CASE STUDY

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Serum Endothelin-1 as biomarker in oral cancer - a case control study

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Abstract: Worldwide, cancers of the oral cavity and pharynx are the 6th most common type & it is a major problem in the Indian subcontinent. Endothelin-1 (ET-1) is a potent vasoconstrictor involved not only in vascular biology but also in carcinogenesis. Serum Endothelin-1 level have been studied in various human cancers like colon & breast. To evaluate significance of serum Endothelin in oral cancer we undertook a case control study. A total of 48 subjects 24 controls and 24 oral cancers were enrolled for the study. Serum Endothelin-1 levels were evaluated by ELISA. The results showed that there was significant difference in serum Endothelin-1 levels in oral cancer and control. Therefore we conclude that serum Endothelin -1 has a potential role as a serum marker for oral cancer progression.

Key words: Oral Squamous cell carcinoma; Endothelin -1; Biomarker

INTRODUCTION

Oral cancer is a major public health problem in the India. It ranks among the top three types of cancer in the country.[1] An age-adjusted rate of oral cancer in India is high, which is, 20 per 100,000 population and accounts for over 30% of all cancers in the country.[2] The prevalence of oral and oropharyngeal lesions is very high in India, due to high prevalence of tobacco chewing habits.[3]

The development of OSCC involves sequential activation of oncogens and inactivation of tumor suppressor gene in clonal population of cells. Oncogenes are altered growth promoting regulatory genes that govern the cells signal transduction pathways.[4] Mutation of these genes leads to either overproduction or increased function of excitatory protein. The malignancy is usually preceded by premalignant lesion like leukoplakia, erythroplakia and oral submucous fibrosis. The long term survival for oral cancer has remained below 50% for past 50 years, despite numerous advances in treatment utilizing the most recent protocol for surgery radiation & chemotherapy.[5,6]

Hickey *et al.,*[7] in 1985 detected an endothelium-derived factor with contractile effects on the smooth muscle. In 1988, it was isolated by Yanagisawa *et al.,* [8] from cultured pig arterial endothelial cells and was named Endothelin-1 (ET-1). ET-1 is a vasoconstrictor peptide composed by 21 amino acids.[9] It belongs to family of multifunctional peptides ET-1,2&3.[10] The ET axis have pivotal role in normal tissue as well as in growth and progression of various cancers.[11,12,13,14] They exert their effects by binding to cell-surface receptors, namely ET-A (ETAR) and ET-B (ETBR), which belong to the G-protein-coupled receptor super-family.[15, 16] Endothelins have been implicated in various pathological conditions, including inflammation, wound healing & carcinogenesis.[17, 18]

Role of Endothelin-1 in tumor invasion has been demonstrated in ovarian carcinoma and melanoma.[19, 20,21,22,23] Increase in serum endothelin-1 has been studied in various human cancers like colon & breast, but its significance in oral cancer has not been studied as yet. The present study was planned with an aim to evaluate serum levels of Endothelin-1 in oral cancer as tumor marker and to compare serum levels of Endothelin-1 in oral cancer and control.

MATERIALS AND METHODS

The present study was undertaken at the Department of Pathology Era's Lucknow Medical College, Lucknow. A total of 48 subjects were enrolled for the study. 24 cases of squamous cell carcinoma & 24 healthy age and gender matched healthy controls.

Clinical samples

The whole blood samples were taken from cases and controls. Tissue biopsy from cases for confirmation of diagnosis. With the consent of the patients approx 2.5ml of whole blood sample was collected from cases and controls. Following collection, samples were centrifuged to separate serum which was subjected to analysis. Serum Endothelin was analyzed with ELISA using kit Endothelin (1-21) Biomedica. Results were tabulated and subjected to analysis by SPSS 15 software. Tissue biopsy was taken from case and it was subjected to routine H & E staining procedures by taking appropriate steps on formalin fixed paraffin embedded tissue. Once the procedure was carried out, their results were compared and noted.

