Original Article - In-Vitro Study

Immunohistochemical Expression of Bcl-2, E-cadherin, CD34 and CD105 in Basaloid Squamous Cell Carcinoma - An *In Vitro* Study

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Abstract

Introduction: Basaloid squamous cell carcinoma (BSCC) is a rare aggressive variant of oral squamous cell carcinoma (OSCC) with a high propensity for distant metastasis. In this article, we present clinicopathological and survival data of eight cases of BSCC and further analyse the behaviour of these tumours with the help of E-cadherin, CD34, CD105 and B cell lymphoma-2 (Bcl-2) immunoexpression. **Materials and Methods:** Histopathologically confirmed cases of BSCC were retrieved from the department archives. Clinicopathological details and survival data of these patients were collected. Immunohistochemical analysis was performed with Bcl-2, E-cadherin, CD34 and CD105 on these cases and compared with different grades of OSCC (well differentiated, moderately differentiated and poorly differentiated). The statistical analysis was done using IBM SPSS software version 23. **Results:** BSCC was seen commonly in males of age group 49–71 years and predominantly reported in the retromolar trigone. Bcl-2 expression was significantly lower in BSCCs when compared to the conventional OSCC groups ($P < 0.05$). E-cadherin expression showed no significant difference between BSCC and well-differentiated OSCC group ($P = 0.487$). The overall mean survival for patients with BSCC was 6.37 months. **Discussion:** BSCCs of the oral cavity show increased CD105, CD34, E-cadherin and low Bcl-2 labelling. A substantial relationship between the tumour neo-angiogenesis, collective cell migration and apoptotic property could be related to the aggressive nature of this tumour and its poor overall survival rate. BSCCs are common in middle to older aged male and show increased expression of CD105, CD34 and E-cadherin.

Keywords: Basaloid oral squamous cell carcinoma, Bcl-2, CD105, CD34, E-cadherin

Introduction

Basaloid squamous cell carcinoma (BSCC) is a high-grade, aggressive malignancy of the head-and-neck region.[1] BSCCs are rare in the oral cavity and represent <1% of all oral squamous cell carcinoma (OSCC).[2] Various genetic mechanisms including tumour angiogenesis, dysregulation of cell cycle regulators and lack of apoptotic mechanisms are assumed to play a significant role in the aggressive behaviour of BSCCs.[3] Histologically, it is a dimorphic tumour with basal and squamous components, along with areas of comedonecrosis and prominent peripheral palisading cells.[3]

Malignant tumours are dynamically shaped by a coordinated bidirectional signalling/communication between the tumour cells and the extracellular matrix (ECM). Apoptotic regulation of the cells, tumour neoangiogenesis and epithelial–mesenchymal transition (EMT) play important roles in the modification of ECM. Angiogenesis, a key hallmark of carcinogenesis, shows complex proliferation of endothelial cells through various growth factors such as fibroblast growth factor, transforming growth factor beta (TGF-β) and vascular endothelial growth factor along with their ligands.[4] Angiogenesis changes the tumour environment by shifting the avascular state to a vascularised phase.[5] This angiogenic switch is well known to initiate EMT by reducing the expression of E-cadherin in the malignant cells. Decreased expression of E-cadherin allows

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the cells to migrate and invade the adjacent stroma, initiating EMT in cancers.[6] This reduction of E-cadherin expression is associated with the apoptotic response of the tumour cells and several other stress-related signalling pathways.[7] Hence, angiogenesis, antiapoptotic mechanisms and EMT are interlinked and play an integral process in carcinogenesis.

Immunohistochemical markers are used to evaluate the significance of angiogenesis, apoptosis and EMT in malignant tumours. CD34 and CD105 are markers used to identify the endothelial cells and understand the process of angiogenesis.[8] CD105 activates angiogenesis through a receptor of the TGF-β pathway. CD105 is considered a superior marker for tumour angiogenesis, as this marker does not stain normal tissues and is expressed only in the endothelial cells involved in tumour neo-angiogenesis.[8,9] B cell lymphoma-2 (Bcl-2) is a very commonly used antiapoptotic marker. Overexpression of this protein promotes cell survival and initiates carcinogenesis in various head-and-neck tumours.[10] The EMT can be studied by evaluating the E-cadherin expression in the tumour cells in the invasive tumour front (ITF). E-cadherin, a 120 kDA transmembrane protein, is a calcium-dependent cell–cell adhesion molecule that is involved in homotypic and homophilic cell adhesion.[11] Combined evaluation of the aforementioned markers could provide an insight into the biological characteristics of BSCC. In the present study article, we presented clinicopathological and survival data of BSCC and further analysed the behaviour of this tumour with the help of immunohistochemical markers through Bcl-2, E-cadherin, CD34 and CD105.

