DOI: 10.7860/JCDR/2021/49249.15223

Original Article

Dentistry Section

Anneroth's Histopathological Grading System in Non Metastatic and Metastatic Oral Squamous Cell Carcinoma- A Pilot Study

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ABSTRACT

Introduction: Prognostic evaluation of Oral Squamous Cell Carcinoma (OSCC) is mainly based on [Tumour (T), Nodes (N), and Metastases (M)] staging. To predict the biological and clinical behaviour of a tumour, histological grading systems plays an important role. Modified Anneroth's grading system includes three parameters each for histologic features and tumour-host relationship.

Aim: To compare and analyse the histological features of tumour center and periphery of non metastatic and metastatic OSCC according to Anneroth's histopathological classification and its association with regional lymph node metastasis.

Materials and Methods: A retrospective pilot study was conducted on 20 histopathologically proven cases of OSCC collected from the Department of Oral Pathology and Oral Microbiology, Vinayaka Mission's Sankarachariyar Dental College, Salem, Tamil Nadu, India. The OSCC cases those reported from March 2018 to June 2020 were included in the study. Among 20 cases, 10 non metastatic and 10 metastatic cases, were retrieved from archival blocks. Scores for the morphology and tumour-host relationship of non metastatic and metastatic tumours was assessed and graded by Anneroth's grading system; then compared with the Broder's histopathological grading system. The histopathological scoring of lymph node was based on TNM staging. Chi-square test and Spearman's correlation coefficient analysis was done.

Results: Anneroth's grading system showed a significant difference in pattern of invasion in tumour periphery of metastatic OSCC (p-value=0.01). Compared to Broder's, Anneroth's classification showed a highly significant corelation with lymph node metastasis (p-value=0.001).

Conclusion: Hence, Anneroth's histopathological grading system can be used to predict the biological changes in metastatic and non metastatic OSCC and its increased possibility of regional lymph node metastasis.

Keywords: Anneroth's grading system, Metastatic oral squamous cell carcinoma, Tumour periphery

INTRODUCTION

Oral cancer ranks sixth among all the types of cancer globally, with 450,000 new cases and 350,000 deaths annually worldwide. In India, oral cancer has been reported with 77,000 new cases and 52,000 deaths annually [1,2]. The prognosis of Oral Squamous Cell Carcinoma (OSCC) depends on clinical, pathological and molecular factors [3]. Among the clinical factors, TNM staging of cervical lymph nodes plays a vital role in determining prognosis of the OSCC [4]. It is the uncontrolled growth rate and variation in pattern of spread by the tumour cells reflects that TNM staging alone is not sufficient in determining the prognosis [5]. For better analysis of cellular and nuclear details by histochemical and immunohistochemical markers; understanding the biological behaviour of tumour is mandatory [6]. Conventional histopathological grading system i.e., Broder's grading system is the gold standard for grading OSCC. The system grades tumour cells based on their differentiation (well/moderate/poorly differentiated and undifferentiated). Hence, differentiation of tumour alone is not sufficient for predicting the biological behaviour of the tumour, because of the heterogenous population.

Anneroth's grading system is based on six parameters for assessing and grading the tumour based on tumour cell population and tumour-host relationship. Thus, the grading system is more specific and helps in predicting prognosis. Many studies have reported increased alterations in the biological behaviour of tumour population in periphery of the tumour [5,7,8] when compared to the tumour center, which determines the invasiveness and metastatic potential of a tumour.

In this study, Anneroth's system was applied to grade the histopathological aspects of tumour center and periphery, to analyse the biological behaviour of the tumour. The study also aimed to look for possible correlation between the Anneroth's grading system and TNM staging.

MATERIALS AND METHODS

A retrospective pilot study was conducted on histopathologically proven cases of OSCC collected from the Department of Oral Pathology and Oral Microbiology, Vinayaka Mission's Sankarachariyar Dental College, Salem, Tamil Nadu, India. The OSCC cases that reported from March 2018 to June 2020 were included in the study. The Institutional Ethical Committee, VMRF (DU) approved the study (VMSDC/IEC/Approval No 185).

