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Could Int-8 Receptors and VEGF be Considered as Potential Prognostic Markers in OSCC?

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Abstract **Objectives:** The goal of this study was to determine the correlation of clinicopathological factors and the up-regulation of Int-8 receptors and vascular endothelial growth factor (VEGF) expression in oral squamous cell carcinoma (OSCC). **Materials and Methods:** Clinical data (age, gender, size of the tumor, location of the tumor, lymph nodes status,..) were collected from 20 patients with OSCC, tabulated and statistically analyzed (SPSS17). Immunohistochemical stainings of Int-8 receptors, CXCR1 and CXCR2 (united states biological, United Bio, USA) and VEGF (Abcam, Cambridge, MA, USA) were done. **Results:** Our immunohistochemical study demonstrated: 1) expression of Int-8 receptors in all cases of OSCC with variable intensities, 2) high-level staining of VEGF In poorly differentiated and invasive oral squamous cell carcinoma. Significant correlation was observed between immunohistochemical expression of Int-8 receptors, VEGF and histologic differentiation and clinical stages (Analysis of variance ANOVA, $P<0.05$). **Conclusion:** Our findings suggest that the reactivity to Int-8 receptors observed in OSCC cases could be used as a parameter for tumor aggressiveness and as potential prognostic marker. Also, up-regulation of VEGF may play a role in the angiogenesis and progression of oral squamous cell carcinoma.

Keywords: *squamous cell carcinoma, int-8 receptors, vascular endothelial growth factor*

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1. Introduction

A crucial step in tumor growth and metastasis is tumor neovascularization (Angiogenesis) [1,2]. Tumor angiogenesis is regulated by aberrant production of angiogenic and anti-angiogenic factors expressed by malignant tumor cells and host cells or both [3]. Angiogenesis is mediated by various angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF) and Interleukin -8 (IL-8) [4].

Highly vascularized tumors showed a poor prognosis and the influence of tumor angiogenesis proved to be independent of conventional prognostic indicators [5]. The controlling mechanisms of tumor progression and metastasis in carcinomas including OSCC are clearly complex. One important cellular process involved in these mechanisms is the relationship between cancer angiogenesis and chemical messengers known as cytokines [6].

Cytokines can act as growth factors and chemokines (chemotactic cytokines) for both endothelial cells and tumor cells. They could play an important role in cancer such as increasing angiogenesis, stimulating tumor progression, enhancing tumor cell migration and facilitating evasion of immune surveillance [7]. Among chemokines CXC family, IL-8 was discovered by Yoshimura et al (1987) [8]. IL-8 protein is encoded by the IL-8 gene that is associated with inflammation, immunity [9], tumorigenicity and pro-angiogenic properties [10].

All biological effects of IL-8 are mediated by 2 receptors designated as receptor 1 (CXCR1) and receptor 2 (CXCR2) [11]. The genes encoding CXCR1 and CXCR2 are located in close proximity to each other on chromosome 2 (2q34-35) [12]. These receptors bind IL-8 with high specificity to CXCR1 and with less specificity to CXCR2 expressed endothelial and tumor cells [13].

The expression of CXCR1 and CXCR2 on endothelial cells, neutrophils, cancer cells and tumor-associated macrophages suggest that the secretion of IL-8 from cancer cells may have a profound effect on the tumor microenvironment. As a consequence of inducing many signaling pathways, activation of CXCR1 and CXCR2 on endothelial cells is known to promote an angiogenic response, inducing the proliferation, survival and migration of vascular endothelial cells [14,15]. The expression of CXCR1 and CXCR2 on cancer cell lines and cancer cells of tumor biopsy tissue also suggests that cancer cells are subject to the effects of autocrine/paracrine IL-8 signaling, which has been associated with stimulating cell proliferation [16,17], migration, and invasion [18,19,20]. In another study, it has received attention in assisting cancer cells to evade stress-induced apoptosis [21].

Many studies have demonstrated a relationship between expression of IL-8 receptors and tumor progression (proliferation, angiogenesis, invasion, migration and metastases), aggressiveness, prognosis and survival rate in human cancers [22-29].

Watanabe et al [7] reported that IL-8 secretion from OSCC cell lines induced migration and invasion of OSCC cells via CXCR1 and CXCR2, but did not change cell proliferation, and overexpression was associated with poor prognosis.

