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Original Article

Dentistry Section

Analysis of Histopathological Parameters of Oral Squamous Cell Carcinoma by Bryne's Grading in Different Age Groups

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ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) commonly seen in 6th to 8th decades of life, when occurring in younger age group under 45 years its prediction and aggression is questioned due to subjective data in literature. Various histopathological grading systems of OSCC have been discussed in the literature, but the reability of such grading systems is controversial. However, Bryne's invasive tumour front grading system has a high prognostic value since it provides diagnostic and predictive information of OSCC.

Aim: To compare the histopathological parameters of OSCC by Bryne's grading in different age groups.

Materials and Methods: This was a retrospective archive study conducted in the Department of Oral Pathology and Microbiology, MIDSR Dental College, Latur, Maharashtra, India, from June 2010 to December 2020. Total 90 histopathologically diagnosed cases with OSCC were included in the study. These patients were divided into three groups: Group I included 30 patients below 40 years of age; Group II included 30 patients who were between 41 to 60 years of age; and Group III included 30 patients who were above 60 years of age. Formalin fixed paraffin embedded blocks of OSCC were used to prepare Haematoxylin and Eosin (H&E) stained section and graded based on Bryne's invasive tumour front grading system. Chi-square test was applied to evaluate the significant difference among different age groups.

Results: Out of 90 cases of OSCC included in the study among different age groups, 70 (77.77%) were males and 20 (22.22%) were females. This study found 93.33% (28 patients) in group III followed by 76.66% (23 patients) in group I and 63.33% (19 patients) in group II had score 2 nuclear polymorphism. When the three groups were compared using chi-square test, a p-value of 0.024 was obtained which was found to be a statistically significant in the nuclear polymorphism among different age groups by Bryne's grading. The comparison between other histological parameters of Bryne's grading degree of keratinisation (p-value=0.169), pattern of invasion (p-value=0.422), number of mitosis (p-value=0.107), and lymphoplasmacytic infiltration (p-value=0.252) revealed no significant differences.

Conclusion: Outcomes of the study result showed differences in nuclear polymorphism were more obvious among old patients (group III) when compared to young patients (group I).

Keywords: Degree of keratinisation, Lymphoplasmacytic infiltration, Morphologic feature, Nuclear polymorphism, Pattern of invasion

INTRODUCTION

The Oral Squamous Cell Carcinoma (OSCC) is the most frequent oral malignancy representing up to 80-90% of all malignant neoplasm of the oral mucosa [1]. It is the most common cancer in men and third most common cancer in women, with incidence rates of 12.8 and 7.5 per 1,00,000 people in men and women respectively [2]. It is one of the causes for the increasing count of cancer associated deaths among the male gender in India [3].

The incidence of oral cancers parallels the longevity, multiplicity and intensity of carcinogenic exposure [4]. It is generally considered that oral cancer is more common in men in the sixth to eighth decades of life and is rare in patients younger than 40 years [1,5]. Epidemiologic analysis have shown disproportionate increase of OSCC incidence in a younger age group (younger than 45-year-old) compared to patients above 45 years representing approximately 4% to 13% of all cases of OSCC has been reported [6]. In India, the incidence of oral cancer is increasing because young people chew pan masala products which usually contain tobacco [7]. Since, young individuals are exposed for a short period of time to risk factors such as consumption of tobacco and alcohol, it has been suggested that the aetiology of OSCC differs in young and old patients. The lack of significant habits in some young patients have prompted many to postulate factors like immune deficiency, genetic factors and dietary factors in the aetiology of these cancers [7,8].

There are numerous studies in literature which have compared the clinical features, aetiology, staging, survival of patients with OSCC in young and old [9-11]. However, there are paucity of studies which have compared the histopathological features of OSCC in different age groups. Bryne M et al., stated that molecular and morphological characteristics at the invasive front area of various squamous cell carcinomas may reflect tumour prognosis better than other parts of the tumour and observed that cells in deep invasive margins are less differentiated than the cells in superficial part of tumour. Thus, they introduced a multifactorial malignancy grading system of the deep invasive margins of OSCC which proved to be of high prognostic value [12]. Their results indicated that features regarding the histologically invasive cells of the tumours may be most crucial for metastasis and prognosis. Several studies have shown that this system is a significantly better predictor of prognosis [12-14]. Therefore, aim and objectives of the present study was to evaluate the differences in the histopathological parameters of OSCC by Bryne's Grading System (invasive tumour front of excision biopsies) (1989) [12] in different age groups.