Inclusion criteria: Archival blocks and slides of histopathological proven cases of OSCC were retrieved along with the clinical details.

Exclusion criteria: Other variants of OSCC, metastatic Squamous Cell Carcinoma (SCC) of oral cavity and surgical specimen after chemotherapy and radiotherapy were excluded.

A total of 70 cases were collected, among them only primary tumours of OSCC confirmed by biopsy and located in any area of oral cavity were included. From the selected cases, slides with proper tumour center and periphery were segregated among metastatic and non metastatic cases.

In this pilot study, 10 cases of non metastatic and 10 metastatic OSCC cases were studied, in each case two samples were collected i.e., one from the tumour center and one from the tumour periphery. Hence, a total number of 40 samples with sufficient tumour periphery were included in this study.

Sample size calculation: Sample size estimation was done using the following formula

$$(Z)^2 \times \text{Std Dev} \times (1-\text{Std Dev})/(\text{Margin of error})^2$$

The sample size was calculated assuming at the most 5% risk, with minimum 80% power and 5% significance level (significant at 95% confidence level. i.e., Z=1.96) and standard deviation of 0.5 and a margin of error (confidence interval).

Necessary sample size=$(1.96)^2 \times (0.5) \times (1-0.5)/(0.10)^2$=40 Sample.

Hence, a total number of 40 samples with sufficient tumour periphery were included in this study.

The Haematoxylin and Eosin (H&E) stained sections were graded by Broder's [9] and Anneroth's Multifactorial grading system. According to Broder's system the gradings were - well differentiated, moderately differentiated and poorly differentiated while that based on Anneroth's grading were: grade I: score of 6-12, grade II: score of 13-16 and grade III: score of 19-24 [6]. The clinical staging of the tumour was done based on TNM staging system [10].

STATISTICAL ANALYSIS

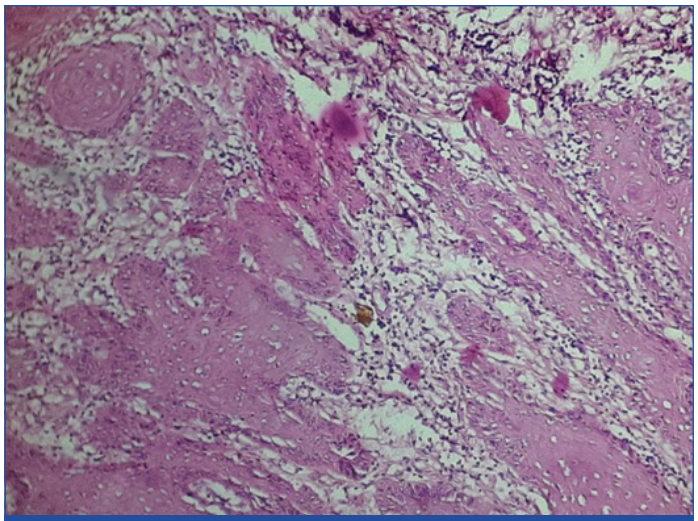
The data was compiled in Microsoft Excel sheet and transferred to Statistical Package for the Social Sciences (SPSS) version 20.0. Both descriptive and inferential statistics have been applied. Chi-square test and Spearman's corelation coefficient test was used. The cut-off p-value was less than 0.05.

RESULTS

Both the grading system showed a significant cellular difference in both tumour center and periphery of metastatic and non metastatic OSCC. Comparatively, Anneroth's multifactorial grading system showed highly significant p-value of 0.007 and 0.001 in tumour center and periphery of non metastatic and metastatic tumours respectively [Table/Fig-1-4]. Among the six parameters of Anneorth's, pattern of invasion showed a significant difference in the periphery of metastatic OSCC with a significant p-value=0.01, respectively [Table/Fig-5].

