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ORIGINAL ARTICLE

Relationship among Different Morphological Parameters and Immunohistochemical Expression of Prostaglandin E2 Receptors EP3 in Oral Squamous Cell Carcinoma

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ABSTRACT
To carry out a descriptive analysis of relationship among different morphological parameters and immunohistochemical expression of EP3 receptors of prostaglandin E2 in oral squamous cell carcinoma (OSCC). The study was performed in the Department of Morbid Anatomy and Histopathology University of Health Sciences (UHS). A total of 40 patients presenting with various histological subtypes and grades of OSCC were recruited from Maxillofacial Surgery Department of Nishtar Institute of Dentistry Multan and Dental Section, Punjab Medical College Faisalabad. Clinical and gross examination findings including age, gender, site of tumor and type of lesion had been recorded. Microscopically tumour subtype and histological grading according to Anneroth's grading system was carried out. Immunohistochemical staining with rabbit polyclonal EP3 receptor antibody was performed and sections were scored for intensity tumour cells. In a total of n=40 patients, 24 were males and 16 were females with a male to female ratio of 1.6:1 was observed. Most of patients were in the age group of 40-60 year. Ulceration was the most common presenting complaintand buccal mucosa was involved predominantly (47.5%). Well differentiated tumours form the largest number (60%) followed by moderately differentiated and poorly differentiated tumours. All the 40 cases were positive for EP3 receptors antibody. Only degree of lymphoplasmacytic inflammation has statistically significant co-relation with the expression of EP3 receptors. All the other morphological parameters had non-significant statistical correlation including degree of keratinization, nuclear pleomorphism, number of mitoses/phf, pattern of invasion and stage of invasion. Only degree of inflammation had statistically significant relationship with the expression of EP3 receptors. All other important morphological parameters had non-significant relation with EP3 receptors antibody positivity in histological sections of OSCC.
Key words: PGE2; EP3 antibody; Prostanoid receptors; Oral Squamous Cell Carcinoma; Morphological parameters of OSCC

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INTRODUCTION

Squamous cell carcinomas (SCC) comprise of more than 90% of oral cancers arising in the mucous membranes of the oral cavity and oropharynx [1]. Some of the important morphological parameters of oral squamous cell carcinoma include degree of keratinization, nuclear pleomorphism, number of mitoses/phf, lymphoplasmacytic infiltration, pattern of invasion and stage of invasion. These morphological parameters are related to histological grading and prognosis of OSCC[2].

The TNM staging system is the conventional method for making treatment decisions for OSCC patients. However, these above mentioned histological parameters of tumour have been ignored in TNM staging system and this drawback of TNM staging system has much been criticized by many researchers. Hence to predict the behaviour and prognosis of the tumour, histological grading and clinical staging should be assessed together and this approach may provide much more accurate options for optimal treatment of each and every patient.Therefore, knowledge about histological parameters of OSCC is of utmost importance [3, 4].

One of the important morphological parameters is degree of keratinization in OSCC. Many researchers have demonstrated multiple cytokeratins expression patterns in OSCC and normal squamous epithelia. It is also well documented that cytokeratins are sole biomarkers to observe the differential of epithelial cells as well as in diagnosing tumours of epithelial origins in surgical pathology [5, 6].

Another important morphological parameter is nuclear pleomorphism, which refers to variations in size, shape and colour of the nucleus. In pleomorphic nuclei are hyperchromatic due to increase in DNA synthesis and condensation of chromatin during interphase of cell division. Nuclear pleomorphism shows occurrence of dysplastic and neoplastic changes in tissues like squamous epithelium.Size of the nucleus is directly proportional to its DNA content and the aggregate of histone proteins, organic and inorganic material and water. Numerous studies have shown a progressive increase in nuclear area in the process of transformation which leads to metaplasia, dysplasia and then ultimately to carcinoma [3, 7].

