Original Article

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Dentistry Section

A Comparison of Clinicopathological Differences in Oral Squamous Cell Carcinoma in Patients Below and Above 40 Years of Age

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ABSTRACT

Introduction: Recent times have revealed an increase in incidence of Oral Squamous Cell Carcinoma (OSCC) in young adults including those who lack association with typical risk factors such as tobacco. There are reported variations in clinical behaviour of tumours in young and older individuals.

Aim: Present study evaluated differences in clinicopathological characteristics between two groups of OSCC, below and above 40 years of age.

Materials and Methods: An analytical study was performed on two groups of OSCC patients, below and above 40 years of age. Clinicopathological parameters of site distribution, type of habit, histological grade, nodal metastasis, margin status, mitotic index and Argyrophilic Nucleolar Organizing Regions (AgNOR) count were compared. Chi-square test and Students t- test were applied for statistical analysis.

Results: Present study revealed that mean AgNOR count was significantly higher in older group (6.38) than younger group (4.27). However, no significant differences were noted in site distribution, tobacco habit, histological grade, mitotic index, nodal metastasis and status of resected surgical margins between the two age groups. A trend for increased metastasis and poor histological differentiation was also observed in the older and younger age group respectively. Most common site was buccal mucosa followed by tongue in both groups.

Conclusion: Reasons for documented variability in tumour characteristics between young and older patients are currently unclear. Difference in AgNOR count found in present study is suggestive of variability in proliferative and ploidy characteristics between different age groups and supports the hypothesis of genetic and epigenetic influences in development of oral cancer.

Keywords: Age factors, Mitotic index, Oral cancer

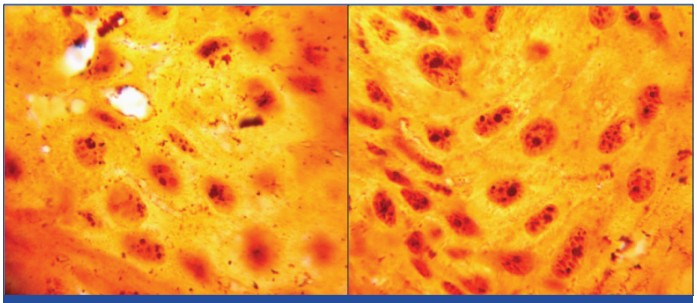
INTRODUCTION

OSCC was until now, chiefly considered to be a disease affecting older individuals, with usage of tobacco being a major causative factor. However, there seems to be a change in the demographic trend, with OSCCs increasingly seen in younger individuals. This has led to increasing prevalence of 'early-onset Squamous Cell Carcinoma (SCC) which may be arbitrarily defined as SCC occurring in individuals younger than 40 years of age [1]. It is observed that there may be certain differences in the biological behaviour of tumours in younger adults. However, there is no known or proven explanation yet, attributable for these differences. In younger adults, OSCCs are sometimes seen to lack the typical association with tobacco and/or alcohol habit in addition to differences in the type and duration of habit. This raises the possibility of association of other etiological or risk factors such as viral infection and genetic susceptibility [1,2]. Differences in clinical behaviour are also observed in terms of recurrence, tendency for metastasis and survival rate. For the present analytical study, the null hypothesis was that there is no difference in clinicopathological characteristics of OSCC between individuals below and above 40 years of age. We compared two groups of individuals with OSCC categorized according to age as, below 40 and above 40 years. The objective was to assess differences in type of habit, histological features (grade of differentiation, mitotic index, and AgNOR count) and prognostic factors (lymph node metastasis and involvement of resected margins) between the two groups.

