant and malignant conditions have been found to have different results regarding CA125 levels and later has been found to have raised levels of antigen<sup>8</sup>. CA125 has also been found to be associated

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with expressing the relapse of the disease.<sup>9</sup>

The tumors of epithelial origin are known to be associated with CA125, it includes the ovarian, gastrointestinal, breast and oral cancer.<sup>20,23</sup> Tumor-immune cell interactions is also a significant factor for evaluating the prognostic value of a marker.<sup>21</sup>

The objective of the study is to analyze the expression of CA125 in squamous cell carcinoma both in serum and tissue expression and identify the potential role of this marker in diagnostic value of the neoplasm.

## METHODOLOGY

### *Study Duration and Patient Consent*

The study was performed in six month time period, after acceptance of synopsis from institution's scientific committee. The time frame from March till August 2018 data collection and research work was performed. The study was approved by institution review board ( IRB) which is the ethical committee of DUHS. A consent form was designed for the patients in two languages; Urdu and English and the patient consent was taken for the study procedure.

### *Study Design*

The study design is analytical cross sectional study carried out in a public sector health facility in Karachi. The individuals that were included in the study were divided into three groups. The control group consists of healthy individuals without any sign or symptom of pre-malignancy or malignancy reported in the OPD, second group was oral squamous cell carcinoma patients (OSCC) patients from oral surgery ward and third group was the individuals clinically diagnosed by dental surgeon for oral sub mucosal fibrosis (OSF).

Sampling was done without discrimination of age and gender. The women with hormonal disturbances and menopause and individuals with any other type of cancer or tumor were excluded from the study. Sampling was done on the basis of convenience.

### *Samples*

**Serum;** Patient coming to dental OPD were given the consent form, after patient's consent blood was collected by venipuncture. Purple top test tubes were used to collect the specimens. The specimens were stored at 4 °C and utilized for analysis of CA125 within 5 days.

**Tissue;** The tissue samples were taken from the histopathology lab of DUHS(Dow university of health sciences) sample were taken from patients biopsy specimen that reported in the lab since last six months . Tissue analysis was performed on sample of different OSCC patients.

Histopathological slides were prepared with proven

cases of OSCC biopsy specimen (n=15) and analyzed for tissue expression of CA125. The slides were certified by the histopathologist for OSCC diagnosis.

Sample size was estimated through OpenEpi with the reference values adopted from Balan J et. al 2012 (6)with confidence interval and power 99%. The sample size obtained was 10 for each group from whom serum will be collected, however, the samples collected were n=14 for control, n=13 for OSF and n=23 for OSCC, including serum specimen 8 and tissue specimen 15 making a total of 50 samples.

## PROCEDURE

**Serum:** The specimen (serum) was preserved at 4°C and it can be stored up to(1-5 days) The Elecsys CA125 II tumor marker assay kit was utilized based on the monoclonal M 11 and OC 125 antibodies according to the manufacturer's guidelines. The kit used was Elecsys CA 125 II tumor marker assay and was based on the monoclonal M 11 and OC 125 antibodies.<sup>17</sup>

**Tissue:** For tissue samples eosin and hematoxylin staining was performed and the diagnosis was verified by the histopathologist. The slide was prepared for immunohistochemistry, the procedure involved initially, treating the slide with xylene and different alcohol concentrations and then epitope retrieval with help of autoclave. Primary and secondary antibodies were administered to the prepared slide immunohistochemical staining was observed under microscope.

The stain obtained as a result of immunohistochemistry was brown. Four grading of the slide that was considered after observation under the microscope are as follows; 0 is negative, 1 is weak positive, 2 is positive, 3 is high positive. The slide was considered as positive for stain if 50% of the slide showed covered area with stain.<sup>4</sup>

**Statistical Analysis:** The levels of CA125 in individuals of three groups were compared by one way ANOVA and Post Hoc was used for pair wise comparison. SPSS21 was utilized for data analysis and results compilation.

## RESULTS

The data included 70% male and 30% female specimens. The age range of subjects was from 25 to 65 years with mean age of individuals being 46 years. The age group 40 to 50 years was dominant. Serum analysis revealed wide range of marker value within threshold level. Whereas in tissue specimen, expression of CA125 was more in poorly differentiated carcinoma than in moderately or well differentiated OSCC. The mean value of the slides covered area observed was 40% which is in the weak positive category.