MATERIALS AND METHODS

This was a retrospective archive study conducted in the Department of Oral Pathology and Microbiology, MIDSR Dental College, Latur, Maharashtra, India, from June 2010 to December 2020 after obtaining Ethical Approval from the Institute (MIDSR/STU/837/919/2021).

Demographic data including age, gender, the type of the lesion and the site involved were collected.

Inclusion criteria: Primary tumours histopathologically diagnosed with OSCC and only excisional biopsy specimens were retrieved along with the clinical details.

Exclusion criteria: Histopathological variants of OSCC, previously treated radiotherapy patients, incompletely registered records and missed pathologic slides were excluded from the study.

Sample size calculation: The sample size was calculated according to the formula: $n=Z^2 \times p \times (1-p)/e^2$

Where: Z=value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI), p=proportion (expressed as a decimal), e= margin of error [15].

Z=1.96, p=0.061, e=0.05

Sample size $(n)=(1.96)^2 \times 0.061 \times (1-0.061)/(0.05)^2=90$ Sample.

Hence, a total number of 90 samples were included in the present study.

Total 90 histopathologically diagnosed cases with OSCC were included in this retrospective study. These patients were divided into three groups:

- Group I (Young patients)- 30 patients below 40 years of age;
- Group II (Middle aged patients)- 30 patients who were between 41 to 60 years of age; and
- Group III (Old patients)- 30 patients who were above 60 years of age. Formalin fixed paraffin embedded tissue blocks of these patients were subjected to soft tissue microtomy for preparation of 5 µm thick tissue specimens. Subsequently, they were stained by standard Haematoxylin and Eosin staining method (H&E) [16]. Detailed histopathological examination was done for each of these lesions under light microscope.

Bryne's Grading

The cases were graded according to histological malignancy grading system given by Bryne M et al., [Table/Fig-1] [12]. This grading system includes five morphological features i.e., degree of keratinisation, nuclear polymorphism, pattern of invasion, number of mitosis, and lymphoplasmacytic infiltration. Each morphologic feature was graded from 1 to 4 based on its severity and a total malignancy score was considered the sum of scores. The score recorded for each morphological feature was summed into total malignancy score and graded as:

- Grade I- 5-8 score: Well Differentiated Squamous Cell Carcinoma (WDSCC);
- Grade II- 9-12 score Moderately Differentiated Squamous Cell Carcinoma (MDSCC);
- Grade III- 13-20 score: Poorly Differentiated Squamous Cell Carcinoma (PDSCC).

Morphologic feature	Scores			
	1	2	3	4
Degree of keratinisation	Highly keratinised (>50% of the cells)	Moderately keratinised (20-50% of the cells)	Minimal keratinisation (5-20% of the cells)	No keratinisation (0-5%)
Nuclear polymorphism	Little nuclear polymorphim (>75% mature cells)	Moderately abundant nuclear polymorphim (50-75% mature cells)	Abundant nuclear polymorphism (25-50% mature cells)	Extreme nuclear polymorphism (0-25% mature cells)
No. of mitosis (HPF)	0-1	2-3	4-5	>5
Pattern of invasion	Pushing well delineated infiltrating borders	Infiltrating, solid cords, bands and or strands.	Small groups or cords of infiltrating cells(n>15)	Marked and widespread Cellular dissociation in small groups of cells (n<15) and or in single cells
Lymphoplasmacytic infiltration	Marked	Moderate	Slight	None

[Table/Fig-1]: Histologic malignancy grading system (Bryne's grading) [12].

STATISTICAL ANALYSIS

Statistical analysis was done using statistical software Statistical Package for the Social Sciences (SPSS) version 16.0. Chi-square test was applied to evaluate the significant difference of histopathological parameters among different age groups. Results with p-value <0.05 was considered to be statistically significant at 95% of confidence interval.