Undoubtedly, it is still necessary to carry out an important effort to determine expression CXCR1 and CXCR2 of cancer cells compared with control tissue and understanding the relationship between expression CXCR1 and CXCR2 with prognostic factors in tumor tissue that may represent potential prognostic biomarkers and therapeutic targets.

VEGF is a 46 kDa heparin-binding homodimeric glycoprotein. To date, in addition to VEGF-A, PIGF-1, VEGF-B, VEGF-C, and VEGF-D are known. These VEGFs respectively bind to VEGF receptor-1 (VEGF-1 or Flt-1) and VEGF receptor-2 (VEGF-2 or KDR/Flk-1) to promote endothelial cell differentiation and proliferation. Many studies have demonstrated increases in the expression of VEGFs in the processes of carcinoma progression and proliferation [30,31].

Oral cancers show lower than 50% long-term survival rates because of their high metastasis and recurrence rates, and their prognoses have not greatly improved, despite the development of various treatment methods, due to their high probability of local recurrence and metastasis. In addition, although the treatment plans and prognoses of patients clinically diagnosed with oral squamous cell carcinoma mainly follow the TNM classification, treatment results do not coincide with these criteria in many cases. Accordingly, studies are needed to identify markers that will enable a more accurate patient prognosis based on the molecular biological characteristics of carcinomas. They are also elements that affect the occurrence, progression, and metastasis of carcinomas.

Therefore, the present study was conducted to examine the expression of IL-8 receptors and VEGF involved in angiogenesis, which is important for carcinoma progression according to the histological characteristics of oral squamous cell carcinoma, and to find a correlation between patient clinicopathologic factors and differences in IL-8 receptors and VEGF expression.

2. Materials and Methods

Clinical data (age, gender, size of the tumor, location of the tumor, lymph nodes status,..) were collected from 20 patients with OSCC, tabulated and statistically analyzed (SPSS17). Immunohistochemical stainings of Int-8 receptors, CXCR1 and CXCR2 (united states biological, United Bio, USA) and VEGF (Abcam, Cambridge, MA, USA) were done. Results: Our immunohistochemical study demonstrated: 1) expression of Int-8 receptors in all cases of OSCC with variable intensities, 2) high-level staining of VEGF In poorly differentiated and invasive oral squamous cell carcinoma. Significant correlation was observed between immunohistochemical expression of Int-8 receptors, VEGF and histologic differentiation and clinical stages (Analysis of variance ANOVA, $P<0.05$).

3. Results

The sample included 14 males (70%) and 6 females (30%) with female to male ratio of 1: 2.3. The age range was between 49-71 years and the mean age was 59.75 years. The OSCC samples were derived from the tongue n= 6 (30%), alveolar mucosa n= 5 (25%), buccal mucosa n= 4 (20%), retromolar area n=2 (10%), palatal mucosa n= 2 (10%) and lip n=1 (5%).

Recurrence of tumor was present in 5 cases (25%), and lymph node involvement was clinically detected in 9 cases (45%). The clinical staging was defined according to the AJCC classification: 2 patients in stage I (10%), 4 patients in stage II (20%), 5 patients in stage III (25%), and 9 patients in stage IV (45%). In addition, 14 cases (70%) showed endophytic growth pattern while 6 cases (30%) were exophytic, forming fungating masses most of which had irregular or papillary surfaces, red or white in color.

The microscopical examination showed: 6 cases (30%) were well differentiated, 10 cases (50%) were moderately differentiated, and 4 cases (20%) were poorly differentiated.