RESULTS

For this purpose an observational case-control study was carried out in which a total of 48 subjects were enrolled. A total of 24 patients comprised the case group and were patients of malignant oral lesions. Remaining 24 subjects were normal healthy controls. Demographic information and clinical data was obtained, estimation for Endothelin-1 was performed in serum (Table 2). Majority of cases with oral lesions were aged >40years. Majority of cases were males. Male to female ratio was 3.8:1. In malignant cases tongue was the most common site involved.

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Table 1: Comparison of serum Endothelin-1 values in Squamous Cell Carcinoma Cases and Control

	No	Mean	Std. Deviation	Minimum	Maximum	Median
SCC Cases	24	4.063	1.002	1	5.55	4.00
Controls	24	2.221	0.497	1	3	2.10
Total	48	2.903	1.196	1	5.55	2.475

Range of Endothelin-1 (Table-1) in SCC cases was 1 to 5.55 (median: 4.00) and in controls it was 1 to 3 pgn (median: 2.10). Endothelin-1 levels of SCC cases (4.063+1.002 pgm) were found to be higher than that of Controls (2.221+0.497 pgm).

Endothelin-1 levels of SCC cases was found to be of higher order as compared to other group (figure 1). An overlap in interquartile values of Control cases was found. Extremes and outlier values were found in SCC cases. The difference in Endothelin-1 values of different grades of oral lesions was found to be statistically significant.

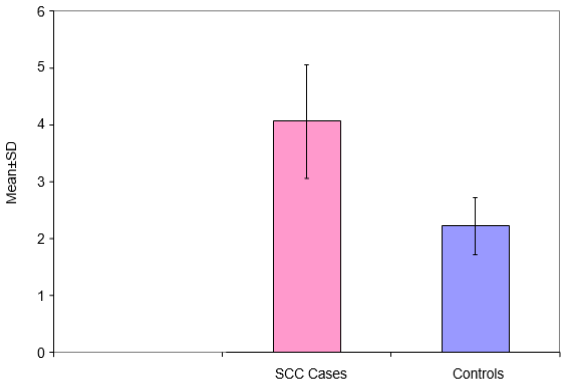

Figure 1: Comparison of serum Endothelin-1 values in Squamous Cell Carcinoma Cases and Control

Table 2: Demographic, Clinical, Histopathological and biochemical data of cases and control; WD- well differentiated MD- Moderately differentiated)

Serial No.	Age	Sex	Chief Complaints	Tobacoo chewing (years)	Clinical Stage	Histolopathology	Grading	Serum ET-1
1	54	Male	Control	control	NA	control		2.2
2	28	Male	Control	control	NA	control		1.98
3	53	Male	Control	control	NA	control		3
4	44	Male	Control	control	NA	control		2.3
5	32	Female	Control	control	NA	control		1.83
6	29	Male	Control	control	NA	control		2.8
7	35	Female	Control	control	NA	control		2.6
8	60	Male	Control	control	NA	control		2
9	34	Female	Control	control	NA	control		1.9
10	40	Male	Control	control	NA	control		3
11	37	Male	Control	control	NA	control		2.6
12	35	Female	Control	control	NA	control		2
13	45	Male	Control	control	NA	control		2.4
14	36	Male	Control	control	NA	control		2.1
15	59	Male	Control	control	NA	control		1.9
16	30	Male	Control	control	NA	control		1.5
17	28	Male	Control	control	NA	control		2.7
18	39	Male	Control	control	NA	control		1
19	42	Male	Control	control	NA	control		2.1
20	30	Female	Control	control	NA	control		2
21	28	Female	Control	control	NA	control		3
22	30	Female	Control	control	NA	control		1.8
23	52	Male	Control	control	NA	control		2.6
24	47	Male	Control	control	NA	control		2
25	30	Male	ulcer right buccal mucosa x 25 days	18	Not done	severe dysplasia		1
26	70	Male	ulceroproliferative growth gingivobuccal sulcus x 5 month	37	T2NOMO	squamous cell carcinoma		4.06
27	65	Male	left tongue ulcer x20 days	45	T2N1MO	squamous cell carcinoma	WD	3.29
28	50	Male	ulcer left lateral border tongue x 3 month	30	T1N0M0	squamous cell carcinoma	WD	3.9
29	34	Male	ulcer over hard palate x 3 month	18	T2NOMO	squamous cell carcinoma	WD	4.6
30	32	Female	ulcer dorsum of tongue x 5 month	15	T2NOMO	squamous cell carcinoma	MD	3.89
31	50	Male	ulcer over lateral border x 1month	30	T2N1MO	squamous cell carcinoma	WD	3.78
32	45	Female	ulcer right tongue x2month	30	T1N0M0	squamous cell carcinoma	WD	3.85
33	27	Male	inability to open mouth x I year	15	T2N1M0	squamous cell carcinoma	WD	3.9
34	48	Female	ulcer right side buccal mucosa X 5 months	30	T2N2MO	squamous cell carcinoma		5.2
35	33	Male	growth right tongue x 4month	14	T2N1M0	squamous cell carcinoma	MD	4.7