Groups		Broder's		Anneroth's	
		Chi-square value	p-value	Chi-square value	p-value
Center	Non metastatic	8.1	0.017*	9.818	0.007**
	Metastatic				
Periphery	Non metastatic	8.1	0.017*	16.44	0.001**
	Metastatic				

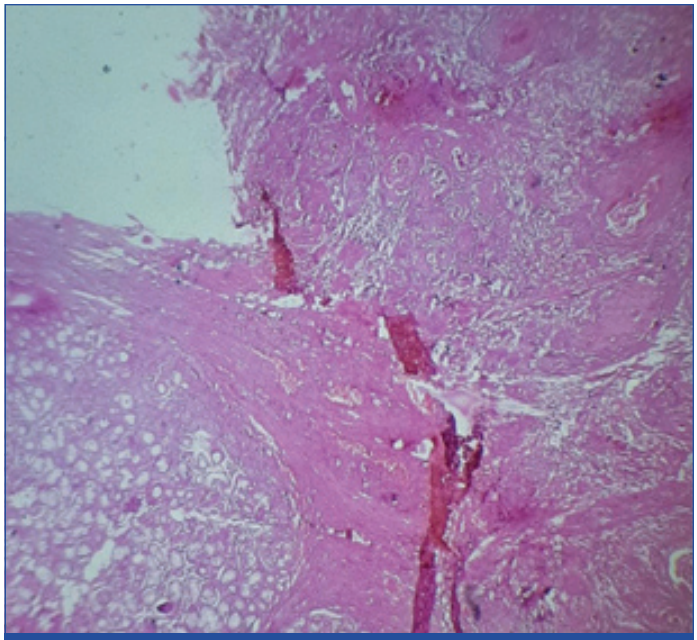
[Table/Fig-1]: Comparison of histopathological gradings of Broder's and Anneroth's grading system.
*Statistically significant at p<0.05; **Highly significant at p<0.001



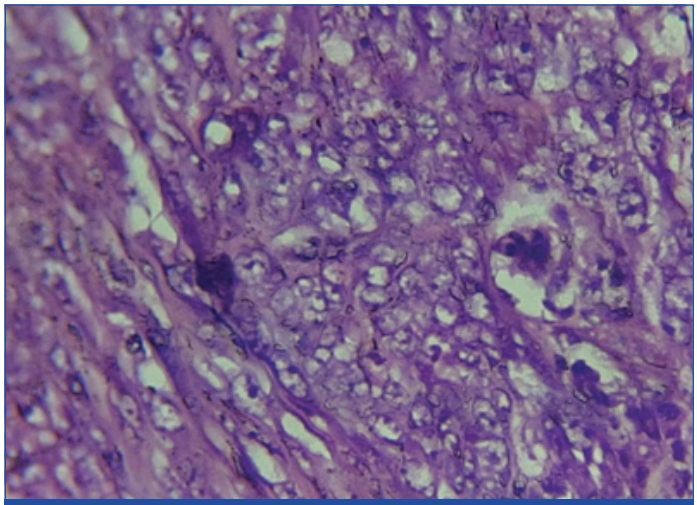
[Table/Fig-2]: The H&E stained section under high power view (40x) showing, infiltration of tumour cells in the form of solid chord and strands.

Among the 20 cases, 7 cases (35%) were of T2 category, 7 (35%) cases were of T3 category and 6 cases (30%) were of T4 category. Only 12.5% of cases were palpable among T2 category, in T3-71% of cases were palpable and in T4-100% of cases were palpable. Histopathologically, nodal positivity was observed in 10 (50%) cases. Statistically, no significant relationship existed between T factors and lymph node metastasis. The relationship between the Anneroth's grading system and TNM staging for non metastatic and metastatic cases are tabulated in [Table/Fig-6,7], respectively. When comparing the association between metastatic and non metastatic groups with TNM staging, metastatic cases of OSCC showed a significant result with p-value=0.001.

Spearman correlation coefficient was done to analyse the correlation between the Anneroth's system and TNM staging. A positive correlation was observed with increased in TNM staging with increased histopathological grading [Table/Fig-8]. Comparatively, Anneroth's grading system showed a significant correlation with TNM staging both in tumour center and periphery with a p-value=0.001 [Table/Fig-9].