Moreover, inflammatory processes are also reported to be related with several types of cancer. Chemokines are a member of the inflammatory-regulator family that has important roles in inflammation processes, angiogenesis, and metastasis. Therefore, measuring the *degree of lymphoplasmacytic inflammation* is also an important parameter in histological grading and prognosis of OSCC [8].

Among the prostanoids, the E-type PGs, particularly PGE2 derived from arachidonic acid, is produced in the body. It is found in most animal species, and exhibits multipurpose actions. PGE2 performsits functions (cell proliferation, angiogenesis, mutagenesis) via four different receptors i.e. EP1 to EP4. These EPs receptors are actually G protein coupled receptors [9].

PGs play vital role in mechanisms involved in carcinogenesis such as cell proliferation, angiogenesis, apoptosis and mutagenesis [10]. Presently researchers have successfully developed some antagonists against these EP receptors of PGE2 to halt these processes which are necessary in development and proliferation of tumours. Studies on knock out animal models have supported their procarcinogenic role [11]. Hence present study was designed to carry out a descriptive analysis of relationship among different morphological parameters and immunohistochemical expression of EP3 receptors of prostaglandin E2 in OSCC.

MATERIALS AND METHODS

After informed consent, biopsy specimens from 40 patients having undergone various diagnostic and/or surgical resection procedures for OSCC were acquired from the Department of Maxillofacial Surgary, Nishtar Institute of Dentistry (NID) Multan and Dental Section, PMC Faisalabad. Socio-demographic information was obtained along with relevant clinical, laboratory and radiological information. To confirm the diagnosis of OSCC 4µm thick, paraffin embedded tissue sections OSCC were cut and mounted on glass slides, sections were stained with haematoxylin and eosin stain and examined by light microscope. Anneroth's histological grading system was applied to determine grade of OSCC by using H&E staining [2].

About 4µm thick tissue sections were cut from all specimens of OSCC and mounted on positively charged glass slides. Sections were deparaffinized in xylene and rehydrated in graded ethyl alcohol, followed by immersion in citrate buffer solution of pH 6.0 and were put in the microwave oven before staining procedures. For immuno-staining, Universal kit (Lab Vision, USA) employing the streptavidin biotin system was used to carry out the peroxidase anti- peroxidase method of immunohistochemistry staining. Tissue sections were then incubated with a primary polyclonal anti EP3 receptor antibody (Anti-PTGER3 antibody; ABCAM, USA) and DAB chromogen was applied to the sections followed by counter staining with hemotoxylin [12]. The intensity of the EP3 immunohistochemical staining was evaluated using a light microscope. The intensity of cytoplasmic staining was graded from 0 to 3 (0: negative, 1: weak, 2: moderate, 3: strong) [13]. All the sections were then evaluated by three experienced histopathologists. The data was entered and analysed using SPSS 20.0.Comparisons between clinical and microscopic parameters were performed with thestudent t-test. A difference of $p \leq 0.05$ was considered as statistically significant. The present study was approved by the Ethical Review Committee and Advanced Studies and Research Board of UHS Lahore.

RESULTS

A total of 40 cases were included in the present study in accordance with the inclusion and exclusion criteria. Some of the important clinical findings, histological grading of OSCC and association between EP3 staining intensity and histological grading is given in tables 1, 2 & 3.

Among 40 cases of OSCC, 27 cases showed >50% degree of keratinisation (figures 1,2 & 5) followed by 20-50% degree of keratinisation (figure 3) in 8 cases and 5-20% degree of keratinisation (figure 4) only in 5 cases (p=0.385). In 40 cases of OSCC, 25 cases showed 25-50% mature cells with high degree of nuclear pleomorphism followed by 50-75% mature cells in 12 cases and only >75% mature cells, 0-25% mature cells in 2 and 1 cases respectively (p=0.234). Most cases (37 cases) showed >5 mitoses/hpf (figure 4) followed by 4-5 mitoses/phf in 2 cases and 2-3 mitoses/phf only in 1 case (p=0.601).While intensity of EP3 antibody staining was randomly distributed in relation with degree of keratinisation, nuclear pleomorphism and number of mitoses/phf.