36	45	Male	ulcer over right side buccal mucosa x 15 days	18	T1N1M0	squamous cell carcinoma	WD	4
37	35	Male	ulcer right buccal mucosa x 1 month	16	T1N0M0	squamous cell carcinoma	WD	4.6
38	31	Male	ulcer left side tongue x 2 month	10	T2NIMO	squamous cell carcinoma	WD	5.3
39	45	Male	ulceroproliferative growth gingivobuccal sulcus x 5 month	12	T2N0M0	squamous cell carcinoma	WD	4.4
40	45	Male	ulcer hard palate x 20 days	20	T2N0M0	squamous cell carcinoma	WD	4.05
41	45	Male	growth left lateral border of tongue x 1 year	17	T1N0M0	squamous cell carcinoma	WD	4.6
42	42	Male	ulcer right inner upper side of lip x 2 month	7	Not done	squamous cell carcinoma	WD	3.9
43	47	Male	ulceroproliferative growth gingivobuccal sulcus x3 month	23	T2N1M0	squamous cell carcinoma		4
44	35	Male	ulcer left lateral border tongue x 3 month	18	T2N0M0	squamous cell carcinoma	WD	5.55
45	35	Male	ulcer hard palate x 20 days	15	T1N0M0	squamous cell carcinoma	WD	5.12
46	49	Male	ulcer left gingivobuccal sulcus	20	T2N1M0	squamous cell carcinoma	WD	5.53
47	55	Male	ulcer right lateral border tongue	20	T2N0M0	squamous cell carcinoma	MD	5
48	26	Male	ulceroproliferative growth rt buccal mucosa	9	Not done	squamous cell carcinoma		3.7

DISCUSSION

Oral cancer is often preceded by pre-malignant lesions such as oral submucous fibrosis, leukoplakia and lichen planus. An early recognition and timely intervention is the key to successful management of oral cancer and its prevention from attaining a malignant stage. The endothelin axis plays a role in mitogenesis, apoptosis inhibition, invasiveness, angiogenesis and bone remodeling. Increase expression of serum Endothelin -1 has been studied in various human cancers like colon & breast.

Various studies have been done to evaluate role of salivary endothelin in oral cancer. Hoffman *et al.,* (2011) [24] observed in their study that salivary endothelin-1 does not help in differentiation between healthy controls and premalignant/malignant cases or between premalignant and malignant cases. However, findings of Pickering *et al.,* (2007)[25] and Cheng *et al.,* (2011)[26] concluded that Endothelin-1 could serve as a biomarker for oral squamous cell carcinoma. To best of our knowledge significance of serum Endothelin-1 in oral cancer is not studied as yet.

Hussein NA *et al.,* [27] in their study evaluated serum big ET-1 and found out that serum levels in newly diagnosed breast cancer patient was significantly higher than control group & showed a significant decrease after surgery and adjuvant therapy. Asham *et al.,* [28] studied endothelin level in plasma of colon cancer patient and found significantly higher values as compared to controls. Similar observation was made in study done by Peeters *et al.,* & Iman A *et al.* [29, 30] Arun *et al.,* [31] observed significantly higher levels of plasma endothelin-1 in cases of colorectal cancer with & without metastasis as compared to controls. However no study is being done to evaluate serum levels of Endothelin-1 in oral cancer & controls.

