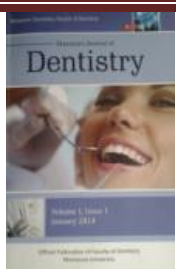

Expression of VEGF-C in Oral Precancerous Lesions and Oral Squamous Cell Carcinoma

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Abstract:

Objectives: Oral squamous cell cacrcinoma(OSCC) comprises 90% of all oral cancers and 16-62% of OSCC develop from oral premalignant lesions (OPLs). Lymph node metastasis is a major prognostic indicator for OSCC progression. It has been revealed that lymphangiogenic growth factor VEGF-C plays an important role in invasion and nodal metastasis of cancer cells. This study was performed to investigate the VEGF-C expression and its clinical significance in OPLs and OSCC.

Methods: The expression levels of VEGF-C was determined immunohistochemically in 10 OPLs cases and 20 OSCC cases and correlations between VEGF-C expression and clinicopathological parameters and prognosis were analyzed.

Results: The statistical analysis revealed high significant correlation between expression of VEGF-C (r =0.737, p=0.015) and degree of dysplasia in OPLs. Also correlations between VEGF-C expression and tumor size(r =0.533, p=0.016),lymph node metastasis(r =0,686, p =0.001),clinical stage of OSCC (r =0.466, p=0.038) showed high significant correlation.

Conclusions: These results suggest that VEGF-C may play an important role in the process of carcinogenesis and can be a strong predictor of lymph node metastasis in OSCC.

Keywords: *VEGF-C,OSCC, lymph node metastasis.*

Introduction

More than 90% of all oral cancers are oral squamous cell carcinomas (OSCCs) [1,2]. Known risk factors include: tobacco use (smoked or chewed), alcohol consumption, diet, viruses such as human papillomavirus (HPV), prevalence of premalignant pathologies and traumatic dental history [3-5]. It was reported that 16–62% of OSCCs develop from PLs [6]. Identifying an accurate biomarker for the premalignant state would aid in diagnosis and also allow premalignancy rather than carcinoma to be an end point in clinical trials and usher new hope to these patients [7].

In HNSCCs, the presence of lymph node metastasis at the time of diagnosis is an indication of poor prognosis [8.9]. Several reports on HNSCC showed that lymphangiogenesis was closely related to lymphatic metastasis [10-12] Among the growth factors, VEGF-C, due to its central roles in lymphangiogenesis and angiogenesis in embryos and tumors, is an important member of the VEGF family [13-14].

VEGF-C has been shown to regulate the growth of lymphatic vessels in various experimental models [15]. It has been shown that lymphangiogenic growth factors correlate to malignant potential of premalignant lesions. Auvinen et al. [16] showed immunohistochemically that VEGF-C expression increases in epithelium as it progresses through dysplasia to adenocarcinoma. Several authors have demonstrated VEGF-C expression using immunohistochemical staining or reverse transcription polymerase chain reaction in various cancers [17-21]. Indeed, VEGF-C, must be more investigated in OSCC, because they may become important therapeutic targets and help to prevent the metastatic spread from these malignancies [22].

Materials and methods

The present study was conducted on paraffin embedded blocks of 10 cases of OPLs, 20 cases of OSCC and 5 specimens of normal oral mucosa. The retrieved paraffin blocks were employed to prepare 4 microns paraffin sections to conduct the following techniques:

1-Haematoxylin and eosin staining: to confirm diagnosis based on the WHO classification of oral and oropharyngeal tumors and premalignant lesions [23,24].

2-Immunohistochemical staining: performed using the Avidin-Biotin Complex (ABC) method according to the manufacturer's instructions [25]. The degree of VEGF-C expression in the epithelial cells of OPMLs and OSCC was evaluated using a modification of the method described by Wang X.L et al. [26] and Sappayatosok K. et al. [27].

Immunostaining assessmen and statistical analysis:

In each case, the positivity and intensity of the cytoplasmic/nuclear immunostaining of the epithelial cells in 5 randomly selected high power fields(x400) were evaluated. A combined score for VEGF-C expression was based on the summation of two scales that reached a maximum score of 6. Both scales graded from 0-3 (scale a: intensity) + (scale b: percentage of positive cells;0:number of positive cells = zero,1:number of positive cells =1–10%,2:number of positive cells =11–49% ,3:number of positive cells > 50%).Final score evaluation was 0=No immunostaining , 2= weak ,3-4= moderate , 5-6= strong.

