



ESTIMATION OF SALIVARY CA-125 IN SQUAMOUS CELL CARCINOMA OF ORAL CAVITY

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KEYWORDS :

INTRODUCTION

Cancers of the oral cavity and oropharynx represent approximately three percent of all malignancies in men and two percent of all malignancies in women. Oral cancer most commonly occurs in middle aged and older individual, although a disturbing number of these malignancies are also being documented in younger adults in recent years.

The most commonly used laboratory diagnostic procedures involve the analyses of the cellular and chemical constituents of blood. Other biological fluids are utilized for the diagnosis of disease, and saliva offers some distinctive advantages. Whole saliva can be collected noninvasively and by individuals with limited training. No special equipment is needed for collection of the fluid. Diagnosis of disease via the analysis of saliva is potentially valuable for children and older adults, since collection of the fluid is associated with fewer compliance problems as compared with the collection of blood. Further, analysis of saliva may provide a cost-effective approach for the screening of large populations.

Due to its many potential advantages, saliva provide an attractive alternative to more invasive, time-consuming, complicated, and expensive diagnostic approaches.

Oral squamous cell carcinoma (OSCC) is a common human malignancy. Circulatory epithelial tumor markers were previously investigated in the serum of OSCC patients but almost never in their saliva, in spite of the fact that there is a direct contact between the saliva and the oral cancer lesion. The purpose of the current study was to examine tumor markers in the saliva of OSCC patients as it has been found a useful diagnostic tool for other distant malignancies, such as breast and ovarian carcinoma also. The purpose of the current study was to examine the saliva of OSCC patients for the most often studied serum circulatory epithelial tumor marker CA-125.

MATERIAL AND METHOD

The present study entitled "Estimation of salivary CA-125 in squamous cell carcinoma of oral cavity" was conducted in department of ENT, Dr. B.R.A.M. Hospital, Raipur in association with Dept. of Biochemistry Pt. J.N.M. Medical College Raipur. Cases were drawn from out patient door and indoor of Department of ENT, Dr. B.R.A.M. Hospital Raipur (C.G.). Analytical and Biochemical work has been conducted in the Department of Biochemistry.

Our study included all the new patients and patients receiving treatment for oral squamous all carcinoma from September 2007 to September 2008.

Patients associated with following conditions like : Carcinoma breast Colorectal carcinoma, Ovarian cyst, Fibroid, Endometriosis, Inflammatory bowel disease, Cirrhosis, Peritonitis, Pancreatitis.

Whenever possible a mid and post treatment sample were also carried out to evaluate the effect of therapy on salivary CA-125 level.

Control Group : Estimation of salivary CA 125 level were also done in healthy controls.

At the time of registration ; name, age, sex, address, education, occupation, religion and socioeconomic status of the patients were asked and following detailed history were taken :-

- **Age of onset of disease :-**
- **Duration of disease :-**
- **Symptomatology :-**
- **Personal history** regarding any addiction with special reference to tobacco, bidi, cigarette, gudakhu & alcohol.
- **Family history** of infertility, any congenital/chromosomal disorder in family, h/o diabetes, hypertension, thyroid disorders were asked.
- **Medical history** Both past and current medical history regarding diabetes, hypertension, TB, thyroid disorder or any long standing medical illness was asked.
- **Surgical history** – Any previous surgery for the tumour and time since surgery and history of recurrence.
- **H/O Radiotherapy / Chemotherapy :-** No of cycles and duration was asked in detail.

General examination : Complete general examination was done regarding Built, nutrition, pulse, BP, pallor, edema, cyanosis, clubbing, icterus and cervical lymph node enlargement.

Local examination :- Lips, Upper/lower Alveolar margins, buccal mucosa gingiva buccal sulcus, floor of mouth, retro molar area, tongue-margins, dorsum and ventral surface were examined.

Examination of neck :- for lymph node enlargement.

Systemic examination :- CVS, RS, per abdomen to rule out any pathology.