Materials and Methods

This retrospective study was conducted in the Department of Oral Pathology and Oral Oncology in a tertiary oral cancer centre in South India. Ethical clearance was obtained from the institutional ethical committee before the commencement of the study (SRB/SDC/FACULTY/23/OPATH/029). Only histopathologically proven cases of BSCC with detailed clinical and demographic details were included in this study. Post-radiotherapy, post-chemotherapy and recurrent cases were excluded from the study. Clinico-demographic details of patients diagnosed with BSCC were collected from the institutional electronic database records. Haematoxylin and eosin (H&E) slides were retrieved and re-evaluated by two pathologists for reconfirmation. The demographic details including age, gender, site, laterality, clinical presentation, histopathological features, follow-up details and the survival data of all the included patients were recorded and tabulated in Microsoft Excel Spread sheet 2021.

Thirty-two cases, eight each of BSCC, well-differentiated squamous cell carcinoma (WDSCC), moderately differentiated squamous cell carcinoma (MDSCC) and poorly differentiated squamous cell carcinoma (PDSCC) were subjected to E-cadherin, CD34, CD105 and Bcl-2 immunohistochemical procedure. Immunohistochemistry (IHC) was performed according to the standard operating protocol of the laboratory.[12] All the slides were analysed independently by two oral pathologists.

The tumour islands were evaluated at the ITF for Bcl-2 and E-cadherin staining. The ITF was analysed as this is considered the active site of the tumour with high proliferative activity. The percentage of positive cells was counted and the intensity of staining was grouped based on the criteria described by Klein *et al*.[13] All the slides were assessed out of a total score of 6. The parameters analysed were the percentage of antibody-labelled cells and the intensity of the IHC staining. The percentage of IHC labelled cells were recorded as 0 (0%), 1 (<30%), 2 (30%–60%) and 3 (>60%). Intensity of staining was evaluated as 0 (nil), 1 (weak), 2 (mild) and 3 (strong). A total score of 0–1 was considered negative, 1–2 signifies mild, >2–4 signifies moderate and >4–6 signifies severe.

Three vascular hotspots at the invasive front were selected after evaluating the entire tumour following which CD34 and CD105 immunohistochemical positivity was evaluated.[14] The number of CD34 and CD105 positive microvessels was counted at ×400. A single positive endothelial cell or its cluster even without proper lumen was considered a separate microvessel. The average of three values denoted the mean vascular density (MVD) of a given case.[14]

All the values were added to the Microsoft Excel spread sheet 2021. The statistical analysis between the two groups was carried out using SPSS version 23 software (IBM Corp., Armonk, New York, United States). Kappa statistics was performed to measure the inter-rater reliability. Descriptive analysis and independent *t*-tests were used for comparison. $P \leq 0.05$ was considered statistically significant. The serial time duration and the event for each subject were noted. The Kaplan–Meier test and log survival function were used to evaluate the overall survival of the included patients.

Results

Clinico-demographic details of the patients reported with BSCC are tabulated in Table 1. All the patients were males of the age group of 49–71 years. The mean age of occurrence was 62.07 years (±9.64: standard deviation; 64: median; 28: range). BSCCs were commonly reported on the retromolar trigone (3 cases), followed by the floor of mouth (two cases). One case each was reported on the soft palate, buccal mucosa and lower alveolus. The left side was more affected than the right (5 cases – left and 3 cases – right). All the patients had a history of tobacco consumption in various forms.

All the cases showed islands of basaloid malignant epithelial cells with peripheral palisading of cells and central areas of comedonecrosis [Figure 1]. The basaloid cells had increased nuclear–cytoplasmic ratio, hyperchromatic nuclei, scanty cytoplasm, pleomorphism and increased mitotic activity. Two cases showed occasional squamous differentiation. Hyalinisation around the tumour islands and clefting between the tumour and the stroma were also noted in many areas.