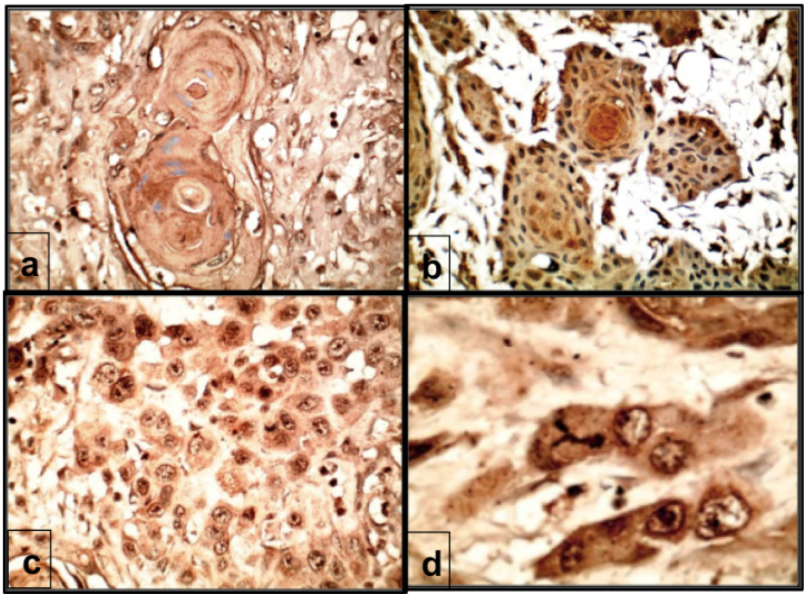

Figure 1. a) Well-differentiated OSCC revealing intense cytoplasmic reaction to CXCR1 in the malignant epithelial cells forming the keratin pearl (x400). b) Cell nests in a moderately differentiated OSCC exhibiting a strong reaction to CXCR1 in both the cytoplasm and nucleus (total cell reactivity). (x400). c) Poorly differentiated OSCC revealing positive immunostaining to CXCR1. (x400). d) Poorly differentiated OSCC revealing intense cytoplasmic and nucleus CXCR1 immunoreaction. Notice: the mitotic figures. (x1000)

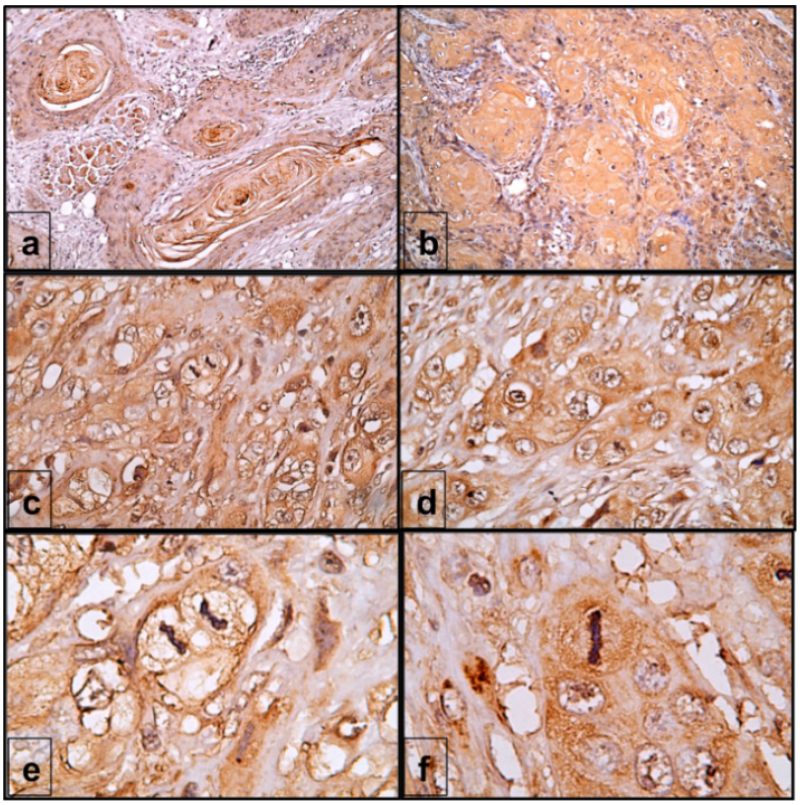

Figure 2). a) Well differentiated OSCC showing keratin and epithelial pearls invading muscle fibers. The malignant epithelial cells are positively reactive to CXCR2 (x200). b) Cell nests in a moderately differentiated OSCC exhibiting a strong reaction to CXCR2 in both cytoplasm and nucleus (total cell reactivity). (x200). c) & d) Poorly differentiated OSCC showing cytoplasmic and nuclear reaction to CXCR2 (x400). e) & f) Higher magnification of the fields revealing normal and abnormal mitotic figures (x1000)