Small groups/cords of infiltrating cells (n>15 cells) with moderate intensity of EP3 staining was the most common (17 cases) pattern of invasion followed by infiltrating solid cords bands, strands type of pattern in 14 cases. As regarding stage of invasion, invasion below lamina propria adjacent to muscles (figure 4), salivary gland tissues and periosteum was the most common (18 cases) stage of invasion followed by distinct invasion, but involving lamina propria (figure 1) only (9 cases), extensive and deep invasion replacing most of the stromal tissue and infiltrating jaw bone (5 cases) andcarcinoma in situ and or questionable invasion in only 1 case of OSCC. These two parameters of histological grading of malignancy of tumour-host relationship could not be assessed in 7 cases because of incisional biopsy samples with missing deeper connective tissue margins.

Moderate degree of inflammation (figure 1) was present in 22 cases followed by marked and slight degrees of inflammation 10 and 8 cases respectively. When we observe the statistical relation between degree of lymphoplasmacytic infiltration and intensity of EP3 receptors antibody, it was found to be statistically significant with p value 0.016 which is <0.05. The table 4 is showing the association among different morphological parameters of OSCC and intensity of EP3 receptors antibody staining (figures 6,7,8 & 9).

Figure 1: Well differentiated OSCC showing abundant keratinization, moderate degree of inflammatory infiltrate and dysplastic squamous epithelial cells encroaching the lamina propria singly and small islands (H & E, x10).

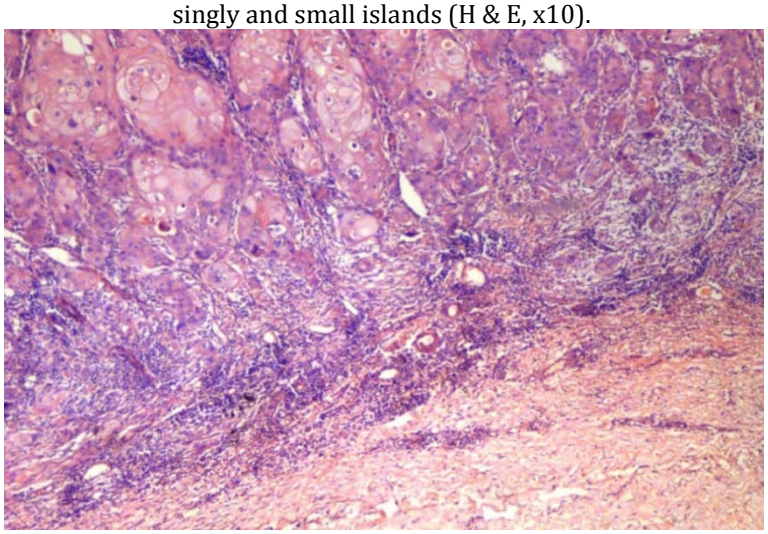

Figure 2: Well differentiated OSCC showing intense inflammatory and abundant keratinization with overlying hyperplastic and hyperchromatic epithelium (H & E, x10).

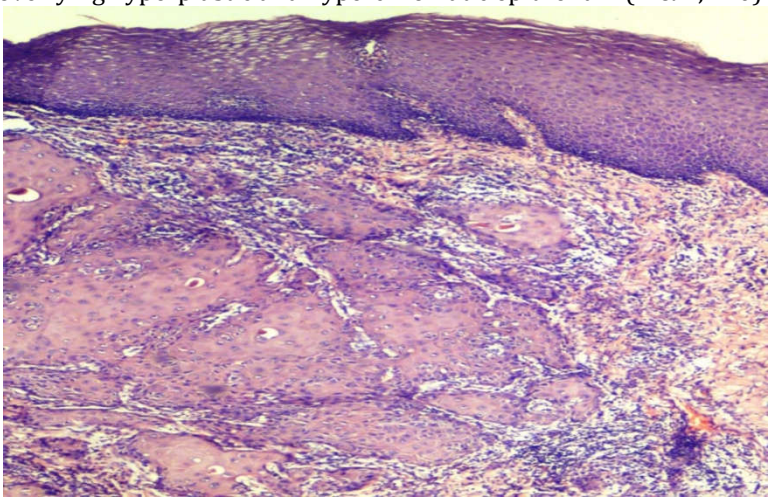

Figure 3: Moderately differentiated OSCC showing nuclear pleomorphism and many atypical mitoses (H & E, x20)