Table 1: Clinico-demographic details of the patients included in the study

Gender	Age	Site	Laterality	Clinical details	Habits	Diagnosis
Male	50	Floor of mouth	Left	Proliferative	Tobacco chewing	BSCC
Male	60	Buccal mucosa	Right	Ulceroproliferative	Cigarette smoking and alcohol consumption	BSCC
Male	49	Soft palate	Left	Ulcerative	Pan chewing	BSCC
Male	77	Lower alveolus	Right	Ulceroproliferative	Tobacco chewing	BSCC
Male	64	Retromolar trigone	Left	Ulceroproliferative	Cigarette smoking	BSCC
Male	64	Floor of mouth	Left	Ulcerative	Pan chewing and gutka chewing	BSCC
Male	67	Retromolar trigone	Left	Ulceroproliferative	Cigarette smoking	BSCC
Male	71	Retromolar trigone	Right	Ulceroproliferative	Cigarette smoking	BSCC
Female	75	Maxillary alveolus	Right	Ulceroproliferative	Pan chewing	WDSCC
Female	79	Lateral border of tongue	Left	Ulceroproliferative	Tobacco chewing	WDSCC
Male	58	Buccal mucosa	Right	Ulceroproliferative	Tobacco chewing	WDSCC
Female	60	Buccal mucosa	Right	Ulcerative	Pan chewing	WDSCC
Female	50	Lateral border of tongue	Right	Ulceroproliferative	Tobacco chewing	WDSCC
Female	50	Lateral border of tongue	Right	Ulcerative	Pan chewing and cigarette smoking	WDSCC
Male	61	Retromolar trigone	Right	Ulceroproliferative	Tobacco chewing	WDSCC
Male	47	Lateral border of the tongue	Right	Ulceroproliferative	Pan chewing	WDSCC
Male	50	Gingivobuccal sulcus	Right	Ulcerative	Pan chewing	MDSCC
Male	62	Gingivobuccal sulcus	Left	Proliferative	Tobacco chewing	MDSCC
Male	54	Gingivobuccal sulcus	Right	Ulceroproliferative	Pan chewing and cigarette smoking	MDSCC
Male	60	Gingivobuccal sulcus	Right	Ulceroproliferative	Pan chewing	MDSCC
Male	62	Lateral border of the tongue	Right	Ulcerative	Tobacco chewing	MDSCC
Male	57	Mandibular alveolus	Right	Proliferative	Pan chewing	MDSCC
Male	55	Buccal mucosa	Left	Ulceroproliferative	Pan chewing and cigarette smoking	MDSCC
Male	54	Buccal mucosa	Right	Ulceroproliferative	Tobacco chewing	MDSCC
Male	48	Buccal mucosa	Right	Ulceroproliferative	Pan chewing	PDSCC
Male	65	Retromolar trigone	Left	Ulceroproliferative	Pan chewing	PDSCC
Male	49	Retromolar trigone	Left	Proliferative	Pan chewing and cigarette smoking	PDSCC
Male	70	Buccal mucosa	Left	Ulceroproliferative	Pan chewing	PDSCC
Male	52	Buccal mucosa	Left	Ulceroproliferative	Tobacco chewing	PDSCC
Male	60	Buccal mucosa	Right	Ulceroproliferative	Pan chewing and cigarette smoking	PDSCC
Male	61	Hard palate	Left	Ulcerative	Pan chewing	PDSCC
Female	80	Mandibular alveolus	Left	Ulceroproliferative	Tobacco chewing	PDSCC

BSCC: Basaloid squamous cell carcinoma, WDSCC: Well differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma

Kappa statistics showed strong agreement between the two observers ($k = 8.3$). It was noted that the Bcl-2 expression was significantly lower in BSCCs when compared to the WDSCC, MDSCC and PDSCC group ($P = 0.003$, $P = 0.003$ and $P = 0.001$, respectively). Four cases of BSCC showed moderate and four showed negative Bcl-2 expression. whereas four cases of WDSCC showed moderate and four cases showed strong Bcl-2 expression. Four cases of MDSCC showed moderate expression and four showed strong Bcl-2 expression. The PDSCC group showed strong Bcl-2 expression in five cases and moderate expression in three cases.

With regard to E-cadherin expression, we observed no significant difference between BSCC and WDSCC groups ($P = 0.487$). Three cases of BSCC showed strong E-cadherin expression, three showed moderate and two cases showed negative expression. Although not statistically significant, the mean value was higher in BSCC when compared to WDSCC (3.50 ± 1.85 and 2.87 ± 1.64, respectively). A significant difference was noted in the E-cadherin expression between BSCC and MDSCC and PDSCC ($P = 0.05$ and $P = 0.019$, respectively). Decreased expression of E-cadherin was noted in MDSCC and PDSCC when compared to BSCC. The mean value of E-cadherin was 2 ± 0.75 in MDSCC and 1.6 ± 0.74 in PDSCC. It was noted that E-cadherin expression decreased from BSCC to WDSCC followed by MDSCC and PDSCC.