[Table/Fig-3]: The H&E stained section under high power view (40x) showing, invasion of tumour cells below lamina propria adjacent to salivary gland



[Table/Fig-4]: The H&E stained section under high power view (40x) showing, invasion of tumour in the form of widespread cellular dissociation in group/individual cells.

Pattern of invasion		Chi-square value	p-value
Center	Non metastatic	6.286	0.099
	Metastatic		
Periphery	Non metastatic	9.176	0.01*
	Metastatic		

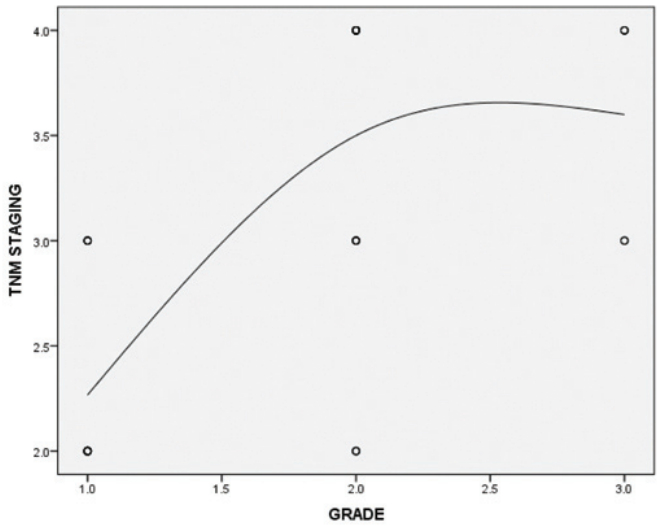
[Table/Fig-5]: Association between non metastatic and metastatic based on Anneroth's pattern of invasion using chi-square test.
*Statistically significant at p<0.05

Non metastatic OSCC	Anneroth's grading system	Scoring of TNM staging		Total
		TNM stage II	TNM stage III	
Center	Grade I (1-12)	5 (83.3%)	1 (16.7%)	6 (60%)
	Grade II (13-18)	2 (50%)	2 (50%)	4 (40%)
	Total	7 (70%)	3 (30%)	10
Periphery	Grade I (1-12)	6 (66.7%)	3 (33.3%)	9 (90%)
	Grade II (13-18)	1 (100%)	0	1 (10%)
	Total	7 (70%)	3 (30%)	10

[Table/Fig-6]: Anneroth's grading of non metastatic OSCC cases and the relationship with the scores of TNM staging

Metastatic OSCC	Anneroth'S grading system	Scoring of TNM staging		Total
		TNM stage III	TNM stage IV	
Center	Grade II (13-18)	1 (14.3%)	6 (85.7%)	7 (70%)
	Grade III (19-24)	1 (33.3%)	2 (66.7%)	3 (30%)
	Total	2 (20%)	8 (80%)	10
Periphery	Grade II (13-18)	1 (12.5%)	7 (87.5%)	8 (80%)
	Grade III (19-24)	1 (50%)	1 (50%)	2 (20%)
	Total	2 (20%)	8 (80%)	10

[Table/Fig-7]: Anneroth's grading of metastatic OSCC cases and the relationship with the scores of TNM staging.



[Table/Fig-8]: Graph shows positive relationship between TNM staging with increase in Anneroth's grading system.

Grade	TNM staging			Total	Chi-square test	p-value	Spearman's correlation coefficient (R-value)	p-value
	2	3	4					
I	11	4	0	15	20.625	0.001	0.662	0.001
II	3	4	13	20				
III	0	2	3	5				
Total	14	10	16	40				

[Table/Fig-9]: Based on correlational analysis, Anneroth's grading system showed a positive correlation between TNM staging using Spearman's correlation coefficient. p-value<0.05 considered significant

DISCUSSION

In head and neck tumours, 90% of cases were OSCC whose unabatable growth and invasive potential increases the locoregional spread [9,11]. Metastasis to lymph nodes signify the next stage in the progression of cancer. TNM staging is a prognostic factor to determine the extent of the diseases and to predict the outcome of cancer patients [12]. However, rate of growth and invasiveness depends not only on the clinical staging, mostly on differences in the degree of differentiation of tumour cells which contributes to the heterogenous population [13,14]. Histological prognostic factors are important in assessing the clinical and biological behaviour of the tumour. In 1920, Broder's quantitative grading system for cancer was initiated still it lacked a correlation with prognosis. To make the morphologic and histologic criteria to be more precise Anneroth and Hansen modified the histopathological grading system for application to OSCC.