Computations were made using the Statistical Package for Social Science(SPSS) version 17.0. Significance was considered when P value ≤.05.

Results

Negative VEGF-C reaction was observed in 60% of normal oral mucosal tissues but some 40% of cases showed weak expression of VEGF-C as cytoplasmic granules at the basal cell layer. Positive expression of VEGF-C was observed in 90% of OPL cases, while only 10% appeared negatively stained. Strong expression was observed in 10% of OPL cases, while moderate expression was noted in 60% and only 20 % showed weak expression. Strong significant correlation was reported between the expression of VEGF-C (*r =0.737, p=0.015*) and degree of dysplasia (Fig.1 left) (Table 1).

Nineteen OSCC cases (95%) showed positive reaction to VEGF-C. Expression was variable between different histological grades of tumors and also within the same tumor. The expression was either strong or moderate. Strong VEGF-C expression was observed in 30% of OSCC cases and moderate expression of VEGF-C appeared in 60%. Weak expression was not detected in any case and only one case (5%) showed no immunereactivity to VEGF-C. It was also noted that tumor cell nests at the invasive edges displayed stronger VEGF-C expression than nests away from the area of invasion (Fig.1 right). Lymph node metastasis with special concern showed high significant correlation with VEGF-C degree of expression (*r = 0,686, p =0.001*).

The statistical analysis in the present study also showed high significant correlation between clinical stage and VEGF-C expression (*r =0.466, p=0.038*).No significant correlation was observed between the VEGF-C expression (*r = 0.306, p=0.189*) and histological grade of OSCC. (Table.1).

Discussion

The present study is a trial to clarify the possible role of VEGF-C in predicting the prognosis of OPLs and OSCCs. The current research observed a significant correlation between VEGF-C expression and the degree of dysplasia .This came in agreement with other studies on oesophageal [28] and cervical carcinogenesis [29] in which increased expression was associated with progressing degrees of dysplasia . This may suggest, in addition to pro-lymphangiogenic activity, the autocrine effects of VEGF-C via receptor VEGFR-3 directly on tumor cells [29].

The current research detected positive VEGF-C reaction in all but one tumor of OSCC cases (95%). Same results were reported in other studies [30,31]. Furthermore, all positive OSCC cases of the present work revealed moderate to strong VEGF-C immunoreactivity , while no cases showed weak VEGF-C reaction. This remarkable increase in the degree of expression than the normal tissues was indicative for a prolymphangiogenic change [32].

Another important observation in the present study was that cell nests at the invasive edges of moderately differentiated SCC cases showed stronger expression than those in the center or away from invasion. This finding was also reported by many other researchers [30,33,34]. Furthermore, Gershenwald team work [35] reported that in prostate cancer, immunohistochemical analysis has shown that expression of genes and proteins associated with angiogenesis and invasion was higher in peripheral zones of cancers than in their centers. On the basis of the present study, VEGF-C expression was highly related to lymph node metastasis. Several other studies supported this finding [36,37]. Thus, in OSCC progression, VEGF-C may be considered a very significant factor that influences regional lymph node metastasis and survival. On the contrary, Alves et al. [38] and other researchers [39,40] reported opposite findings. This discrepancy in results could be attributed to the different antibodies used, number of studied cases as well as evaluation methods of VEGF-C expression.

Regarding the clinical stage of tumors , the current study demonstrated high significant correlation with VEGF-C expression. Several investigators were in agreement with this finding [33,36,41]. On the contrary, Yang et al. [42] and Watanabe et al. [43] reported absence of statistically significant correlation with clinical stage. Finally, Wang et al. [44] and other researcher [45] observed a significant correlation between increased VEGF-C expression and advanced histological grades, suggesting that poorly differentiated tumor cells may be more capable to secrete VEGF-C, which induced lymphangiogenesis [46]. On the contrary, the present study in addition to many previous studies [10,36,40] showed no significant correlation between the VEGF-C expression and histological grade of OSCC. This discrepancy in findings could be due to small number of studied cases.

Conclusion

From the findings of the present study it can be concluded that VEGF-C may play an important role in the process of carcinogenesis and may be used as a prognostic marker in predicting progression of OPLs and lymph node metastasis in OSCCs.