RESULTS

Out of 90 cases of OSCC included in the study among different age groups, 70 (77.77%) were males and 20 (22.22%) were females. On studying demographic details pertaining to gender, a definite male predominance with 83.33% (25 patients) among group III followed by 80 % (24 patients) in group I and 70 % (21 patients) in group II was observed among different age groups. On analysing the histologic grades of OSCC, the study results showed that the majority of the patients in the different age groups were diagnosed with MDSCC 73.33% (66 patients) followed by WDSCC 24.44% (22 patients) and PDSCC 2.22% (2 patients). When the three groups were compared for histopathological grading using Chi-square test, a p-value of 0.106 was obtained which was not found to be statistically significant [Table/Fig-2].

Histo-logical grades of OSCC	Group I	Group II	Group III	Overall (%)	Chi-square value	p-value
WDSCC	8 (26.66%)	10 (33.33%)	4 (13.33%)	22 (24.44%)	7.636	0.106
MDSCC	20 (66.66%)	20 (66.66%)	26 (86.66%)	66 (73.33%)		
PDSCC	2 (6.66%)	0	0	2 (2.22%)		
Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)		

[Table/Fig-2]: Distribution of OSCC cases according to Bryne's grading in different age groups.
WDSCC: Well differentiated squamous cell carcinoma; MDSCC: Moderately differentiated squamous cell carcinoma; PDSCC: Poorly differentiated squamous cell carcinoma

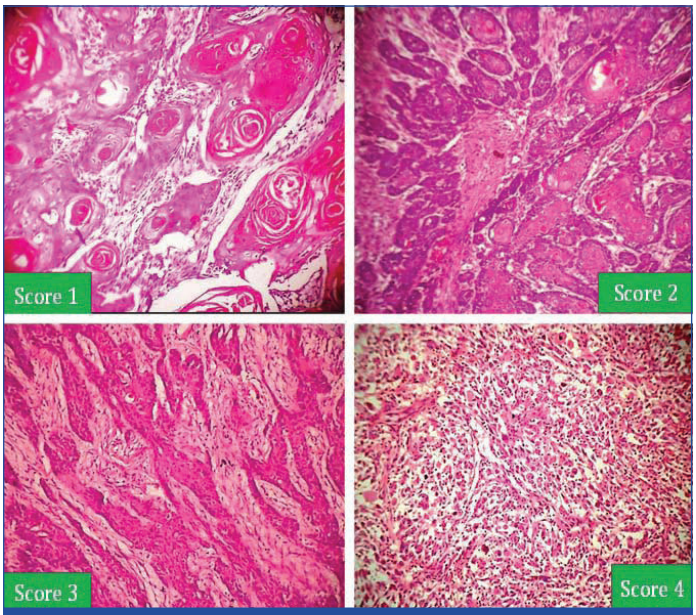
In the present study, score 2 degree of keratinisation (moderately keratinised 20-50% of the cells) was most common in 73.33% (66 patients) followed by 21.11% (19 patients) in score 1, 3.33% (3 patients) in score 3 and 2.22% (2 patients) in score 4 among different age groups. About 80% (24 patients) in both group II and group III followed by 60% (18 patients) in group I had score 2 degree of keratinisation. When the three groups were compared for degree of keratinisation, using Chi-square test, a p-value of 0.169 was obtained which was not statistically significant [Table/Fig-3,4].

Degree of keratini-sation	Group I	Group II	Group III	Overall (%)	Chi-square value	p-value
Score 1	9 (30%)	6 (20%)	4 (13.33%)	19 (21.11%)	9.091	0.169
Score 2	18 (60%)	24 (80%)	24 (80%)	66 (73.33%)		
Score 3	1 (3.33%)	0	2 (6.66%)	3 (3.33%)		
Score 4	2 (6.66%)	0	0	2 (2.22%)		
Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)		

[Table/Fig-3]: Degree of keratinisation in different age groups.

