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A comparative account of COX-2 expression in normal oral mucosa, oral leukoplakia and oral squamous cell carcinoma

[1]Dr Gargi Chaudhuri, Assistant Professor, Jagannath Gupta Institute of Medical Sciences and Hospital Kolkata, West Bengal, India

[2]Dr Sanjeet Das, Associate Professor, Department of Oral And Maxillofacial Pathology, Guru Nanak Institute Of Dental Sciences And Research Kolkata, West Bengal, India; Ph. D Scholar JIS University, Kolkata, West Bengal, India

[3]Subhadipa Majumder, Department of Zoology, Head, State Aided College Teacher Category-I, Surendranath College, Kolkata, West Bengal, India

[4]Sanghamitra Sengupta, Professor, Department of Biochemistry, University of Calcutta, Kolkata, West Bengal, India

[5]Mousumi Pal, Professor and Head, Department of Oral And Maxillofacial Pathology, Guru Nanak Institute Of Dental Sciences And Research, Kolkata, West Bengal ,India

[6]Ranjan Rashmi Paul, Professor Emeritus cum Director Research, Department Of Oral And Dental Sciences, JIS University, Kolkata, West Bengal, India

Corresponding Author: Dr Gargi Chaudhuri, Assistant Professor, Jagannath Gupta Institute of Medical Sciences And Hospital Kolkata, West Bengal, India

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Abstract

Background: Oral leukoplakia is a potentially pre malignant disorder which develops in oral epithelial surfaces which is chronically exposed to oral carcinogens including tobacco and alcohol. Literatures suggest a role of inflammatory mediators like COX-2 in oral carcinogenesis.

Aim: The present study compares COX-2 mRNA expression in oral leukoplakia, oral squamous cell carcinoma and normal oral mucosa to understand its possible diagnostic significance.

Method: A total of 32 patients were selected for the study, out of which 27 were Oral leukoplakia patients and, 5 were OSCC patients. Normal tissues were collected from unaffected mucosa. Confirmed diagnosis was made after histopathological analysis of the patients' tissue sample affected with oral leukoplakia and squamous cell carcinoma. COX-2 mRNA expression was determined in normal, oral leukoplakia

and oral squamous cell carcinoma associated tissue samples using qRT-PCR. The data were analyzed with Mann-Whitney test.

Results: An increased level of COX-2 expression in oral leukoplakia (4.3 fold, p value of 0.001) and oral squamous cell carcinoma (2.56 fold, p value of 0.01) compared to normal mucosa were identified.

Conclusion: Elevated levels of COX-2 in oral leukoplakia and oral leukoplakia associated oral squamous cell carcinoma tissue sample suggests that COX-2 may be used as adjunct molecular marker to facilitate early diagnosis and treatment of oral cancer.

Keywords: Oral Leukoplakia, Squamous Cell Carcinoma, dysplasia, COX-2.

Introduction

The term leukoplakia refers to a clinical entity defined by the "World Health Organization" (WHO) as "a white patch or plaque that cannot be characterized clinically or histologically as any other disease"[1]. The incidence of the lesion varied from 1.3 to 2.1 per 1000 individuals in different parts of India but the prevalence of the lesion in the country was found to be 17 per 1000 tobacco users[2].

Leukoplakia is by far the most common oral precancer, representing 85% of such lesions with a worldwide prevalence of 1.5% to 4.3%[3]. Factors indicated for the occurrence of leukoplakia include tobacco, alcohol, chronic infection by Candida, Human Papilloma Virus (HPV), and more recently Epstein Barr virus (EBV), sanguinaria, ultra violet radiation and chronic trauma[4].

Oral leukoplakia has always been a predominantly male disease. Clinically leukoplakia appears as homogeneous and non-homogeneous. The homogeneous pattern refers to lesions with a regular, smooth or rough fissured whitish surface and well-defined edges and the non-homogeneous pattern includes leukoplakias that are associated with an erythematous component (erythroleukoplakia) or a nodular or granular leukoplakia and proliferative verrucous leukoplakia, which has a greater propencity to turn into malignancy[5,6,7,8].