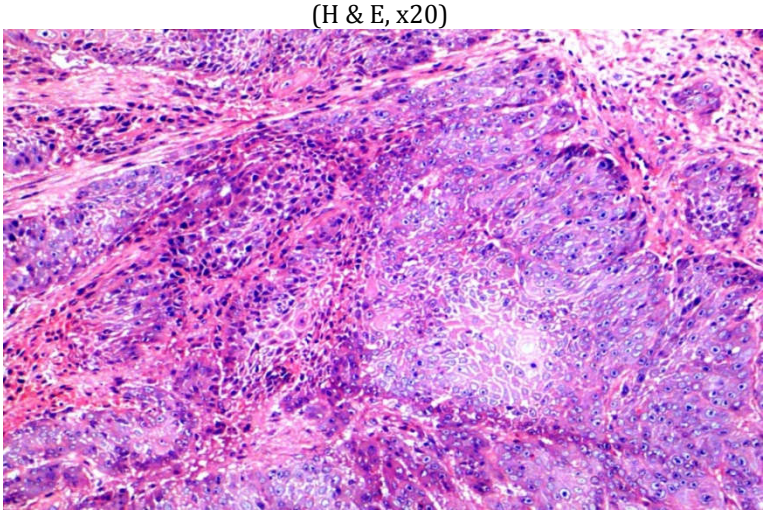

Figure 4: Poorly differentiated OSCC high degree of nuclear pleomorphism, intense inflammatory infiltrate and dysplastic squamous epithelial cells invading the skeletal muscle fibers (H & E, x20)

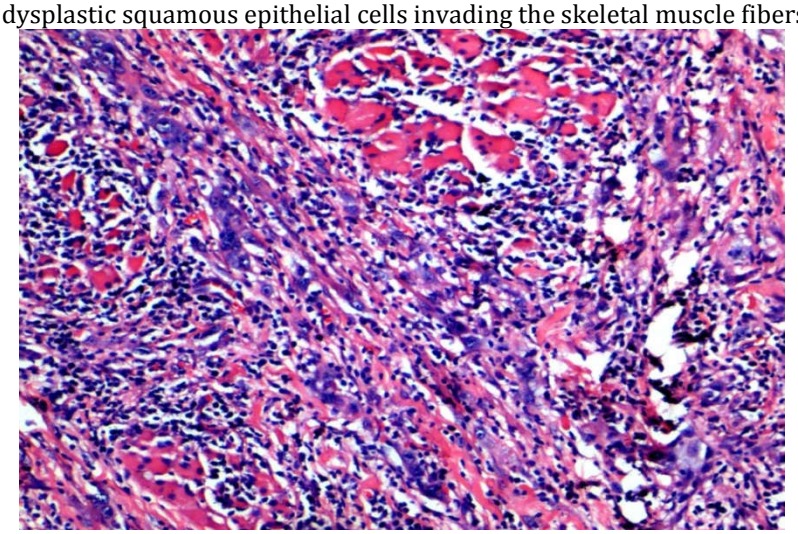

Figure 5: Verrucous subtype of OSCC showing prominent keratin pearl formation (H & E, x10)