Quantification of CD34 and CD105 expression was performed by calculating the MVD in the ITF of the carcinoma. The MVD score of CD34 was statistically higher in BSCC when compared to the WDSCC ($P = 0.002$; mean score: 16.62 ± 1.99 and 12.62 ± 2.32, respectively). When BSCC was compared with MDSCC and PDSCC, no significant difference was noted between the two groups ($P = 0.086$ and $P = 0.116$, respectively). The mean score of MDSCC was 14.1 ± 3.27 and PDSCC was 18.62 ± 2.72.

Furthermore, the MVD expression score of CD105 was also higher in BSCC when compared to the WDSCC group (10.87 ± 2.03 and 6.62 ± 3.2, respectively). This difference was statistically significant with $P = 0.007$. There

was no significant difference in the expression of CD105 between BSCC and MDSCC and PDSCC (P = 0.544 and P = 0.884). The mean score of MDSCC was 10 ± 3.42 and PDSCC was 11.12 ± 4.32. Figure 2 shows Bcl-2, CD34, and CD105 immunoexpression in BSCC, WDSCC, MDSCC and PDSCC.

The overall mean survival of 32 patients was 19.64 months and median 20 months. Individually, the overall survival for patients with BSCC (mean: 6.37 and median: 5.0) was significantly lower than WDSCC (mean: 32.79 and median: 31), MDSCC (mean: 19.81 and median: 20) and PDSCC (mean: 13.16 and median: 11). The log rank (Mantel–Cox) showed a statistically significant difference between the groups with P = 0.00. The Kaplan–Meier curve is represented in Figure 3.

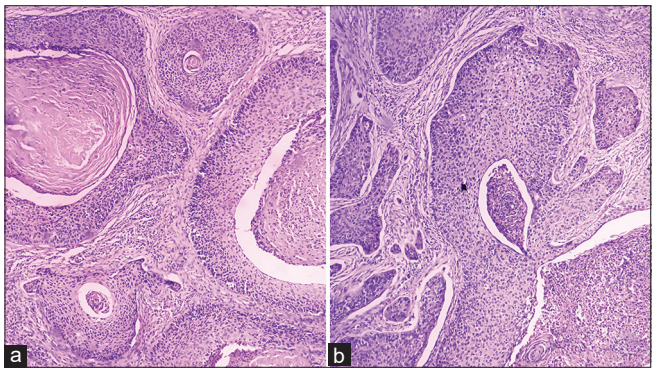

Figure 1: Haematoxylin and Eosin stained sections (a and b) showing basaloid malignant epithelial neoplasm with peripheral palisading of cells and central areas of comedonecrosis (×40)

Discussion

BSCC is an uncommon malignancy of oral cavity with an unfavourable clinical outcome.[1] Multimodality treatment including surgical excision, radial neck dissection, chemotherapy and radiotherapy is preferred for these patients as they show poor clinical prognosis with early metastasis. In this study, the clinicopathological details and survival data of eight BSCCs are reported. We found that BSCC is common in middle-to-older-aged males. A similar preponderance was obtained by Fritsch *et al*.[15] These authors reported the median age of BSCCs to be 64.7 years.[15] Site-specific analysis of BSCCs by Fritsch *et al*., revealed differences in disease-specific survival (DSS) of the patients.[15,16] Patients with BSCCs in the oropharynx showed better DSS than the larynx.[15,16] Out of eight patients presented here, three patients reported the lesion on the retromolar trigone. Clinically, patients report with ulcerative or ulceroproliferative lesions. The aetiology of BSCC is similar