TABLE 1: MEAN SERUM LEVELS OF CA125 AMONG THE GROUPS

Serum- levels	Min	Max	Mean $\pm$ SD	95% Confidence Interval
Oral sub-mucous fibrosis	8.3	22.5	15.037 $\pm$ 4.98	12.02-18.05
Oral squamous cell carcinoma	5.0	17.1	11.161 $\pm$ 4.17	7.66-14.65
Control	2.6	28.00	18.573 $\pm$ 7.23	14.39-22.75

One way ANOVA P-value = 0.025

TABLE 2: POST HOC ANALYSIS

Groups	Mean Difference	p-value
Oral sub-mucous fibrosis & Oral squamous cell carcinoma	3.876	0.318
Oral sub-mucous fibrosis & Control	-3.535	0.275
Oral squamous cell carcinoma & Control	-7.412	0.020*

\*The mean difference is significant at 0.05 level

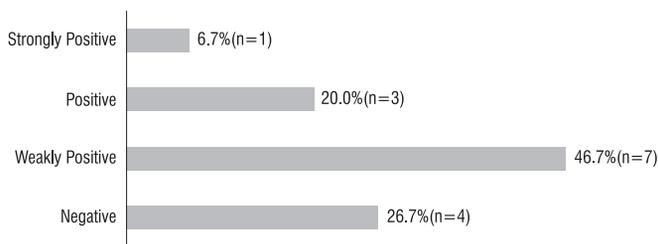


Fig.1: Surface expression in oral squamous cell carcinoma patients.

Parametric test One Way ANOVA was used as the data was normally distributed. Variance and mean of three groups was evaluated.

In table 2 mean difference can be seen between the groups which is not significant.

According to 0-3 rating criteria majority of the slides were among the weak positive category i.e the covered area with immunohistochemical stain was less than 50%. The majority of cases of OSCC specimen with positive and weak positive ranking laid in the class of poorly differentiated carcinoma.

## DISCUSSION

The current study does not support CA125 of diagnostic significance as the serum values have been significantly different in three research groups but were found less than threshold level. A similar study support CA125 as a diagnostic tool in OSCC patients as its levels were significantly high in saliva of these patients.<sup>5</sup>

Weak positive expression of CA125 is observed in the present study in immunohistochemical analysis of OSCC specimen. In another study on oral cancer involving salivary gland adenocarcinoma, concludes a strong positive expression of CA125 on tumor surface.<sup>16</sup>

However a study on endometrial cancer concludes epithelial expression of marker in every stage of cancer and the result show strong association of CA125 with tissue expression. Another study with saliva analysis supports CA125 as significant marker for OSCC along with other markers including Cyfra 21-1 and TPS.<sup>1A</sup> A similar study also report differential levels of CA125 along with other markers in patient's saliva.<sup>22</sup>

In present study the levels of CA125 were not significantly raised in OSCC patients but there was a difference in the values of healthy and carcinoma patients. OSCC patient CA125 levels were towards the upper limit near threshold. A study performed to compare healthy and OSCC patients reveals a significant difference in CA125 levels in oral neoplastic and non neoplastic conditions with raised levels in former.<sup>20</sup>

CA125 has been found to be associated with other malignancies as reported in a study on epithelioid sarcoma that supports strong tissue expression of CA125 and narrates as malignancy marker along with other markers.<sup>23</sup>

Results of a study performed on serum of OSCC patients also narrated no relevance of CA125 with OSCC, the results of present study are similar to the these findings; serum CA125 is not concluded as a credible biomarker for diagnosis of OSCC.<sup>8</sup>

Variable results have been found regarding the status of CA125, yet in another study it is found to be a tool for determining the relapse of oral cancer.<sup>9</sup> Association between CA125 marker and lung cancer has also been found.<sup>10,11</sup> It has low specificity regarding diagnosis in patients with pleural effusion and malignancy so it is used with other markers in combination.<sup>14</sup> The classic strong tissue expression of CA125 is observed in ovarian cancer.<sup>15</sup>

CA125 role in OSCC diagnosis is not found significant as concluded according to present study. Serum levels of patients were not raised to defined threshold level. However the expression of CA125 on tissue surface was categorized predominantly as weak positive. It can be observed in above mentioned studies that

CA125 levels in saliva were found to be significantly raised in OSCC patients as compare to serum CA125 levels. Utilization of multiple salivary markers simultaneously is regarded as efficient screening tool for oral cancer.<sup>13</sup>The immediate contact of lesion with saliva makes it a strong medium for multiple marker detection.<sup>12</sup>This supports saliva CA125 in contrary to serum, as diagnostic tool.

### LIMITATIONS

The tests for CA 125 levels in serum have high cost. Single person handling can result in data loss .Tissue expression procedure is not affording for patients. A consultant approval for every case is necessary to continue the whole procedure.

### RECOMMENDATIONS

Other markers specific to OSCC should be explored to get a quick diagnosis of the OSCC.The studies should be focused on tissue expression of different markers as well and increase sample size can also affect the result. The research carried out in a facility having lab and patient ward in same vicinity can prevent data loss.

### CONCLUSION

The CA125 expression on specimen tissue surface is weak positive. The covered area of the slide with stain was increased in high grade OSCC. Serum levels of CA125 in participants (cases and controls) of three groups' i.e. OSCC, OSF and control group were found below threshold levels but there was a difference in values of case and control groups.

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