Examination was followed by investigations

All cases were investigated for-

- Hb, TLC, DLC, ESR
- Urine R/M
- Blood urea / Sugar
- X-ray chest & mandible
- Punch Biopsy report
- FNAC

TNAM Classification for staging of tumour

Saliva collection and estimation of CA-125

Saliva collection: - Whole saliva was collected under not

stimulatory conditions in a quiet room between 8 a.m. and noon, at least 1 hour after eating. Patients were asked to generate saliva and to spit into a wide test tube for 10 minutes as described previously. Following collection, the saliva was immediately centrifuged at 800 x g at 4°C for 10 minutes to remove squamous cells debris. The resulting supernatant fluid was used for the further biochemical analysis.

Serum collection :- Ten milliliters of blood was collected from each individual and centrifuged at 800 x g for 10 min and the serum fraction was separated, aliquotted, stored at 80°C and assessment of tumour marker was done.

Assessment of salivary tumor marker :- Salivary samples were stored at -70°C until analyzed, CA-125 was determined with a micro particle enzyme-linked immunoassay.

Procedure :- In present study we have used a kit-Accu-Bind ELISA Microwells CA-125 assay kit.

The kit consists of:-

1. Micro well strips :- Streptavidin coated wells.
2. Biotinylated capture antibody solution in BSA and phosphate buffer.
3. Reference standards calibrated to 0, 15, 50, 100, 200 and 400 U/ml.
4. Washing buffer concentrate-containing a surfactant in buffered saline.
5. Chromogen solution A, containing Tetramethylbenzidine.
6. Substrate solution B, containing hydrogen peroxide.
7. Stop solution :- 1NHCL.
8. Well holder.

ASSAY PROCEDURE

Before proceeding with the assay, all reagents, serum references and controls to room temperature (20-27) were accumulated.

1. The desired numbers of coated wells were secured in the holder with marking for identification.
2. 25 mL of sample diluents standards (blank well), samples or controls were dispensed into the assigned wells. Immediately 100 mL of biotinylated capture antibody was dispensed into assigned wells.
3. Incubated for 60 min at room temperature.
4. Incubation mixture was removed and rinse the wells five times with washing buffer (300 ml / well / each times).
5. 100 ml enzyme conjugate was dispensed into each well except the blank well.
6. Incubated for 60 minute room temperature.
7. Incubation mixture removed and rinse the wells five times with washing buffer (300 mL/well/each times).
8. 100 mL of solution A and 100 mL of solution B were dispensed into each well including blank well.
9. Incubated for 15 minute at room temperature.
10. 50mL of stop solution was added into each well to stop the reaction.
11. The result were read within thirty minutes of adding the stop solution by reading the absorbance in each well at 450nm in a microplate reader.

CALULATION OF RESULTS

A dose response curve was used to ascertain the concentration of CA-125 in unknown specimens.

1. The absorbance obtained from the printout of the microplate reader was recorded.

2. Graph was plotted for the absorbance for each duplicate serum reference versus the corresponding CA-125 concentration in U/ml on liner graph paper.
3. Best fitted curve was drawn.
4. The average absorbance of the duplicates for each unknown was located on the vertical axis and find out the intersecting point and read the concentration in U/ml from the horizontal axis.

Analysis : All the data collected were analysed to find out values in controls as well as in diseased group and sensitivity and specificity of serum and salivary levels of CA-125 were assessed.

In present study – for saliva, level of CA-125 <500 U/ml was considered to be within normal.

For serum, level of CA-125 0.872U/ml was considered to be within normal.

Both the above values were obtained from healthy control group.

OBSERVATIONS

The present study “Estimation of Salivary CA – 125 in squamous cell carcinoma of oral cavity” was conducted in 24 cases of oral squamous cell carcinoma and 10 healthy persons (control) attending the Department of ENT, Dr. BRAM Hospital Raipur (C.G.) from September 2007 to September 2008. Estimation of salivary and serum CA-125 was done in all cases and compared with healthy control group.