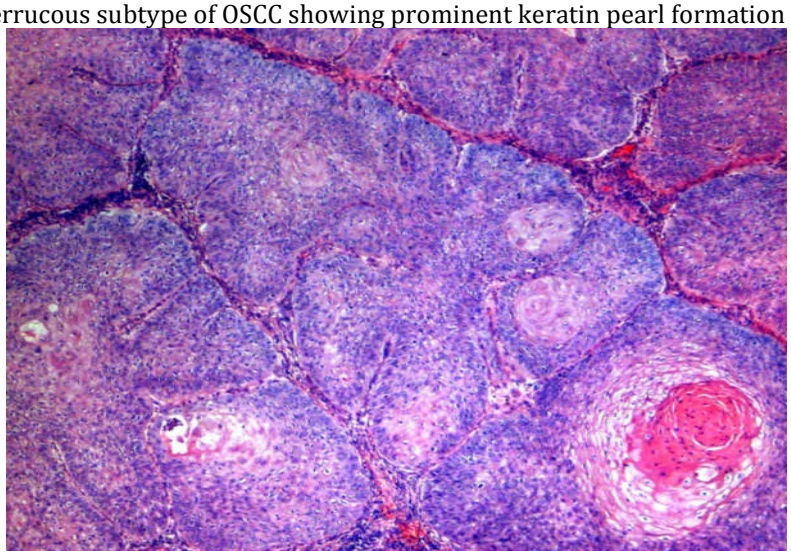

Figure 6: Well differentiated OSCC showing prominent keratin pearl and mild degree of nuclear pleomorphism (H & E, x10)

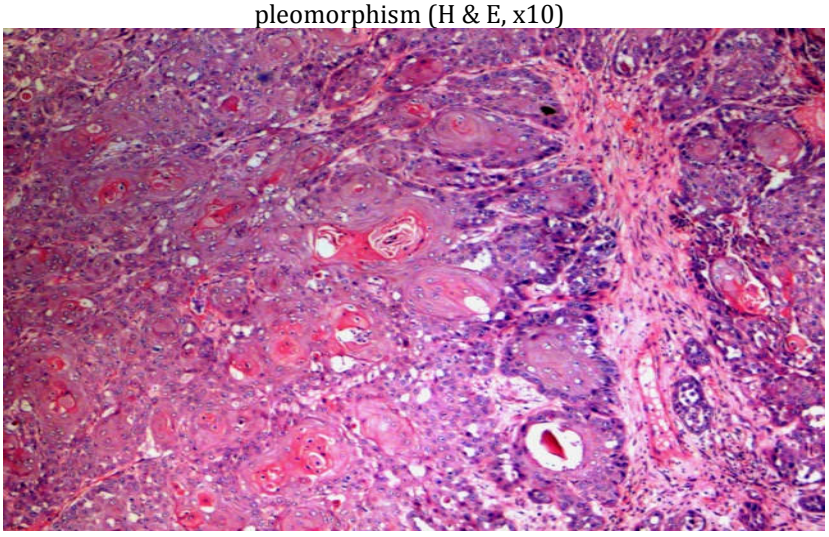

Figure 7: Well differentiated OSCC showing moderate intensity of EP3 receptors antibody staining (IHC, x20)

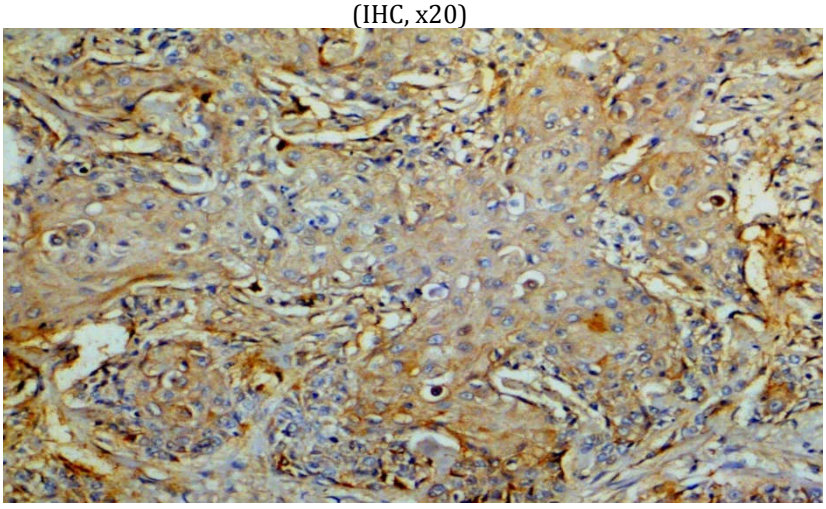

Figure 8: Well differentiated OSCC with moderate degree of inflammatory infiltrate showing moderate degree of EP3 receptor antibody staining (IHC, x10)