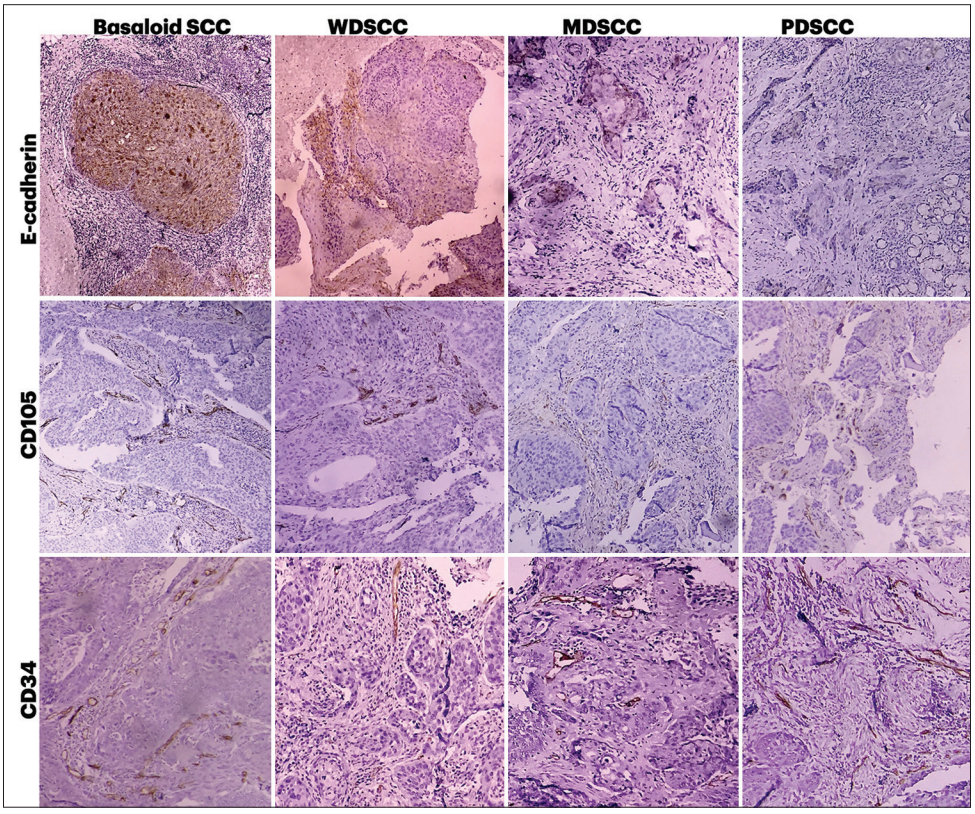

Figure 2: Immunohistochemical expression of E-cadherin, CD105 and CD34 in BSCC, WDSCC, MDSCC and PDSCC (100X). Column 1 shows E cadherin, CD105 and CD34 in BSCC. Column 2,3 and 4 shows E-cadherin, CD105 and CD34 immunoexpression in WDSCC, MDSCC and PDSCC respectively

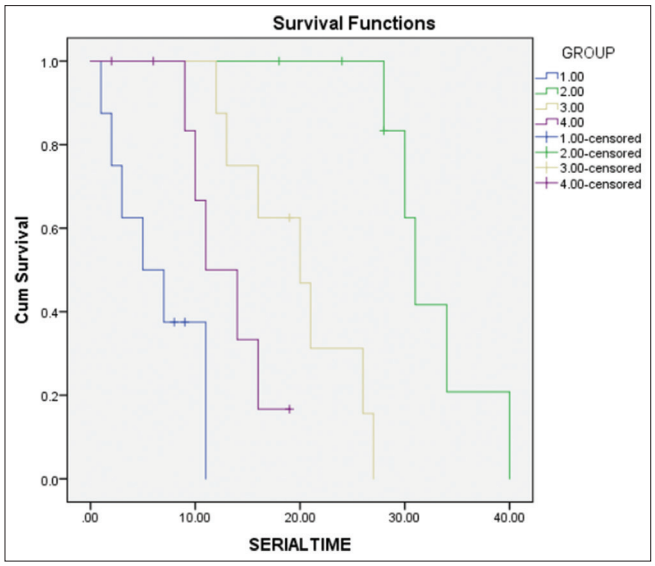

Figure 3: Kaplan–Meier curve for basaloid squamous cell carcinoma (denoted as 1), well-differentiated squamous cell carcinoma (denoted as 2), moderately differentiated squamous cell carcinoma (denoted as 3) and poorly differentiated squamous cell carcinoma (denoted as 4)

to the conventional OSCC.[17] All the patients reported here had a history of tobacco consumption in various forms.

BSCCs show basaloid cells with increased nucleo–cytoplasmic ratio, scanty cytoplasm and hyperchromatic nuclei. These show central areas of comedonecrosis and focal keratinisation.[17] Rarely, patients with BSCC with substantial spindle cell components have also been reported in the literature.[1] A panel of immunohistochemical markers was performed on BSCC samples to provide an insight into the behaviour of these tumours.