TABLE NO. 1 AGE & SEX WISE DISRIBUTION OF CASES & CONTROL

C A S E	S.NO.	AGE (Yrs.)	NO. OF CASES	%	SEX			
					MALE	%	FEMALE	%
S	1	20-30	1	4.16	1	100	0	0
	2	31-40	7	29.16	7	100	0	0
	3	41-50	11	45.83	5	45.45	6	54.54
	4	51-60	4	16.66	4	100	0	0
	5	>60	1	4.16	1	100	0	0
	TOTAL		24	100	18	75	6	25
C O N T R O L	S.NO.	AGE (Yrs.)	NO. OF CONT.	%	SEX			
					MALE	%	FEMALE	%
N T R	1	20-30	1	10	1	100	0	0
	2	31-40	3	30	3	100	0	0
	3	41-50	4	40	2	50	2	50
	4	51-60	1	10	1	100	0	0
	5	>60	1	10	1	100	0	0
	E TOTAL		10	100	8	80	2	20

In present study maximum number of cases belonged to age group 41-50 years (45.83%). Next common group was 31-40 years (29.16%). Youngest patient was 26 years and oldest was 62 years old. The male : female ratio was 3:1. The commonest age group among male was 31-40 years whereas among female was 41-50 years.

TABLE NO. 2 DISTRIBUTION OF CASES ACCORDING TO FREQUENCY OF SYMPTOMS

S.NO.	TYPE OF LESSION	CASES	
		NO.	%
1	Swelling	10	41.16
2	Ulcer	24	100
3	Other	1	4.16

Above table shows that ulceration was the most frequent symptom present in all 24 cases. Swelling in neck was seen in 10 (41.16%) cases. Discharging sinus was present in only 1 (4.16%) case.

TABLE NO. 3 DISTRIBUTION OF CASES ACCORDING TO DURATION OF LESSION

S.NO.	DURATION OF LESSION	CASES	
		NO.	%
1	Less than 3 month	8	33.33
2	3-6 month	11	45.83
3	6-12 month	2	8.33
4	More than 12 month	3	12.5
Total		24	100

Most of case presented within 3-6 month of illness. Minimum duration was 1 month and maximum was more than 1 year.

TABLE NO. 4 DISTRIBUTION OF CASES ACCORDING TO SITE OF LESSION

S.NO.	SITE OF LESSION	CASES	
		NO.	%
1	Buccal Mucosa	7	29.16
2	Alveolar margin	9	37.5
3	Tongue	5	20.83
4	Floor of mouth	0	0
	Lip	2	8.33
	Hard palate	1	4.16
Total		24	100

Alveolar margin was accounted for maximum number of cases i.e. 9(37.5%) followed by buccal mucosa 7(29.16%); anterior 2/3rd of tongue 5(20.83%); lip 2(8.33%); and hard palate 1(4.16%).

TABLE NO. 5 DISTRIBUTION OF CASES ACCORDING TO ADDICTION

S. NO.	ADDICTION	CASES	
		NO.	%
1	Tobacco	4	16.66
2	Tobacco+Alcohol	11	45.83
3	Tobacco+Alcohol+Smoking	5	20.83
4	Smoking+Tobacco	7	29.16
5	Alcohol+Smoking	8	33.33
6	Gudakhu	8	33.33
7	Betal leaf with betal nut	2	8.33

Above table shows maximum cases i.e. 11(45.83%) cases were in habit of tobacco and alcohol. The incidence of gudakhu addiction and combined addiction of alcohol and smoking was found to be in equal number of cases i.e. 8(33.33%) followed by combined addiction of smoking and tobacco in 7(29.16%), combined addiction of tobacco alcohol and smoking in 5(20.83%) case, addiction of tobacco alone in 4(16.66%) and addiction of betal nut and betal leaf chewing in minimum number of cases i.e. 2*8.33%) respectively. It was found that all the cases were addicted to tobacco in one or other form.