Microscopically, oral leukoplakias are characterized by various degrees of epithelial dysplasia which is characterized by both cellular and nuclear atypia such as: enlarged nuclei and cell, large and prominent nucleoli, increased nuclear cytoplasmic ratio, hyperchromatic nuclei, pleomorphic nuclei and cells, dyskeratosis (premature keratinisation of individual cells), increased mitotic activity, abnormal mitotic figures, bullous or tear-drop shaped rete pegs, loss of polarity and loss of typical epithelial cell cohesiveness.[1]

Dysplasia is universally accepted as one of the predictive factors of malignancy[7] with overall malignant transformation rates of 11 to 36%[9], although its ability to turn into malignancy is still unpredictable. 15% of malignant transformation has been reported in cases without epithelial dysplasia[10,11].

Thus dysplasia is regarded as an important predictor of malignant transformation of leukoplakia, although this transformation is not indispensable for OSCC to occur. Since most oral leukoplakias are asymptomatic, the need for treatment is primarily based on the precancerous properties of the lesion. Prevention of malignant transformation is particularly important in view of the poor prognosis associated with oral cancer, with only 30% to 40% of patients still alive 5 years after the diagnosis. Till date neither the clinical nor the histopathological characteristics made it possible to unravel the mystery of malignant potentiality of leukoplakia. On one hand, epithelial dysplasia does not necessarily progress to squamous cell carcinoma in all cases; on the other, absence of dysplasia histopathologically does not rule out the possibility of malignant transformation and a rate of about 15% of

malignant transformation has been reported in cases without epithelial dysplasia. Thus there is an urgent need for predictive molecular markers that may aid in the analysis of malignant potentiality of oral leukoplakia. In the recent past there is a paradigm shift of research from clinicopathological to molecular assays for assessment of the likelihood of malignant transformation of Leukoplakia and one such marker is COX-2. There are two forms COX-1 and COX-2. COX-1is believed to be involved in the "house-keeping" functions of the body while COX-2, the inducible form is expressed only under special conditions like inflammation, angiogenesis, and tumorigenesis[15,16].

Current research indicates that the malignant process is based on overexpression of COX-2 and prostanoids in transformed cells and malignant tissue of various carcinomas including gastric carcinoma[12], lung cancer, breast cancer and also head and neck cancer[13,14].

Keeping this in mind the present study was designed to evaluate the involvement of COX-2 in the progression of oral leukoplakia and its malignant transformation.

Materials and Methods

The research study was conducted in the Department of Oral and Maxillofacial Pathology, Guru Nanak Institute of Dental Sciences and Research, (GNIDSR), Kolkata in collaboration with Department of Biochemistry, University of Kolkata. The study was approved by BioSafety and Ethics Committee of Guru Nanak Institute Of Dental Sciences And Research.

A total number of 2400 (approximately) patients, visiting Out Patient Department of GNIDSR, Kolkata were screened thoroughly for the presence of Oral Leukoplakia and Oral Leukoplakia associated OSCC according to the clinical criteria laid down by Neville et al., 2009. In the process of this clinical screening a total number of 40 Oral Leukoplakia patients and 5 Oral Leukoplakia associated OSCC patients could be detected. All these patients were subjected to the medical and routine hematological check-up. Two of the patients were severely medically compromised, and excluded from the study. After the above medical screening a total number of 5 Oral Leukoplakia associated OSCC patients and 38 Oral Leukoplakia patients were selected for the study.

Tissue samples (both pathological and normal tissues) were collected from the patients during biopsy procedure. Formalin preserved specimen were subjected to histopathologic evaluation or confirmation of the lesion. Some tissues (both normal and diseased) were preserved in RNAlater (Ambion) for further processing. Histologically validated oral leukoplakia and OSCC were selected for molecular analyses. Relevant clinical parameters were obtained for each sample.