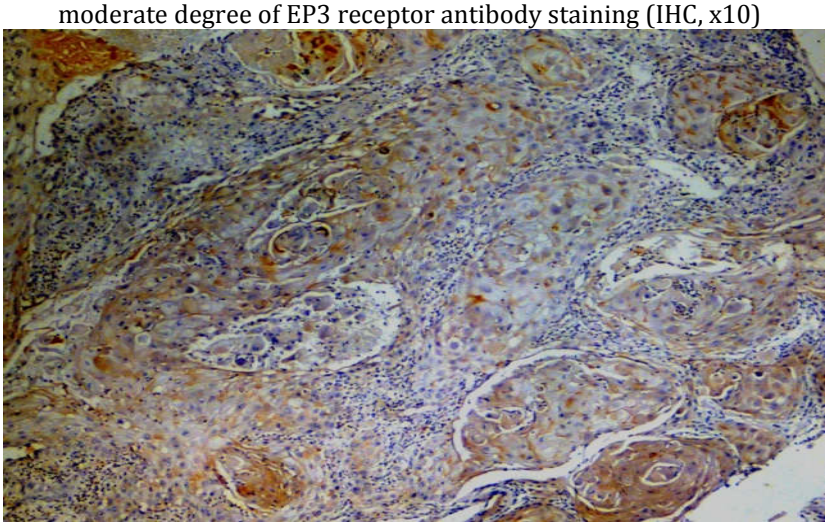

Figure 9: Moderately dfferentiated OSCC with moderate degree of nuclear pleomorphism showing moderate intensity of EP3 antibody staining (IHC, x20)

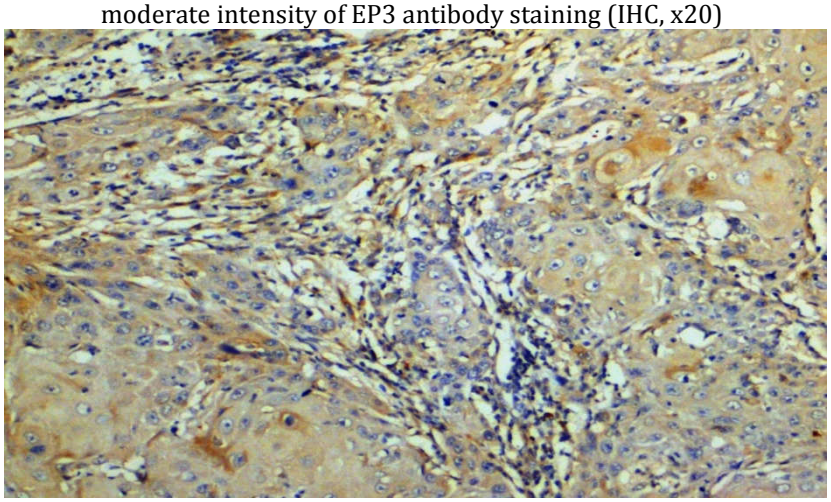

Table 1: Clinical background of the patients
Total Cases=40

Characteristics	Number of cases
Sex	
Male	24 (60%)
Female	16 (40%)
Age	
Below 40 years	6 (15%)
40 Years or above	34 (85%)
Std. Deviation	±14.865
Primary site of the tumor	
Buccal mucosa	19 (47.5%)
Tongue	12 (30%)
Lip	6 (15%)
Floor of mouth	1 (2.5%)
Palate	2 (5%)
Clinical presentation	
Ulceration	29 (72.5%)
Fungating mass	8 (20%)
Plaque like lesion	3 (7.5%)