TABLE NO. 6 TABLE SHOWING STAGING ACCORDING TO TNM CLASSIFICATION

S.NO.	STAGE	CASES	
		NO.	%
1	I	6	25
2	II	2	8.33
3	III	9	37.5
4	IV	7	29.16
Total		24	100

Above table shows out of 24 cases maximum number of cases i.e. 9 (37.9%) were of stage III followed by stage IV i.e. 7(29.16%) cases; stage I & II accounted 6(25%) & 2(8.33%) respectively.

TABLE NO. 7 DISTRIBUTION ACCORDING TO CERVICAL LYMPH NODES METASTAS

S. NO.	SITE	NO.OF CASES	N0(%)	N1(%)	N2(%)	N3(%)
1	Buccal mucosa	7	5	1	1	0
2	Alveolus	9	5	2	2	0
3	Tongue	5	2	0	2	1
4	Floor of mouth	0	0	0	0	0
5	Hard Palate	1	0	1	0	0
6	Lips	2	2	0	0	0
TOTAL		24	14(58.33)	4(16.66)	5(20.83)	1(4.16)

Above table shows out of 24 cases, 14(58.33%) cases had no neck node metastasis and 10(41.16) had neck node metastasis at the time of presentation.

TABLE NO. 8 HISTOPATHOLOGICAL GRADING OF CASES

S. NO.	HISTOPATHOLOGICAL TYPE	CASES	
		NO.	%
1	Well differentiated	22	91.66
2	Mod. differentiated	0	0
3	Poorly differentiated	2	8.33
Total		24	100

Present study was done in cases of squamous cell carcinoma only. Out of 24 cases, well differentiated carcinoma was according maximally in 22 (91.66%) cases followed by poorly differentiated carcinoma in 2(8.33%) cases while none of the case of moderately differentiating carcinoma was found in our study.

TABLE NO. 9 DISTRIBUTION OF CASES ACCORDING TO PREVIOUS TREATMENT HISTORY

S.NO.	TREATMENT HISTORY	CASES	
		NO.	%
1	Surgical	2	8.33
2	Radiotherapy	0	0
3	Chemotherapy	4	16.66
4	No. t/t	18	75
Total		24	100

Above table shows out of 24 cases 4(16.66%) had undergone of chemotherapy and 2(8.33%) for surgery. 18(75%) cases had no history of any sort of treatment.

TABLE NO. 10 TABLE SHOWING PRE TREATMENT SALIVARY CA-125 LEVEL

S. NO.	CA-125 (U/ML)	CASE		CONTROL	
		NO.	%	NO	%
1	0-100	6	25	2	20
2	101-102	3	12.5	0	0
3	201-300	2	8.33	2	20
4	301-400	3	12.5	1	10
5	>400	8	33.33	5	50
6	>500	2	8.33	-	-
Total		24	100	10	100

Above table shows that out of 24 cases, maximum level of pretreatment salivary CA-125 (>500 U/ml) showed by 2 (8.33%) cases only. While CA-125 level >400 U/ml found in 8(33.33%) cases, level between 301-400U/ml, 201-300 U/ml, 101-200 U/ml and minimum 0-100 U/ml were found in 3 (12.5%), 2(8.33%), 3(12.5%) and 6(25%) cases respectively.

Out of 10 healthy control, >400 U/ml showed by 5(50%) persons ; range between 100-400 U/ml showed by 3(30%) persons; > 100 U/ml by 2(20%) of healthy persons.

TABLE NO. 11 PRE TREATMENT SERUM CA-125 LEVEL

S.NO.	CA-125 (U/ML)	CASE		CONTROL	
		NO.	%	NO	%
1	<10	22	91.66	10	100
2	11-20	1	4.16	-	-
3	>20	1	4.16	-	-
Total		24	100	10	100

Maximum level of pre treatment serum CA-125 i.e.25.45 U/ml showed only by 1(4.16%) cases ; while between 11-20 U/ml showed by 1(4.16%) case; and 22(91.66%) cases showed level below 10U/ml.