On comparison of nuclear polymorphism among different age groups, score 2 nuclear polymorphism (moderately abundant nuclear polymorphism i.e., 50-75% mature cells) was commonest in 77.77% (70 patients) followed by 13.33% (12 patients) in score 1, 7.77% (7 patients) in score 3 and only 1.11% (1 patient) in score 4. 93.33% (28 patients) in group III followed by 76.66% (23 patients) in group I and 63.33% (19 patients) in group II had score 2 nuclear polymorphism. When the three groups were compared for nuclear polymorphism,

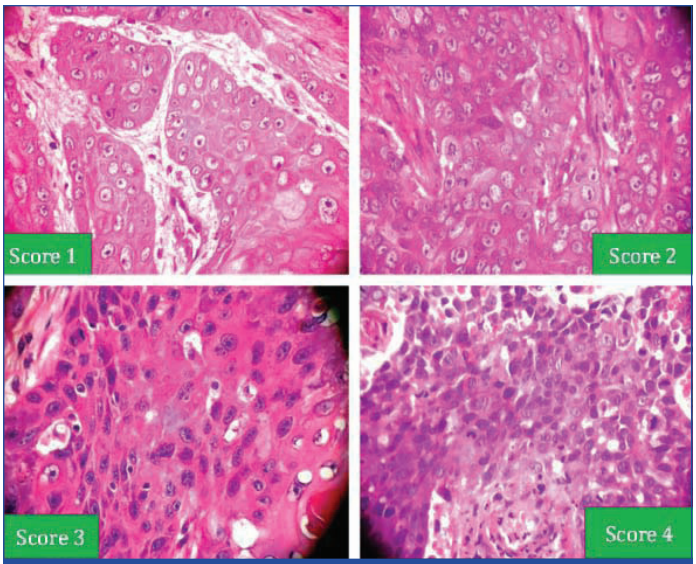
using Chi-square test, a p-value of 0.024 was obtained which was found to be statistically significant [Table/Fig-5,6].



[Table/Fig-4]: Photomicrograph showing different grades of degree of keratinisation in OSCC with Bryne's criteria (H&E stain, 100X); Score 1: Highly keratinised (>50% of the cells); Score 2: Moderately keratinised (20-50% of the cells); Score 3: Minimal keratinisation (5-20% of the cells); Score 4: No keratinisation (0-5%).

Nuclear polymorphism	Group I	Group II	Group III	Overall (%)	Chi-square value	p-value
Score 1	3 (10%)	9 (30%)	0 (0.00%)	12 (13.33%)	14.529	0.024*
Score 2	23 (76.66%)	19 (63.33%)	28 (93.33%)	70 (77.77%)		
Score 3	3 (10%)	2 (6.66%)	2 (6.66%)	7 (7.77%)		
Score 4	1 (3.33%)	0	0	1 (1.11%)		
Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)		

[Table/Fig-5]: Nuclear polymorphism in different age groups.
*Significant at p-value <0.05

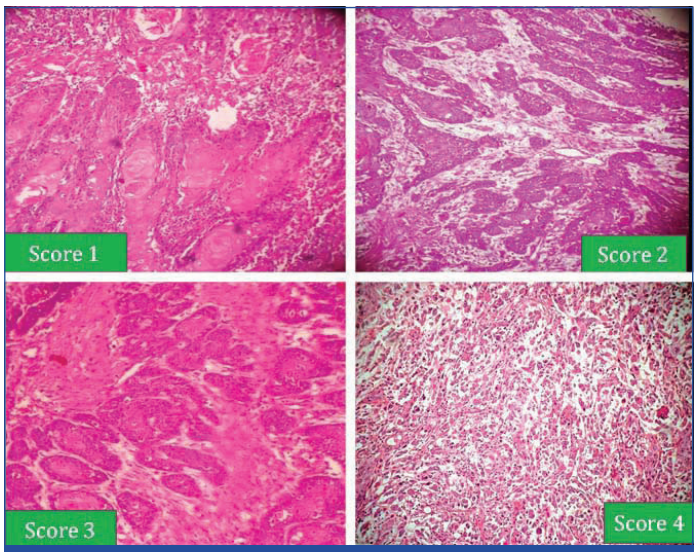


[Table/Fig-6]: Photomicrograph showing different grades of Nuclear polymorphism in OSCC with Bryne's criteria (H&E stain, 400X); Score 1: Little nuclear polymorphim (>75% mature cells); Score 2: Moderately abundant nuclear polymorphim (50-75% mature cells); Score 3: Abundant nuclear polymorphism (25-50% mature cells); Score 4: Extreme nuclear polymorphism (0-25% mature cells).