PCR assay

Total RNA was extracted from tissue (>50 mg) using TRI reagent (Sigma Aldrich) according to manufacturer's protocol. One microgram of total RNA was used for cDNA synthesis using random hexamers and High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Inc.). The condition for cDNA preparation was 10 minutes at 25°C, 120 minutes at 37°C followed by heating at 85°C for 5 minutes in a thermal cycler (Applied Biosystems GeneAmp PCR System 9700) and stored at -20°C. A 1:10 fold dilution of cDNA samples were used as template in all quantitative PCR reactions which were carried out in a 10 ml reaction volume with 5 ml of (2X) Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) using Applied Biosystems 7900HT Fast Real-Time PCR System. The thermal cycler was programmed for an initial denaturation step of 5 minutes at 95°C followed by 40 thermal cycles of 30 seconds at 95°C, 30 seconds

at 60°C and 30 seconds at 72°C. The experiments were carried out in triplicate to ensure best reproducibility. 18S rRNA gene was used as endogenous control. Specificity of PCR amplification for each primer pair was confirmed by melting curve analysis. The data were subjected statistical analysis using the standard $2^{-\Delta\Delta Cq}$ method taking normal tissue as reference and 18S rRNA gene expression as endogenous control.

Statistical Analysis

Delta Cq of each group is represented by Box Plot in GraphPad Prism Software. The difference of delta Cq distribution was analysed by Kruskal-Wallis test. Fold change in gene expression was quantified in terms of RQ (Relative Quantification=$2^{-\Delta\Delta Cq}$) value where the ΔCq value of leukoplakia and OSCC was normalized to normal samples and represented by bar diagrams. The difference of expression was analysed by Student's t-test in Graphpad Prism software.

Results

Box plots represent the delta Cq comparing the three conditions; normal, leucoplakia and OSCC. Horizontal bars represent the median and range of the delta Cq for all the samples (fig.1). The results obtained showed that relative transcript level of Cox-2 was significantly elevated (4.3 fold high) in leucoplakia in comparison to normal oral mucosa (fig.2). Similarly, an increase in fold change up to 2.56 was

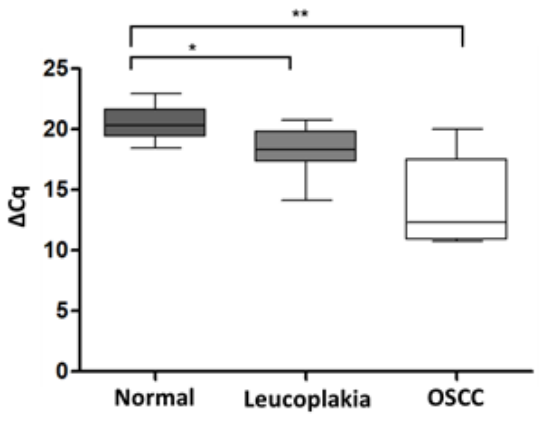

Figure 1: Delta Cq values distribution graphs of Cox-2 in normal and different clinical stages (Leukoplakia and OSCC).

The box covers the two centre quartiles, and the median value is represented as a horizontal line in the box.

*P value <0.001

**P value <0.001

Observed when expression of Cox-2 was compared with normal oral mucosa and squamous cell carcinoma.

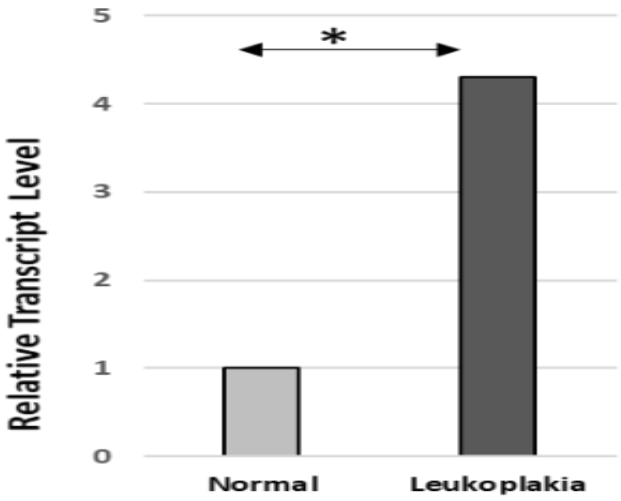

Figure 2: Comparison of Cox-2 gene expression in normal and leucoplakia tissues

Column plots represent the relative transcript level of COX-2 gene in oral leucoplakia with respect to normal oral mucosa.