Table 2: Histological Grading of OSCC
Total cases=40

Grades of tumor	Number of Cases
Well Differentiated OSCC	24 (60%)
Moderately Differentiated OSCC	13 (33.3%)
Poorly Differentiated OSCC	3 (6.6%)

Table 3: Association of intensity of EP3 receptors antibody staining with histological grades of OSCC

Anneroth's Histological Grade	Intensity of EP3 staining in tumor tissue			Total
	weak	moderate	strong	
Well differentiated	8	6	9	23
Moderate differentiated	6	5	3	14
Poor differentiated	0	2	1	3
Total	14	13	13	40

*Intensity of EP3 anti body staining was insignificantly associated with histological grade of OSCC (p=0.615).

Table 4: Association of Morphological parameters of OSCC with intensity of EP3 receptors antibody staining
Total cases=40

Morphological parameters of OSCC	*p* value
Degree of keratinisation	0.385
Nuclear pleomorphism	0.234
Number of mitosis/phf	0.601
Pattern of invasion	0.898
Stage of invasion	0.687
Lymphoplasmacytic infiltration	**0.016**

DISCUSSION

Although there are advancements in preventive, diagnostic, prognostic and treatment modalities for OSCC but researchers still do not have complete knowledge about the underlying mechanisms involved in the progression of normal squamous epithelium towards squamous cell carcinoma. Highest percentage of OSCC was observed in the age group of 40-60 years where males (60%) outnumbered females (40%). Quite similar findings were reported in studies carried out by Khandekar (2006) and Mohammad (2011) in India. They reported a male predominance and highest incidence in the age group of 40 to 65 years o [14, 15]. Other studies carried out in Sri Lanka by Dissanayaka (2012) and Tahir (2013) in Pakistan reported that the most common site of involvement for OSCC was buccal mucosa followed by tongue. A similar finding has been observed in the present study [16, 17]. The study by Dissanayaka in 2012 also reported that degree of keratinisation was not significantly associated with the five years survival rate while stage and pattern of invasion of OSCC and host response were associated significantly with the survival rate [16].

A study carried out by Abrahao and his co-worker in 2010reported that all the four receptors of PGE2 are widely expressed both in HNSCC cell lines and paraffinized tissue sections with expression of EP3 receptors in 94% of the samples [13]. While in the present study 100% of the paraffinized OSCC samples are positive for EP3 receptors. When we observed the association among immunohistochemical expression of EP3 receptors and morphological parameters of OSCC, only degree of lymphoplasmacytic inflammation was associated with EP3 positivity significantly with p value 0.016 (<0.05), all other morphological parameters including degree of keratinization, nuclear pleomorphism, number of mitoses/phf, pattern and stage of invasion were not significantly associated (p=>0.05). COX-2 which is a rate limiting enzyme involved in the production of PGE2 in HNSCC, tumoural and stromal cells may widely express COX-2 due to inflammatory processes in tumour micro environment but it has also been elucidated in many studies that HNSCC cells which express EP receptors, do not always express COX-2 but

still they can respond to PGE2 mitogenically [13, 18, 19]. Therefore, significant association of PGE2 receptor EP3 with the degree of lymphoplasmacytic inflammation was not just due to inflammatory reaction rather it might be due to some mitogenic signals as have been reported in different studies as mentioned previously. Non-significant associations among other morphological parameters of OSCC and expression of EP3 receptors positivity in the present study might be due to small sample size. Therefore, further studies, are needed on larger sample size that may suggest some significant associations among immunohistochemical positivity of EP3 receptors and morphological parameters of OSCC.

CONCLUSION

Immunohistochemical findings of the present study predicted that prostanoid EP3 receptors are widely but variably expressed in OSCC but only degree of inflammation has a significant statistical relationship with the expression of EP3 receptors. All other important morphological parameters have non-significant relationship with EP3 receptors antibody positivity in histological sections of OSCC. Further studies are needed on a larger sample size that should focus on molecular and genetic pathways to explore underlying molecular mechanisms to assess the role of PGE2 in OSCC.

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CONFLICT OF INTEREST

None to declare

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