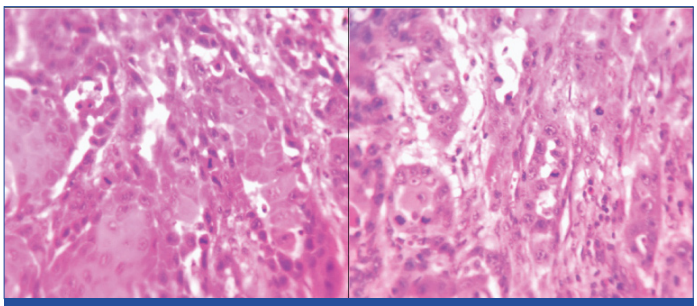
MATERIALS AND METHODS

An analytical study was performed on archival formalin-fixed, paraffin-embedded tissue specimens of cases histologically diagnosed as OSCC at the Department of Oral Pathology, Manipal College of Dental Sciences, Mangalore, Manipal University, Karnataka, India. The samples were selected by convenience sampling. Inclusion criteria were histopathological diagnosis of OSCC and availability of data (age and site of tumour). Exclusion criteria were lack of data (age and site of tumour) and insufficient archival tissue. The sample comprised of 21 cases below 40 years of age and 19 cases above 40 years of age.The study was performed between July 2014 to December 2014 after approval from the Institutional Ethics Committee.

For histopathological assessment, two sections each of 4 µ thickness were obtained for each case. One section was stained with Haematoxylin and Eosin (H&E) and the other, using silver staining method for assessing AgNORs [3]. Medical records were reviewed for: histologically-proven tumour metastasis to lymph nodes, status of surgically resected margins, predominant type of tobacco habit (smoking or chewing forms). All cases were histologically categorized as well, moderately or poorly differentiated according to Bryne M et al., grading at invasive tumour front [4]. Proliferative activity in each case was assessed by two methods: AgNORs count and mitotic index. Silver staining of tissue sections was performed using a modification of the technique suggested by Kahn MA et al., using freshly prepared colloidal silver nitrate solution (50% aq. silver nitrate and gelatine in 1% aq. formic acid) and incubating the same at 50^{0}C for 45 minutes under dark conditions [3]. For each case, 50 nuclei at the invasive tumour front were assessed at 400x magnification. Each discrete dot was counted as one AgNOR and mean AgNOR count was thus calculated for each case [Table/Fig-1]. The mitotic index was determined as number of mitotic figures in ten consecutive High Power Fields (HPF) in H&E stained



[Table/Fig-1]: AgNORs visualised as black dots within the OSCC tumour cells (Silver stain for AgNORs, ×400) in a) <40 years and b) >40 years. (Images left to right).



[Table/Fig-2]: Number of mitoses assessed in 10 high power fields to calculate mitotic index (H and E stain, ×400) in OSCC in a) <40 years and b) >40 years of age. (Images left to right)

sections [Table/Fig-2].

STATISTICAL ANALYSIS

Descriptive statistics were used to summarize the data. Qualitative variables (type of habit, grade of differentiation, lymph node status and margin status) were assessed between the two age groups using Chi-square test; and quantitative variables (mitotic index and AgNOR count) using Independent samples t-test. In addition, Pearson's correlation was assessed between AgNOR count and mitotic index. The level of significance was fixed at 5% and the power of the study at 80%. The data was analysed using Statistical Package for Social Sciences version 16.0.

RESULTS

The common sites for OSCC in both age groups were buccal mucosa and tongue. Both categories of age showed a higher prevalence of males and a predominance of chewing type of tobacco habit. [Table/Fig-3] denotes comparison of clinicopathological characteristics of OSCC in young and old groups. The null hypothesis was rejected as there was a significant difference in mean AgNOR count between the two age groups. Mean AgNOR count was found to be significantly higher in older patients (mean±SD= 6.38±1.41), in comparison to the younger group (mean±SD = 4.27±0.96) (t-test, p<0.001). Pearson's correlation analysis did not show correlation between AgNOR count and mitotic activity index (Pearson's Rho= 0.162). Though not significant, a higher number of cases with lymph node involvement were noted among younger individuals (27.3%) as compared to older group (6%).