*P value <0.001

Discussion

Oral cancer is the most common type of cancer of head and neck region, with an annual worldwide incidence in excess of 300000 cases. The disease is an important cause of death and morbidity, with a 5-year survival of less than 50%[17].

Most of oral cancers arise from a pre-cancerous lesion or condition like oral leukoplakia which is present in patients diagnosed with oral squamous cell carcinoma[18].

One of the new approaches for control of this cancer is early detection of oral leukoplakia and its propenicity to convert into malignancy. Various researches have showed that deregulation of COX-2 gene expression occurs in early dysplastic oral tissues. This up-regulation of COX-2 in premalignant cells predisposes the cells to further deregulation of several progression-related gene expression markers and can be rapidly diagnosed by RT-PCR assays[19]. The COX-2 gene is an immediate, early-response gene that is induced by growth factors, oncogenes, carcinogens, and tumor-promoting phorbol esters[20].

Hyper-expression of COX-2 has been implicated in the pathogenesis of colon-rectal cancer in humans but it appears to play a significant role as a tumour progression factor also in other forms of human cancer, including oral cancer[21]. The possible factors responsible for increased expression of COX-2 are shown in the figure 3.

Fig. 3

It has been known through earlier studies that COX regulates expression of various genes in colon cancer[22], non-small cell lung cancer[23], and prostate cancer[24]. Various studies also confirm these earlier reports that constitutive COX-2 expression is an early causal event for tumor initiation through conversion of procarcinogens to carcinogens because of its peroxidase activity, as well as tumor progression because of its COX activity-related gene expression changes[17]. This study also demonstrated that COX-2 was markedly up-regulated in oral squamous cell carcinoma, where a 2.56 fold change was observed when compared to the normal[25]. Several different mechanisms could provide an important link between COX-2 and OSCC. Enhanced synthesis of PGs, a consequence of up-regulation of COX-2 can increase cell proliferation[26], promote angiogenesis[27], and inhibit immune surveillance[28]. All of these effects favour the growth of malignant cells. Additionally, over expression of COX-2 inhibits apoptosis[29] and enhances invasiveness[30]. In extra hepatic tissues, like the head and neck; which have low mixed function oxidase activity[31], COX-2 may also be important for activating xenobiotics to reactive electrophiles that are carcinogenic. For example, COX catalyzes the oxidation of the tobacco procarcinogen benzo[a]pyrene-7, 8-dihyrodiol to benzo [a] pyrene-diol epoxide, which is a highly reactive and strongly mutagenic carcinogen[32]. Thus it is imperative to say a comparative evaluation of COX-2 between normal and OSCC plays a pivotal role in assessing the role of COX-2 in cancer progression.

Further from the above study, it was also found that 60 fold (?) change was observed when comparing expression of COX-2 between leukoplakia and OSCC samples. Although review of English literature failed to reveal any study in this particular aspect relating to differential expression of COX-2 between oral leukoplakia and OSCC, very many literatures had elucidated upregulation of COX-2 in gastric cancer with respect to gastric pre- cancer and its role in malignant transformation[33,34]. In this respect this study was found to be very encouraging. But still further studies are suggested in this field.

In the present study from the results obtained we can clearly see that there was an increase in trend of mRNA expression for COX-2 in Oral leukoplakia affected tissue and in the case of OSCC samples which indicates its role in tumor microenvironment also.

Conclusion

Malignant neoplasms are major causes of fear, morbidity and mortality all over the world. Globally oral cancer is the sixth most common cause of cancer-related death. Oral cancer accounts for approximately 30-40% of all cancers in India. Leukoplakia is the most common oral potentially malignant disorder. It should therefore be perfectly characterized in order to define the high risk variant with greatest potential for malignant transformation. In view of this, identification of various molecular markers should help to more accurately identify high-risk leukoplakias and allow a more aggressive therapeutic approach to be taken in these cases. COX-2 is one such marker which is expressed in both leukoplakia as well as OSCC and it has been paid attention because it could play an important role in initiation and progression of oral carcinoma.