The pattern of invasion among different age groups noted that score 2 pattern of invasion (infiltrating, solid cords, bands and or strands) was commonest in 74.44% (67 patients) followed by 16.66% (15 patients) in score 1, 7.77% (7 patients) in score 3 and 1.11% (one patient) in Score 4. When the three groups were compared for pattern of invasion, a p-value of 0.422 was obtained which was not found to be statistically significant [Table/Fig-7,8].

Pattern of invasion	Group I	Group II	Group III	Overall (%)	Chi-square value	p-value
Score 1	6 (20%)	7 (23.33%)	2 (6.66%)	15 (16.66%)	6.011	0.422
Score 2	20 (66.66%)	21 (70%)	26 (86.66%)	67 (74.44%)		
Score 3	3 (10%)	2 (6.66%)	2 (6.66%)	7 (7.77%)		
Score 4	1 (3.33%)	0	0	1 (1.11%)		
Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)		

[Table/Fig-7]: Pattern of Invasion in different age groups.

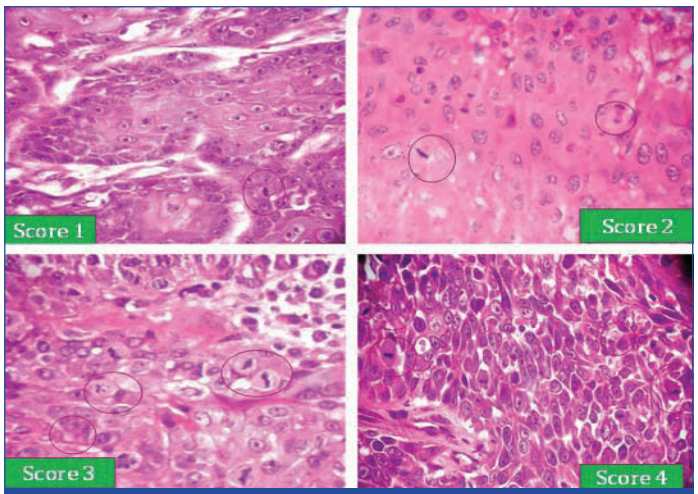


[Table/Fig-8]: Photomicrograph showing different grades of different grades of Pattern of invasion in OSCC with Bryne's criteria (H&E stain, 100X); Score 1: Pushing well delineated infiltrating borders; Score 2: Infiltrating, solid cords, bands and or strands; Score 3: Small groups or cords of infiltrating cells (n>15); Score 4: Marked and widespread cellular dissociation in small groups of cells (n<15) and or in single cells.

Further, on comparison of the number of mitosis in different age groups, 63.33% (57 patients) had score 1 mitosis followed by 32.22% (29 patients) had score 2 and 4.44% (4 patients) had score 3 mitosis. When the three groups were compared for number of mitosis in different age groups a p-value of 0.107 was obtained which was not statistically significant [Table/Fig-9,10].

Number of mitosis	Group I	Group II	Group III	Overall (%)	Chi-square value	p-value
Score 1	17 (56.66%)	18 (60%)	22 (73.33%)	57 (63.33%)	7.616	0.107
Score 2	12 (40%)	12 (40%)	5 (16.66%)	29 (32.22%)		
Score 3	1 (3.33%)	0	3 (10%)	4 (4.44%)		
Score 4	0	0	0	0		
Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)		

[Table/Fig-9]: Number of mitosis in different age groups.