Table1: Correlations among degree of dysplasia of OPL cases ,histological grades of OSCC cases, clinical stage , lymph node metastasis and degree of VEGF-C expression.

Clinicopathologic parameter		Degree of expression
Degree of dysplasia	r	.737*
	p	.015
Histologic grade	r	.306
	p	.189
L.N metastasis	r	.686**
	p	.001
Clinical stage	r	.466*

	p	.038

*: Significant (at P ≤0.05) , r: Spearman's rho Correlation coefficient, p : Probability

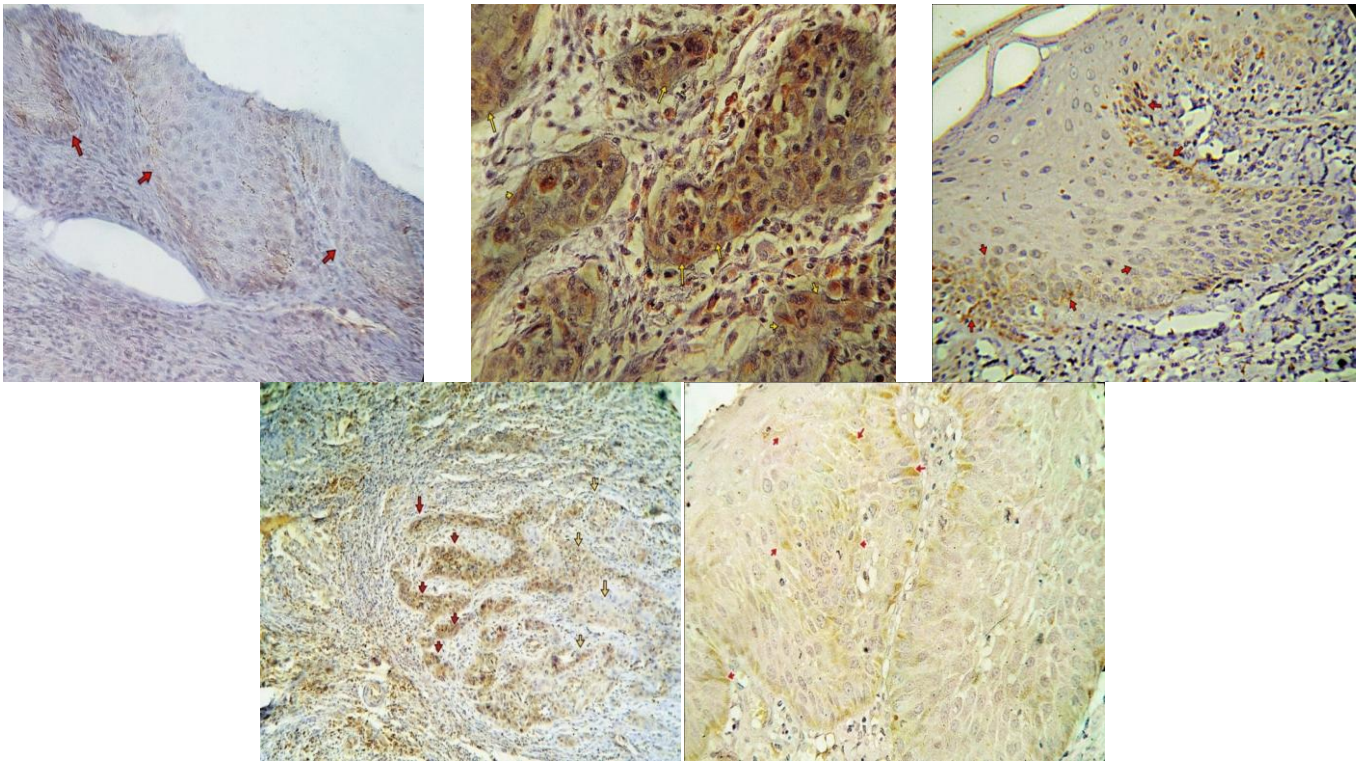

Figure 1: Left: VEGF-Cexpression in OPLs (Top)Weak expression.(Middle) Moderate expression.(Bottom)Strong expressionRight:VEGF-C expression in OSCC.(Top);Strong expression.(Bottom):Moderate expression with more intensity at invasive edge(ABC, DAB X400).

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