References

1. Subdho J, Reith A. The evolution of predictive oncology and molecular based therapy for oral cancer prevention. Int J Cancer.2005;115:339-45
2. Shafer, G, et al;2006; Benign and Malignant tumours of the Oral cavity. In “Shefer Text Book of Oral Pathology”, Edited by R.Rajendran et al, published by Elsevier; 5th Ed 121-128.
3. Neville BW, Damn DD, Allen CM, Bouquot JE.Oral and Maxillofacial Pathology.3rd ed.India:Elsevier Publishers;2009.p 388-397.
4. Oral Leukoplakia: Clinical, Histopathologic, and Molecular Features and Therapeutic Approach by A.Martorell-Calatayud, R.Bortella-Estrada et al published by Elsevier, 2011 pg 669-684.
5. Bouquot JE, Whitaker SB: Oral leukoplakia-Rational for Diagnosis and Prognosis of its clinical subtypes or “Phrases”Quintessence Int 25:133-140, 1994
6. Silverman S Jr, Gorsky M, Lozada F. Oral Leukoplakia and malignant transformation. A follow up study of 257 patients. Cancer. 1984;53:563-8.
7. Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med 2003;14:47-62.
8. Hansen LS, Olson JA, Silverman SJ. Proliferative verrucous leukoplakia. A long term of study of thirty patients. Oral Surg Oral Med Oral Pathol Pral Radiol Endod.1985;60:285-98.
9. Holmstrup, P, Vedtofte,P, Reibel, J. and Stoltze, K (2006) Long term treatment outcome of oral pre-malignant lesions. Oral Oncol, 42, 461-474.
10. Banoczy J. Follow up studoes in oral leukoplakia. J Maxillofac Surg.1977;5:69-75.
11. Mehta FS, Pindborg JJ, Gupta PC, Daftary DK. Epidemiological and histologic study of oral cancer and leukoplasia among 50915 villagers in India.Cancer.1996;24:832
12. Liu W, Wang YF, Zhou HW, Shi P, Zhou ZT, Tang GY. Malignant transformation of oral leukoplakia: a retrospective cohort study of 218 Chinese patients. BMC cancer. 2010 Dec;10(1):1-6.
13. Wang Z, Chen JQ, Liu JL. COX-2 inhibitors and gastric cancer. Gastroenterology research and practice. 2014 Jan 1;2014.
14. Khan Z, Khan N, P Tiwari R, K Sah N, Prasad GB, S Bisen P. Biology of Cox-2: an application in

cancer therapeutics. Current drug targets. 2011 Jun 1;12(7):1082-93.