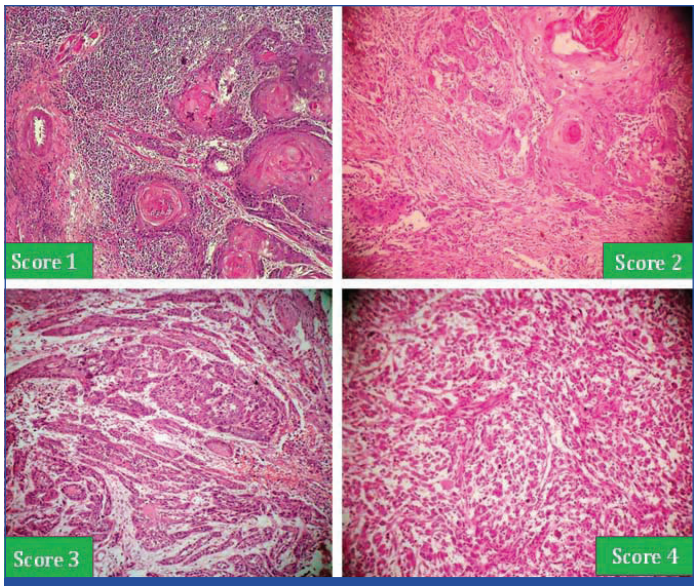


[Table/Fig-10]: Photomicrograph showing different grades of number of mitosis in OSCC with Bryne's criteria (H&E stain, 400X); Score 1: 0-1 high power field; Score 2: 2-3 high power field; Score 3: 4-5 high power field; Score 4: >5 high power field.

On evaluation of the lymphoplasmacytic infiltrate among different age groups, score 2 lymphoplasmacytic infiltrate (moderate) was most common in 83.33% (75 patients) followed by 7.77% (seven patients each) in both score 1 and score 3 and 1.11% (1 patient) in score 4. In the present study, score 2 lymphoplasmacytic infiltrate (moderate) was commonest with 96.66% (29 patients) in group III followed by 76.66% (23 patients each) in both group II and group I. When the three groups were compared for lymphoplasmacytic infiltrate a p-value of 0.252 was obtained which was not found to be statistically significant [Table/Fig-11,12]. Oral squamous cell carcinoma comparison between different age group of patients didn't demonstrate any relevant difference.

Lymphop-las-macytic infiltrate	Group I	Group II	Group III	Overall (%)	Chi-square value	p-value
Score 1	3 (10%)	4 (13.33%)	0	7 (7.77%)	7.817	0.252
Score 2	23 (76.66%)	23 (76.66%)	29 (96.66%)	75 (83.33%)		
Score 3	3 (10%)	3 (10%)	1 (3.33%)	7 (7.77%)		
Score 4	1 (3.33%)	0	0	1 (1.11%)		
Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)		

[Table/Fig-11]: Lymphoplasmacystic infiltrate in different age groups.



[Table/Fig-12]: Photomicrograph showing different grades of Lymphoplasmacystic infiltrate in OSCC with Bryne's criteria (H&E stain, 100X); Score 1: Marked; Score 2: Moderate; Score 3: Slight; Score 4: None.

DISCUSSION

Oral cancer is considered a serious public health problem that causes great mortality and morbidity in the population. Oral squamous cell carcinoma is a major oncological problem in many regions of the world where tobacco habits are practiced in the form of chewing or smoking [10]. However, an increased incidence of OSCC among young adults was first highlighted in the 1970's with the average age of cases ranging between 30.8-34.2 years with a male predominance [17]. Cusumano RJ and Persky MS reported that the incidence of OSCC in young patients ranged from 0.4% to 3.9% of all cases [18]. There is a long standing debate over the aggression and prognosis of OSCC in young patients as compared to older patients and studies have suggested the need for further research.

Currently, diagnosis and treatment are based on clinical and histopathological characteristics. Histologic grading has been used for many decades in an attempt to predict the clinical behaviour of squamous cell carcinomas of head and neck [9,19]. Bryne M et al., stated that several molecular events occur at the tumour host interface (invasive front) and showed that grading in the invasive sites of the tumour had a highly significant prognostic value. Their results indicated that features regarding the histologically invasive cells of the tumours may be most crucial for metastasis and prognosis. Several studies have shown that this system is a significantly better predictor of prognosis. Therefore, all the cases of OSCC in the present study were graded by Bryne M et al., grading system [12].

In this study, majority of the patients in the different age groups were diagnosed with MDSCC 73.33% (66 patients) followed by WDSCC 24.44% (22 patients) and PDSCC 2.22% (2 patients). These results were comparable to the studies done by Ur Rahaman SM and Ahmed Mujib BR, and Udeabor SE et al., who reported that most of the patients among younger age group were diagnosed with MDSCC (40%) followed by WDSCC (36%) and PDSCC (24%) [5,20,21]. Kuriakose M et al, did not find statistically significant difference in the histopathological grading of tumours between younger and older age groups and noted that majority of the tumours were diagnosed with WDSCC [11].