In this study, tumour center and periphery of metastatic and non metastatic tumours were graded by the Anneroth's and Broder's grading system to know the difference in the cellular details and invasiveness of the tumour. Comparatively, Anneroth's system showed significant cellular difference in tumour periphery of metastatic OSCC with increased potential to regional LN metastasis.

Baba AI and Câtoi C found that there are cellular differences between tumour center and periphery, where the cell population in tumour center has normal intercellular connections, with desmosomes and junctional complexes, while these are absent or reduced at the periphery [15]. Sharma M et al., stated that areas with a high invasive rhythm shows cells that are completely detached from the tumour mass, and whose interconnections disappear altogether [16]. Bankfalvi A and Piffko J and Tumuluri V et al., reported that the tumour population in the invasive front determines the actual behaviour of the tumour, which provides information for assessing the clinical aggressiveness and thereby the prognosis of the tumour [17,18]. It is also stated that the tumour cells in the invasive front were in less differentiated state when compared the differentiated cell population in the center. Tumuluri V et al., and Byers RM et al., reported that cell population in the invasive tumour front has an affirmative correlation between the multifactorial histopathological grading system, prognosis and risk factors in OSCC [19,20].

In Anneroth's classification, the total scoring of the six parameters gives an idea about the extension, involvement and aggressiveness of the tumour. Though, Broder's system exhibits a significant relationship between the tumour center and periphery, it is based only on one parameter i.e., degree differentiation of a cell. In this study, Anneroth's grading showed highly significant results in both center and periphery of metastatic and non metastatic OSCC. Similar findings were observed in the study conducted by Akhter M et al., where both the grading system showed significant results but Anneroth's grading system was more significant when compared to Broder's grading system [7].

Okada Y et al., and Dantas DD et al., observed no significant difference between and the nodal positivity and the T factors [21,22]. Study also reported a positive relationship between the Anneroth's grading system and cervical node metastasis and postulated that the histopathological findings could serve as a predictor in cervical lymph node metastasis. In addition, Haque MA et al., also reported a significant relation between the multifactorial grading system and palpable lymph nodes [23]. The present study differs from the above investigations by analysing the cellular changes in both tumour center and periphery of metastatic and non metastatic OSCC. The results also favour to predict, higher the alteration in tumour cell population in periphery of the tumour higher is the possibility of metastasis to regional lymph node.

Limitation(s)

It includes that only one area of the tumour was analysed for periphery. The mean score calculated from two or more periphery of whole tumour may represent the accurate biological behaviour of the tumour and can be used to assess early metastasis.

CONCLUSION(S)

Anneroth's multifactorial grading system of OSCC can be taken as a valuable diagnostic and predictive tool for assessing regional lymph node metastasis. This was a retrospective study and in future a prospective study with a larger sample size has to be carried for confirming the above results. For further improving the clinical value of the histological grading system, use of proliferative and vascular immunohistochemical markers can be accounted to predict the biological behaviour of the tumour.

Acknowledgement

The authors would like to thank Dr C Bharath, Assistant Professor of Department of Community Dentistry and Dr Balamanikandan, Associate Professor of Department of Oral and Maxillofacial Surgery for their expert advice, support and valuable suggestions while conducting this study.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 29, 2021
- Manual Googling: Jul 05, 2021
- iThenticate Software: Jul 17, 2021 (14%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Mar 03, 2021**
Date of Peer Review: **May 10, 2021**
Date of Acceptance: **Jul 06, 2021**
Date of Publishing: **Aug 01, 2021**