Tumour characteristics	Number (%) of people in young age group (≤ 40 years)	Number (%) of people in older age group (>40years)	Test value	p-value
Type of habit ‡ Predominantly smoking Predominantly chewing	4 (19) 9 (43) NA† 8(38)	3 (15.8) 8 (42.1) NA† 8(42.1)	χ^2=0.035	1.00
Grade of differentiation ‡ Well Moderate Poor	1 (4.7) 13 (62) 7 (33.3)	3 (15.8) 12 (63.2) 4 (21)	χ^2=1.690	0.508
Gender ‡ Male Female	18 (85.7) 3 (14.3)	14 (73.7) 5 (26.3)	χ^2=0.902	0.442
Lymph node involvement ‡ Free Involved	8 (38.1) 3 (14.3) NA† 10(47.6)	15 (79) 1 (5.3) NA† 3(15.7)	χ^2=2.283	0.273
Margin involvement ‡ Free involved	5 (23.8) 6 (28.6) NA† 10(47.6)	8 (42.1) 8 (42.1) NA† 3(15.8)	χ^2=0.054	1.0
AgNOR count §	Mean,SD= 4.27,0.96	Mean,SD= 6.38,1.41	t= -5.437	0.0001
Mitotic index §	Mean±,SD 2.29, 2.17	Mean±,SD 2.63,1.89	t= -0.535	0.59

[Table/Fig-3]: Tumour characteristics in 'young' and 'old' age groups in present study.
†Data not available
‡ Chi-square test
§T-test

DISCUSSION

OSCC is generally known to be a disease predominantly affecting older males having a history of tobacco use and alcohol intake. In recent years, an increasing number of cases of OSCC in younger individuals have been documented, with studies in India paralleling the international trend [5-11]. The incidence of OSCC in patients younger than 40 years of age has been reported in various studies to range from 1% to 6% [2]. A similar trend has been observed in our institution, with the percentage of OSCC cases below the age of 40 years increasing from 7.7% between 1990-2000 to 11.7% in the time period between 2001-2013, with the youngest case reported being that of a 24 year old male. While the categorisation of 'young' patients being less than 40 years of age is somewhat arbitrary, it can be justified for the purpose of comparison with older individuals since the incidence of OSCC is reported to be much higher in individuals >40 years of age [2,5,8,12].

A literature search revealed that there are conflicting reports regarding differences in tumour characteristics in the aforementioned age groups [Table/Fig-4]. Some authors found tongue to be the most frequent site in younger cases [5,6,8,11,13-15]. In our study, the buccal mucosa was the most common site in both age groups, followed by tongue, mirroring findings of two other studies on Indian population [10,16]. Interestingly, different studies have observed that OSCC in younger individuals seems to be associated with fewer etiological factors such as smoking, chewing tobacco and alcohol consumption and lacks typical risk factors [1,2,5,8,12,14,17-21]. However, these factors need to be explored in the Indian context. Agrawal KH and Rajderkar SS found that 41.7% of their Indian study subjects did not consume tobacco in any form [11]. Iype EM et al also reported a similar trend, where 73% of cases below age of 31 years did not consume tobacco [22]. In addition, duration of consumption of tobacco and/or alcohol in younger individuals is considerably lesser than that in older persons, leading to scepticism as to whether these factors alone, are capable of inducing carcinogenesis [2,19,23]. This raises the question of other possible etiologic and risk factors, and leaves room for potential contribution of genetic susceptibility, immunodeficiency or viral infection in oral carcinogenesis [1,5,14,24,25]. Various studies support the role of genetic factors in carcinogenesis. [Table/Fig-4] enlists genetic differences found by various authors between OSCC in young and older individuals [24,16-31]. It has also been suggested that presence of both genetic susceptibility of individuals along with exposure to carcinogens may be responsible for increased risk of cancer [32].

There is no consensus yet regarding the clinical course and prognosis in younger individuals [33]. There are various factors that influence the outcome of OSCC. [Table/Fig-4] presents a brief summarization of clinicopathological observations of OSCC by various authors. Reports of better prognosis in younger patients could be attributed to increased awareness, detection of tumour at an earlier stage and

better palliative care [2,12,18]. On the other hand, increased co-morbidities, late detection of malignancy, higher grade of tumour, genetic predisposition and increased tendency to metastasize and recur are factors that contribute to worsened prognosis in others [1,34]. In the present study, we compared lymph node metastasis and involvement of surgical resection margins by tumour between the two age groups. These are histologically assessable prognostic determinants [13,35]. We did not find any substantial difference in surgical margin status between the two age groups. However, though not statistically significant, an increasing trend for lymph node metastasis was seen in younger individuals, with a similar finding reported by some authors [17,18,23]. On the other hand, some investigators did not find any difference in prognosis of OSCC in younger individuals [7,8,13,14,21,22,33]. The possibility of distinct clinicopathological profile of OSCC in young adults warrants future research.