Further, when five histopathologic parameters were evaluated in the invasive front of tumours by Bryne M et al., it was found that score 2 degree of keratinisation (moderately keratinised 20-50% of the cells) was commonest in 80% (24 patients) in group II and group III followed by 60% (18 patients) in group I which was not statistically significant among different age groups [12]. Similar results were discussed in the studies done by Acharya S and Tayaar AS, and Ur Rahaman SM and Ahmed Mujib BR [1,5].

On comparison of nuclear polymorphism among different age groups, found that score 2 nuclear polymorphism (moderately abundant nuclear polymorphism i.e., 50-75% mature cells) was most common 93.33% (28 patients) in Group III followed by 76.66% (23 patients) in Group I and 63.33% (19 patients) in group II. When the three groups were compared for nuclear polymorphism, p-value of 0.024 was obtained which was found to be statistically significant. Similar trends were reported by Ur Rahaman SM and Ahmed Mujib BR [5]. However, Siriwardena BS et al., reported that higher nuclear aberration (48.2%) was seen in the younger age group whereas this study found higher nuclear polymorphism in the older age group [22]. This study results shows differences in nuclear polymorphism were more obvious among old patients when compared to young patients.

Furthermore, when evaluated pattern of invasion maximum number of patients fell under score 2 pattern of invasion in group III i.e., 86.66% (26 patients) followed by 70% (21 patients) in group II and 66.66% (20 patients) in group I. When the three groups were compared a p value of 0.422 was obtained which was not statistically significant. On comparison of the number of mitosis in different age groups, score 1 number of mitosis was common in 73.33% (22 patients) in group III followed by 60% (18 patients) in group II and 56.66% (17 patients) in group I and there was no statistically significant difference among different age groups (p-value=0.107) [Table/Fig-9,10]. Similarly, Siriwardena BSMS et al., found a significantly higher number of mitosis in the older patients compared with the younger patients [22]. Lymphoplasmacytic infiltrate among different age groups showed score 2 lymphoplasmacytic infiltrate was commonest. Among different age groups no statistically significant difference was obtained (p-value=0.252) [Table/Fig-11,12]. The results showed the lower host immune response in the younger age group. Considering the important role of immune response in control to tumour cell proliferation, lower immune response in younger age group may be reason for more aggressive behaviour in this age group. These results were comparable to the studies done by Ur Rahaman SM and Ahmed Mujib BR, Sasaki T et al., Razavi SM and Khalesi S, who reported that higher grades of lymphoplasmacytic infiltrate in the older patients compared with the younger patients [5,23,24].

In the present study, nuclear polymorphism was found to be the only statistically significant parameter among different age groups. Similar results were obtained in the studies done by Sasaki T et al., [23] who used five of the six parameters given by Bhargava A et al., which

excluded nuclear polymorphism as a parameter and revealed that none of the individual histopathologic parameters showed statistically significant difference between younger and older age groups [19]. In contrast, Ur Rahaman SM and Ahmed Mujib BR, [5] in their study found statistically significant difference between the younger and older groups in three of the six parameters of the grading system given by Bhargava A et al., i.e., differences in nuclear polymorphism, mitosis index and depth of invasion were more obvious among young patients when compared to old patients in their study [5,19]. Immunohistochemical markers like Proliferating Cell Nuclear Antigen (PCNA), p53 and Ki-67 have also been assessed in young and old patients but no significant difference was noted. [22,25,26], So, analysis of multiple molecular targets by microarray technology will better elucidate the difference between young and old patients with OSCC and thereby help in evaluating prognosis and survival of these patients.

Limitation(s)

The present study was a preliminary study, so a study with a large sample size is mandatory. Since the study was carried out at our Institute, it represents a specific patient population and not a community as a whole therefore, it constituted a relatively smaller sample size.

CONCLUSION(S)

Outcomes of the study result showed differences in nuclear polymorphism were more obvious among old patients (group III) when compared to young patients (group I). Bryne's invasive tumour front grading system of OSCC can be taken as a valuable predictive and diagnostic tool as molecular and morphological characteristics at the invasive front area reflect tumour prognosis better than other parts of the tumour and is more effective in predicting survival in OSCC. The present study was a retrospective study and in future a prospective study has to be carried for confirming the above results. Further, multicentric studies will help us in identifying prevalence pattern of this alarming increasing disease, so that prevention activities can be carried out in order to decrease the incidence and mortality rates.

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