In the present study we evaluated and compared the expression of serum Endothelin-1 with an objective to differentiate normal and cancerous lesions. We found out that serum levels Endothelin-1 were maximum in oral cancer (4.063 ±1.002pgm) & minimum in control group (2.221 ± 0.497pgm). Serum Endothelin-1 showed a potential to be a marker for oral squamous cell carcinoma, but the knowledge about serum endothelin-1 level in oral cancer is very limited. Critical serum levels necessary for diagnosis of oral squamous cell carcinoma are yet to be determined as Endothelin level is influenced by various non-neoplastic conditions like gastrointestinal, cardiovascular disorders, respiratory and other metabolic disorders.[32,33]

Shuji Awano *et al.,* [34]did a study to find out role of endothelin system in oral squamous cell carcinoma. They concluded that ET-1, ETAR, ETBR and ECE are expressed in oral squamous cell cancer. They found that ET-1 was produced in higher levels in oral squamous cell cancer cells as compared to normal. ET-1 induces proliferation via ETAR and ETBR.

The present work is inceptive of the study for evaluating possible role serum Endothelin -1 in oral cancer. Further studies with large sample size are recommended in future.

CONCLUSION

Endothelin axis plays a role in growth & progression of various cancers. Significance of serum Endothelin -1 has been studied in various human cancers but its utility in diagnosis and prediction of oral cancer is very little. The present study explored the level of serum Endothelin-1 in oral squamous cell carcinoma and found out that endothelin-1 has a potential to be used as a marker for oral squamous cell carcinoma.