Parameter	Studies	Findings	Inferences
Common site	Muller S et al., [5] O'Regan EM et al., [6] Martin-Granzio R et al., [8] Agrawal KH and Rajderkar SS [11] Komolmalai N et al., [12] Manuel S et al., [13] Bodner L et al., [14] Fan Y et al., [15] Sun Q et al., [21]	Tongue, in younger individuals	Present study also showed highest frequency in buccal mucosa followed by tongue
	Ramachandra NB [9] Kiran G et al., [10] Taranikanti M and Das B [16] Present study	Buccal mucosa	
Grade of differentiation	Manuel S et al., [13] Sasaki T et al., [33]	Predominantly well-differentiated tumours in younger patients	
	Udeabor SE et al.,[2]	Moderately differentiated tumours in less than 40 years age group	Present study showed higher tendency of poorly differentiated tumour in younger individuals (not statistically significant)
Prognosis - survival	Bragelmann J et al., [1] Sarkaria JN and Harari DM [34]	Young have reduced overall survival; poor prognosis	
	Udeabor SE et al., [2] Komolmalai N et al., [12] Lacy PD et al., [18]	Younger patients have better overall survival; better prognosis	
	Myers JN et al., [7] Martin-Granzio R et al., [8] Manuel S et al., [13] Bodner L et al., [14] Sun Q et al., [21] Iype EM et al., [22] Sasaki T et al., [33]	No difference in prognosis between young and old	Conflicting reports of difference in prognosis in young and old age groups
Prognosis – lymph node metastasis	Sun Q et al., [21] Siriwardena BS et al., [36]	More in older patients	Present study showed a trend for higher incidence of metastasis in older age group
	Kuriakose M et al., [17] Lacy PD et al., [18] Hilly O et al., [23]	Higher in younger individuals	
	O'Regan EM et al., [6]	Similar in both age groups	
Proliferative activity - Mitotic index, PCNA	Siriwardena BS et al., [36]	Proliferative activity (PCNA index and number of mitoses) was higher in the older group whereas, younger persons showed a significantly greater number of nuclear aberrations histologically	Though higher number of nuclear aberrations are seen in young, OSCC in older is more proliferative
	Present study	No significant difference in mitotic activity index in young and old	Larger sample needed to validate
AgNORs	Present study	Significantly higher in the older age group ($p<0.001$)	AgNOR may be representative of nuclear aberrations and ploidy variations as stated by some authors [37,41,42]
Genetic differences	Schantz SP et al., [24]: Chromosomal damage	Young population showed mutagen-induced chromosomal damage. (Bleomycin-induced chromosome breaks per cell) chromosome sensitivity was pronounced in non-tobacco users and in patients below 30 years of age.	Genetically controlled sensitivity to environmental carcinogens may be part of etiology.
	Majchrazk C et al., [25]	OSCC in younger patients exhibits a different genotype	
	Santos-Silva AR et al., [26]: Ploidy status of cells	Majority of cases in the younger group exhibited aneuploidy	
	Lingen MW et al., [27]: P53 expression and mutation	81% of cases <40 years of age overexpressed p53 but did not show mutations in exons 5-9 of the p53 gene, which are known to exist in at least 50% of older cases.	Difference in genetic mutations in young and old
	Gawecki W et al., [28] Kostrzewska-Poczekaj M et al., [29]: Genotype	Younger individuals had higher co-occurence of risk genotypes (GSTM1(-) and Nat 2*4/6A) and reduced occurrence of XPD genotype which is responsible for DNA repair. Increased chromosomal fragility in young	Difference in the alleles involved in the metabolism of carcinogens in young and older individuals
	Sorenson DM et al., [30]	P53 mutation was less common in young without history of alcohol and tobacco use	Molecular mechanisms behind OSCC without substance use are still unknown
	Hafkamp HC et al., [31]	HPV more commonly detected in young head and neck cancer patients. This was related to pRb downregulation, overexpressed p16, wild type p53 expression.	Possible viral etiopathogenesis

[Table/Fig-4]: Observations of various studies on comparison of differences in tumour characteristics between young and older patients of OSCC.