Bcl-2 is a proto-oncogene that promotes carcinogenesis by blocking apoptosis.[12] We found that Bcl-2 expression was lower in BSCC when compared to conventional OSCC. As mentioned earlier BSCC is considered an aggressive neoplasm, but the decreased Bcl-2 expression in this tumour is contentious. Similar results were reported by Deniz *et al*., in BSCC of the larynx. The authors opine that there was no difference in the Bcl-2 expression between the basaloid variant and the conventional squamous cell carcinoma.[18] In addition, tumours such as Kaposi sarcoma and leukaemia also show low Bcl-2 labelling.[19,20] Krishna *et al*., reported that the Bcl-2 labelling is not related to the grade or type of glial tumours.[21] Bcl-2 expression is known to suppress the tumour cells by a cell cycle inhibitory function and reduce the proliferation of malignant cells. Overexpression of Bcl-2 increases the half-life of Bax which further causes death of normal photoreceptor cells.[22] Caspase 3 is also known to cleave Bcl-2 at Asp 34 and induce cell death.[22] The downregulation of Bcl-2 in BSCC might occur due to similar possibilities.

The reduced expression of E-cadherin has been correlated with increased cell motility and increased expression of mesenchymal cadherins such as N-cadherin, suggesting initiation of EMT. E-cadherin expression was found to be reduced at the ITF of conventional OSCCs, but interestingly E-cadherin expression was maintained by the tumour cells of BSCCs. The aggressive behaviour of BSCCs and the increased expression of E cadherin at the invasive front of the tumour may be explained by the mechanism of collective cell migration (CCM). CCM is the mechanism by which a group of malignant cells with prominent intercellular bridges move through the ECM.[23] Elisha *et al*., have reported that the E-cadherin expression is maintained in the process of CCM and a moderate level of E-cadherin labelling is required by mammary carcinoma cells to promote carcinogenesis.[24] Re-expression of E-cadherin was also noted in cell lines like AGS gastric cancer cells.[25] Another possible explanation for the maintenance of E-cadherin is the importance of interaction between the cells for nutrition, polarisation of border cells and directed movement of malignant clusters. These results suggest that E-cadherin expression is essential for the malignant cells to move in clusters in BSCC.

Tumour-associated angiogenesis orchestrates carcinogenesis by allowing the dissemination of malignant cells and promotes metastasis. CD105 is considered to be a superior marker, as this is a coreceptor for TGF β and is expressed only on activated endothelial cells. On the activated endothelial cells, TGF β signals through ALK-5 and ALK-1, inducing the phosphorylation of SMAD-2, SMAD-3, SMAD-1, SMAD-5 and SMAD-8.[26] Increased CD105 is associated with poor overall survival, poor patient outcome and worse prognosis.[26] Increased expression of CD105 in OSCC has been reported in the literature.[9] A progressive increase in the CD105 expression was also noted from premalignant lesions, such as leukoplakia and oral submucous fibrosis to OSCC.[8,27] CD34 is a glycosylated transmembrane protein used to evaluate angiogenesis.[28] In the present study, increased MVD of CD34 and CD105 was noted in BSCC when compared to WDSCC; however, the value of BSCC was similar to PDSCC. This indicates increased tumour angiogenesis in BSCCs and determines the aggressive nature of these tumours. Furthermore, the MVD of CD34 was > CD105 in BSCCs. CD34 is a pan-endothelial marker and stains all the blood vessels in the vicinity; however, CD105, a potent pleiotropic angiogenic factor, stains only the activated endothelial cells involved in tumour-associated angiogenesis. Therefore, anticancer treatments targeting CD105-related tumour neo-angiogenesis can further help these patients in addition to the conventional treatment modalities.

The overall survival of patients with BSCC was significantly lower than conventional OSCCs. A similar result was reported by Winzenburg *et al*.[29] These authors reported the survival rate of BSCC to be less than half of conventional OSCCs.[29] The survival of patients with BSCC depends upon various factors like the consumption of tobacco, duration, age, lifestyle, stage of disease at the time of detection, treatment provided, and tumor characteristics.[30] In addition to these, as mentioned earlier apoptotic resistance, increased tumour neo-angiogenesis and CCM might also play a major role.

Conclusion

- BSCC is common in middle- to older-aged males
- BSCCs of the oral cavity show increased expression of CD105, CD34, E-cadherin and concomitant lower Bcl-2 immunolabelling
- A substantial relationship between the tumour neo-angiogenesis, CCM and apoptotic property could be related to the aggressive nature of this tumour and its poor overall survival rate.

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Conflicts of interest

There are no conflicts of interest.

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