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REFERENCES

1. Elango JK, Gangadharan P, Sumithra S, Kuriakose MA. Trends of head and neck cancers in urban and rural India, Asian Pacific Journal of Cancer Prevention 7.1 (2006): 108–112.
2. Sankaranarayanan R, Ramadas K, Thomas G, *et al.,* Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial. The Lancet 9475(2005): 1927–1933.
3. Shenoi R, Devrukhkar V; Chaudhuri, Sharma BK, Sapre SB, Chikhale A. Demographic and clinical profile of oral squamous cell carcinoma patients: a retrospective study. Indian J Cancer 49.1(2012): 21-6.
4. Bishop JM. Molecular themes in oncogenesis. Cell 64(1991):235–48.
5. Vokes EE *et al.,* Head & neck cancer. N Eng J Med. 324(1993):184.
6. Forastiere A etal: Head and neck cancer. N Eng J Med. (2001):1890.
7. Hickey KA, Rubanyi G, Paul RJ, Highsmith RF. Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. Am J Phisiol 248 (1985):C550–6.
8. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, *et al.,* . A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332 (1988):411–5.
9. Levin ER. Endothelins. N Eng J Med 333(1995):356–63.
10. Joel Nelson, Anna Bagnato, Bruno Battistini, Perry Nisen. The endothelin-1 axis: Emerging role in cancer. Nature Reviews Cancer3 (2003): 110-116 .
11. Bagnato A, Tecce R, DiCastro V, Catt K: Activation of mitogenic signaling by endothelin-1 1 in ovarian carcinoma cells. Cancer Res57 (1997): 1306-1311.
12. Pristivishalil G, Nelson JB. Endothelium-derived factors as paracrine mediators of prostate cancer progression. Prostate44 (2000): 77-87.
13. Masaki T. The endothelin-1 family: An overview. J. Cardiovasc. Pharmacol 35(2000): S3-S5.
14. Motte S, Mcentee K, Naeije R. Endothelin receptor antagonists. Pharmacol Ther 110 (2006):386–414.
15. Bhalla A, Haque S, Taylor I, Winslet M, Loizidou M. Endothelin receptor antagonism and cancer. Eur J Clin Invest39.S2 (2009):74–7.
16. Shah R. Endothelins in health and disease. Eur J Intern Med18.4 (2007):272–2.
17. Khimji AK, Rockey DC. Endothelin – biology and disease. Cell Signal 22.11 (2010):1615.
18. Mangahas CR, dela Cruz GV, Friedman-Jimenez G, Jamal S. Endothelin-1 induces CXCL1 and CXCL8 secretion in human melanoma cells. J Invest Dermatol 125.2 (2005):307–11.
19. Mangahas CR, dela Cruz GV, Schneider RJ, Jamal S. Endothelin- 1 upregulates MCAM in melanocytes. J Invest Dermatol 123.6(2004):1135–9.
20. Salani D, Rosano L, Di Castro V, Spinella F, Venuti A, Padley RJ, *et al.,* . ABT-627, a potent endothelin receptor A antagonist, inhibits ovarian carcinoma growth in vitro. Clin Sci 103.48S (2002);:318S–21S.
21. Rosano L, Salani D, Di Castro V, Spinella F, Natali PG, Bagnato A. Endothelin-1 promotes proteolytic activity of ovarian carcinoma. Clin Sci 103.48S (2002):306S–9S.
22. Rosano L, Varmi M, Salani D, Di Castro V, Spinella F, Natali PG, *et al.,* . Endothelin-1 induces tumor proteinase activation and invasiveness of ovarian carcinoma cells. Cancer Res66.22 (2001): 8340–6.
23. Hoffmann RR, Yurgel LS, Campos MM. Evaluation of salivary endothelin-1 levels in oral squamous cell carcinoma and oral leukoplakia. Regul Pept. 166.1-3 (2011):55–58.
24. Pickering V, Jordan RC, Schmidt BL. Elevated salivary endothelin levels in oral cancer patients–a pilot study. Oral Oncol43.1(2007): 37–41.
25. Cheng YS, Rees T, Jordan L, Oxford L, O'Brien J, Chen HS, Wong D. Salivary endothelin-1 potential for detecting oral cancer in patients with oral lichen planus or oral cancer in remission. Oral Oncol47.12 (2011):1122–6.
26. Hussein NA, Anwar MM, Elkerm Y, Abozaid NA, Rashad MM Evaluation of serum big endothelin-1 for the diagnosis and prediction of disease recurrence in breast cancer patients. J Cancer Res Ther 1 (2013):178–185.
27. Asham E. Increased endothelin-1-1 in colorectal cancer and reduction of tumour growth by ETA receptor antagonism. Br J Cancer.85 (2001): 1759-1763.
28. Peeters CF, Thomas CM, Sweep FC, Span PN, Wobbes T, Ruers TM. Elevated serum endothelin-1-1 levels in patients with colorectal cancer, relevance for prognosis. Int J Biol Markers15.4 (2000): 28- 93.

29. Iman A. Abdel-Gawad, M.D., Hala M.R. Hassanein, M.D., Nahla A. Bahgat, M.D.,Mona A. Abdel Sattar, M.D.,Azza H. El-Sissy, M.D., Maha A. Altaweel, M.D.AndAmany M. Helal, M.D. Study of Endothelin-1 and Vascular Endothelial Growth Factor in Patients with Cancer Colon. Journal of the Egyptian Nat. Cancer Inst20 (2008): 216-223.

30. Arun C, Swift B, Porter KE, West KP, London NJ, Hemingway DM. The role of big endothelin-1-1 in colorectal cancer. Int J Biol Markers17.4 (2002): 268-74.

31. Chalmers GW, Thomson L, Macleod KJ, McGinn BG, McSharry C, Patel KR, Thomson NC. Endothelin-1 levels in induced sputum samples from asthmatic and normal subjects. Thorax 52 (1997):625-627

32. Bovenzi M, D'Agostin F, Rui F, Ambrosi L, Zefferino R. Salivary endothelin and vascular disorders in vibration-exposed workers. Scand J Work Environ Health 34.2(2008):133–141.

33. Shuji Awano, Louise A. Dawson, Alison R. Hunter, Anthony J. Turner and Badar A. Usmani. Endothelin system in oral squamous carcinoma cells: Specific siRNA targeting of ECE-1 blocks cell proliferation Int. J. Cancer 118(2006): 1645–1652.

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