Tumour proliferation characteristics portray a picture of disease aggressiveness. To determine possible differences in proliferative capacity of tumours in younger patients, we histologically evaluated two surrogate markers, mitotic activity index [Table/Fig-2] and AgNORs [Table/Fig-1]. Higher number of mitotic figures is indicative of greater proliferative activity. In the present study, mean mitotic index was marginally higher in the older age group (2.29 and 2.63 in young and old patients respectively). A similar study by Siriwardena BS et al., also indicated a higher proliferative index (PCNA and mitosis) in older individuals [36]. AgNORs are loops of DNA occurring within nucleoli, in acrocentric chromosomes (chr.13, 14, 15, 21, 22) that encode for ribosomal RNA and are involved in ribosomal and protein synthesis. They are associated with non-histone proteins (NOR-associated proteins) which are argyrophilic in nature and thus, can be demonstrated as black dots by silver staining [3,27-40].On comparison between the two age groups in the present study, we found a significantly higher number of AgNORs in older adults ($p<0.001$). To the best of our knowledge, difference in AgNORs count between young and old patients of OSCC has not been compared previously.

AgNORs and mitotic activity are generally considered to be markers for proliferation. While both parameters were observed to be lower in younger adults in the present study, the AgNOR count and mitotic index did not statistically correlate with each other. Proliferating cells exhibit increased biosynthesis, resulting in increased rRNA, increased nucleolar activity and hence, higher number of AgNOR dots [22,41]. Keeping in view that majority of the evidence suggests that AgNORs are indicative of proliferation, the lower AgNOR count in younger individuals in the present study suggests that tumours have a less proliferative phenotype in younger individuals. On the other hand, a review of AgNORs by Underwood JC suggests that they are more representative of ploidy rather than proliferative nature, as there are differences in their presentation and assessment, besides the fact that AgNORs are found only on five chromosomes [42]. The number of AgNORs depends upon:

a. NOR-bearing chromosomes in the karyotype: Presence of higher number of AgNORs in older age group suggests that acrocentric chromosomes bearing NORs may be undergoing mutation in such persons;

b. Level of transcriptional activity, and

c. Stage of cell cycle: Nucleolus disperses immediately before division, causing dispersion and a relatively higher number of visible AgNOR dots [42].

However, it has also been suggested that increased number of AgNOR dots could also be due to gene amplification or chromosomal segregation [37,41]. These conflicting interpretations of AgNORs may explain the lack of correlation between AgNOR count and mitotic index. Higher AgNOR counts along with higher incidence of metastasis in older adults could indicate a difference in the genetic makeup of OSCC of older age group. Various studies have revealed differences in genotype of OSCC between young and old adults. In the future, treatment decisions and specific targeted therapy can be guided by characterising individual molecular profiles of tumour [20].

LIMITATION

Due to the limited sampling frame and sample size, the findings cannot be generalized to the entire population. Further large scale research is warranted to explore possible differences in OSCC between young and older individuals.

CONCLUSION

Many controversies surround OSCC in young adults. The present comparative study on tumour characteristics in young and older patients with OSCC did not show any significant difference in type of habit, site, grade of differentiation, lymph node status, margin status and mitotic index. A significantly higher number of AgNORs was observed in the older group. There is still ambiguity regarding the possibility of OSCC in young adults being a distinct clinicopathological entity. Large scale studies will help to shed light of differences in tumour characteristics between young and older adults. Better understanding of these differences will also potentially enable judicious employment of customised treatment modalities.

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