15. Celenk F, Bayramoglu I, Yilmaz A, Menevse A, Bayazit Y. Expression of cyclooxygenase-2, 12-lipoxygenase, and inducible nitric oxide synthase in head and neck squamous cell carcinoma. Journal of Craniofacial Surgery. 2013 Jul 1;24(4):1114-7.
16. Luo C, Urgard E, Vooder T, Metspalu A. The role of COX-2 and Nrf2/ARE in anti-inflammation and antioxidative stress: Aging and anti-aging. Medical hypotheses. 2011 Aug 1;77(2):174-8.
17. Khan Z, Khan N, P Tiwari R, K Sah N, Prasad GB, S Bisen P. Biology of Cox-2: an application in cancer therapeutics. Current drug targets. 2011 Jun 1;12(7):1082-93.
18. Zini A, Czerninski R, Sgan-Cohen HD. Oral cancer over four decades: epidemiology, trends, histology, and survival by anatomical sites. Journal of oral pathology & medicine. 2010 Apr;39(4):299-305.
19. Liu W, Shi LJ, Wu L, Feng JQ, Yang X, Li J, Zhou ZT, Zhang CP. Oral cancer development in patients with leukoplakia–clinicopathological factors affecting outcome. PLoS One. 2012 Apr 13;7(4):e34773.
20. Prado SM, Cedrún JL, Rey RL, Villaamil VM, García AÁ, Ayerbes MV, Aparicio LA. Evaluation of COX-2, EGFR, and p53 as biomarkers of non-dysplastic oral leukoplakias. Experimental and molecular pathology. 2010 Oct 1;89(2):197-203.
21. Seyedmajidi M, Shafaee S, Siadati S, Khorasani M, Bijani A, Ghasemi N. Cyclo-oxygenase-2 expression in oral squamous cell carcinoma. Journal of cancer research and therapeutics. 2014 Oct 1;10(4):1024
22. Wang D, DuBois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. Oncogene. 2010 Feb;29(6):781-8.
23. Roelofs HM, Te Morsche RH, van Heumen BW, Nagengast FM, Peters WH. Over-expression of COX-2 mRNA in colorectal cancer. BMC gastroenterology. 2014 Dec;14(1):1-6.
24. Sharma SD, Meeran SM, Katiyar SK. Proanthocyanidins Inhibit In vitro and In vivo Growth of Human Non–Small Cell Lung Cancer Cells by Inhibiting the Prostaglandin E2 and Prostaglandin E2 ReceptorsProanthocyanidins Target COX-2 and PGE2 receptors. Molecular cancer therapeutics. 2010 Mar 1;9(3):569-80.
25. Mandal RK, Mittal RD. Polymorphisms in COX-2 gene influence prostate cancer susceptibility in a northern Indian cohort. Archives of medical research. 2011 Oct 1;42(7):620-6.
26. McCormick DL, Phillips JM, Horn TL, Johnson WD, Steele VE, Lubet RA. Overexpression of cyclooxygenase-2 in rat oral cancers and prevention of oral carcinogenesis in rats by selective and nonselective COX inhibitors. Cancer Prevention Research. 2010 Jan;3(1):73-81.
27. Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. International journal of cell biology. 2010 Oct;2010.
28. Salvado MD, Alfranca A, Haeggström JZ, Redondo JM. Prostanoids in tumor angiogenesis: therapeutic intervention beyond COX-2. Trends in molecular medicine. 2012 Apr 1;18(4):233-43.
29. Göbel C, Breitenbuecher F, Kalkavan H, Hähnel PS, Kasper S, Hoffarth S, Merches K, Schild H, Lang KS, Schuler M. Functional expression cloning identifies COX-2 as a suppressor of antigen-specific cancer immunity. Cell death & disease. 2014 Dec;5(12):e1568-.

30. Khan Z, Khan N, P Tiwari R, K Sah N, Prasad GB, S Bisen P. Biology of Cox-2: an application in cancer therapeutics. Current drug targets. 2011 Jun 1;12(7):1082-93.
31. Bocca C, Ievolella M, Autelli R, Motta M, Mosso L, Torchio B, Bozzo F, Cannito S, Paternostro C, Colombatto S, Parola M. Expression of Cox-2 in human breast cancer cells as a critical determinant of epithelial-to-mesenchymal transition and invasiveness. Expert opinion on therapeutic targets. 2014 Feb 1;18(2):121-35.
32. Masood N, Malik FA, Kayani MA. Expression of xenobiotic metabolizing genes in head and neck cancer tissues. Asian Pac J Cancer Prev. 2011 Jan 1;12(2):377-82.
33. Huovinen M. Effects of benzo (a) pyrene in human breast cancer cell lines related to chemical carcinogenesis (Doctoral dissertation, Itä-Suomen yliopisto).
34. Hong L, Li S, Liu L, Shi Y, Wu K, Fan D. The value of MG7-Ag and COX-2 for predicting malignancy in gastric precancerous lesions. Cell biology international. 2010 Sep;34(9):873-6.
35. Wu WK, Sung JJ, Lee CW, Yu J, Cho CH. Cyclooxygenase-2 in tumorigenesis of gastrointestinal cancers: an update on the molecular mechanisms. Cancer letters. 2010 Sep 1;295(1):7-16.