All 10 healthy control showed serum level <10U/ml.

TABLE NO. 12 CO-RELATION OF SALIVARY CA-125 LEVEL WITH TNM STAGING

S.NO.	CA-125 (U/ML)	STAGING			
		I	II	III	IV
1	0-100	6	-	-	-
2	101-200	-	1	-	1
3	201-300	-	1	-	1
4	301-400	-	-	4	-
5	>400	-	-	5	3
6	>500	-	-	-	2
Total		6	2	9	7

Above table shows that maximum level i.e. > 500 & >400U/ml showed relation with advanced stage IV and III. Stage II and I related with >300 and >100 respectively.

TABLE NO. 13 CO-RELATION OF SERUM CA-125 LEVEL WITH TNM STAGING

S.NO.	CA-125 (U/ML)	STAGING			
		I	II	III	IV
1	>10	5	2	9	6
2	11-20	-	-	-	1
3	>20	-	-	-	1
Total		5	2	9	8

Above table shows that Serum CA-125 level was correlate with staging of tumour.

TABLE NO. 14 CO-RELATION OF SALIVARY CA-125 LEVEL BEFORE & AFTER CHEMOTHERAPY IN SAME PATIENTS

S.NO.	PRE TREATMENT	POST TREATMENT
1	>400	302
2	>400	97.21
3	178	47.18
4	317	157.8

Above table shows that after 1st cycle of chemotherapy, all 4 cases showed decrease in salivary level of CA-125.

TABLE NO. 15 CO-RELATION OF SERUM CA-125 LEVEL BEFORE & AFTER CHEMOTHERAPY IN SAME PATIENTS

S.NO.	PRE TREATMENT	POST TREATMENT
1	6.92	3.4
2	0.00	3.03
3	2.14	1.48
4	4.46	2.14

Above table shows that after 1st cycle of chemotherapy, all 4 cases showed decrease in salivary level of CA-125.

TABLE NO. 16 CO-RELATION OF SALIVARY CA-125 LEVEL BEFORE & AFTER SURGERY IN SAME PATIENTS

S.NO.	PRE TREATMENT	POST TREATMENT
1	>400	>400 (after 15 days)
2	>400	>200 (after 30 days)

Out of 2 cases, 1 case showed marked decrease in salivary CA-125 level after 30 days of surgery, whereas 1 case does not show any change after 15 days spot surgery.

TABLE NO. 17 CO-RELATION OF SERUM CA-125 LEVEL BEFORE & AFTER SURGERY IN SAME PATIENTS

S.NO.	PRE TREATMENT	POST TREATMENT
1	2.3	1.47 (after 15 days)
2	0.00	<0.00

Out of 2 cases both cases showed decrease in serum level of CA-125 either 15 days or 30 days post surgery.

Statistical analysis

Sensitivity -Ability of a test to identify correctly all those who have the disease.

Specificity -Ability of a test to identify correctly those who do not have the disease.

Salivary CA-125

Estimation of 24 cases and 10 healthy controls was carried out. Maximum number of patients were in the age group of (41-50 years). The "mean" for salivary CA-125 could no be calculated as the values reported to be > 400 U/ml. taking this into consideration a Salivary CA-125 value of < 500 U/ml was considered to be within normal. Study showed following calculation

	Cases	Control
Positive (>500U/ml)	2(a)	0(b)
Negative (>500 U/ml)	22(c)	10(d)
Total	24(a+c)	10(b+d)

Sensitivity = a/a+c x 100
= 2/24 x 100 = 8.33%

Specificity = d/b+d x 100
= 10/10 x 100 = 100%

Serum CA-125

Study included estimation of serum CA-125 in 24 cases and 10 healthy controls. A mean value of serum CA-125 of 10 healthy controls was calculated; turned to be 0.872U/ml.

	Cases	Control
Positive (>0.872U/ml)	20(a)	3(b)
Negative (>0.872 U/ml)	04(c)	7(d)
Total	24(a+c)	10(b+d)

Sensitivity = a/a+c x 100
= 20/24 x 100 = 83.33%
= 83.3%

Specificity = d/b+d x 100
= 7/10 x 100 = 100%
= 70%

Interpretation : Estimation of serum CA-125 is more sensitive then salivary CA-125.

Salivary CA-125 estimation is more specific index for oral malignancy.

SUMMARY

The present study "**Estimation of SALIVARY CA-125 IN Squamous**

cell carcinoma of oral cavity” Was conducted in 24 cases of oral Squamous cell carcinoma and 10 healthy persons (control) attending the Department of ENT, Dr. BRAM Hospital Raipur (C.G.) from September 2007 to September 2008. Estimation of salivary and serum CA-125 was done in all cases and compared with healthy control group.

1. Carcinoma of oral cavity occurs most commonly between 41-50 years.(45.839%)
2. The male:female ratio was 3:1
3. Ulceration was most frequent symptom, presented within 3-6 month of illness.
4. Most common site of involvement was lower alveolus comprising 9(37.5%) followed by buccal mucosa 7(29.16%: anterior 2/3rd of tongue 5(20.83%); lip 2(8.33): and hard palate 1(4.16%).
5. It was found that all the cases were addicted to tobacco in one or other form, maximum cases i.e. 11(45.83 %) were in habit of tobacco and alcohol. The incidence of gudakhu addiction and combined addiction of alcohol and smoking was found to be in equal number of cases i.e. 8(33.33 %) followed by combined addiction of smoking and tobacco n 7 (29.16%), combined addiction of tobacco alcohol and smoking in 5 (20.83%) cases, addiction of tobacco alone in 4(16.66%) and addiction of betel nut and betel leaf chewing in minimum number of cases i.e. 2(8.33%) respectively.
6. Most of the patients came late for examination, so maximum number of cases i.e. 9 (37.5%) were of stage III followed by stage IV i.e. 7(29.16%) cases; stage I & II accounted 6(25%) & 2(8.33) respectively.
7. Cervical node metastasis was encountered in 10(41.16%) cases, it was most frequent in carcinoma of alveolus.
8. Present study was done in cases of squamous cell carcinoma only. Out of them well differentiated carcinoma was accounting maximally in 22 (91.66%) cases followed by poorly differentiated carcinoma in 2(8.33%) cases.
9. Out of 24 cases and 10 healthy control 2 cases only showed pretreatment **salivary** CA-125 level >500 U/ml. In present study salivary levels were 8.33% sensitive and 100% specific.
10. Out of 24 cases and 10 healthy controls, 20 cases and 3 controls showed pretreatment **serum** level >. 872U/ml. Serum levels were 83.33% sensitive and 70% specific.
11. Raised Salivary CA-125 levels >500U/ml were correlated with advanced stage ie.stage IV, same correlation was seen with serumCA-125 levels also.
12. Out of 24 cases, 6 cases undergone for treatment (surgery/chemotherapy), when compared, pre and post treatment Salivary and serum CA-125 levels, significant response to treatment was noticed in the form of decreased levels.

CONCLUSION

On the basis of observations and analysis of present study following conclusions can be drawn:

1. Carcinoma of oral cavity occurs most commonly between a 41-50 Years.
2. Males are more prone to develop oral carcinoma than females.
3. The important known life style factors for oral cancer are smoking, chewing tobacco and alcohol consumption and collectively they have lethal synergistic effects.
4. Serum CA-125 is more sensitive but less specific tumour marker in comparison to salivary CA-125
5. A high CA-125 level both in saliva and serum at diagnosis correlates with advanced disease and poor outcome.
6. Repeated CA-125 measurements are useful in monitoring response to treatment.
7. A Salivary and serum determination of CA-125 seems to be useful as a prognostic risk factor for disease outcome.

However, further study of a larger series is essential to confirm conclusions of the present study.