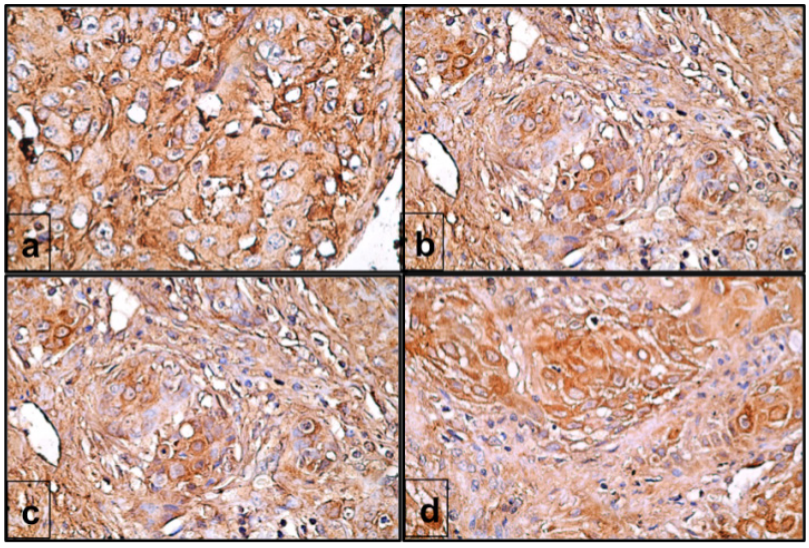

Figure 3. a) Poorly differentiated OSCC showing cytoplasmic immunoreactivity to VEGF.(x400). b) Poorly differentiated OSCC showing cytoplasmic immunoreactivity to VEGF. Note the abnormal mitotic figures. (x400). c) Moderately differentiated OSCC showing intense reaction to VEGF in the cytoplasm of malignant cells. (x400). d) Moderately differentiated OSCC cell nests showing intense cytoplasmic immunoreactivity to VEGF. (x400)

Immunohistochemical findings: Reaction for Il-8 receptors CXCR1 (Figure 1) and CXCR2 (Figure 2) was detected in all cases with variable intensity. The pattern of expression was in both the cytoplasm and nucleus of malignant epithelial cells. The immunohistochemical reaction was mainly concentrated in the keratin pearl of the well-differentiated OSCC. In moderately differentiated OSCC, the immunoreactivity was concentrated in all malignant epithelial cells forming epithelial pearls and cell nests. In poorly differentiated OSCC, the immunostaining was concentrated in all anaplastic malignant cells. VEGF immunoreactivity was observed in all included cases of OSCC (Figure 3). The reaction was noted in the cytoplasm of malignant epithelial cells of well, moderate and poorly differentiated cases of OSCC. The immunoexpression of CXCR1 was statistically significant with different histopathologic grades and clinical stages of OSCC, while it was not statistically significant in CXCR2 (Figure 4, Figure 5). Significant correlation was found between the expression of VEGF and the different histologic grades and clinical stages of OSCC.

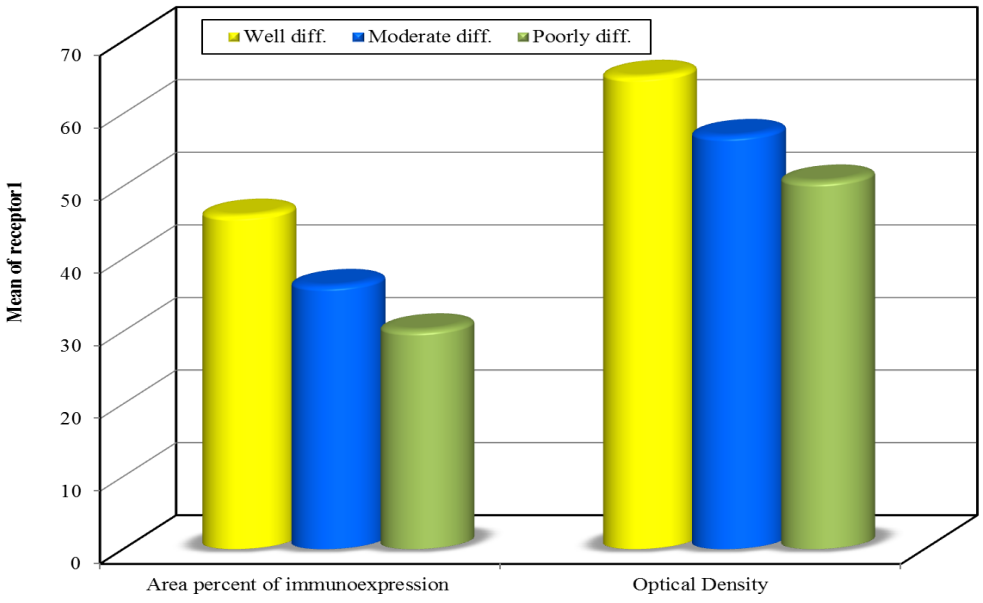

Figure 4. Immunoreactivity of CXCR1 in different histopathological grade

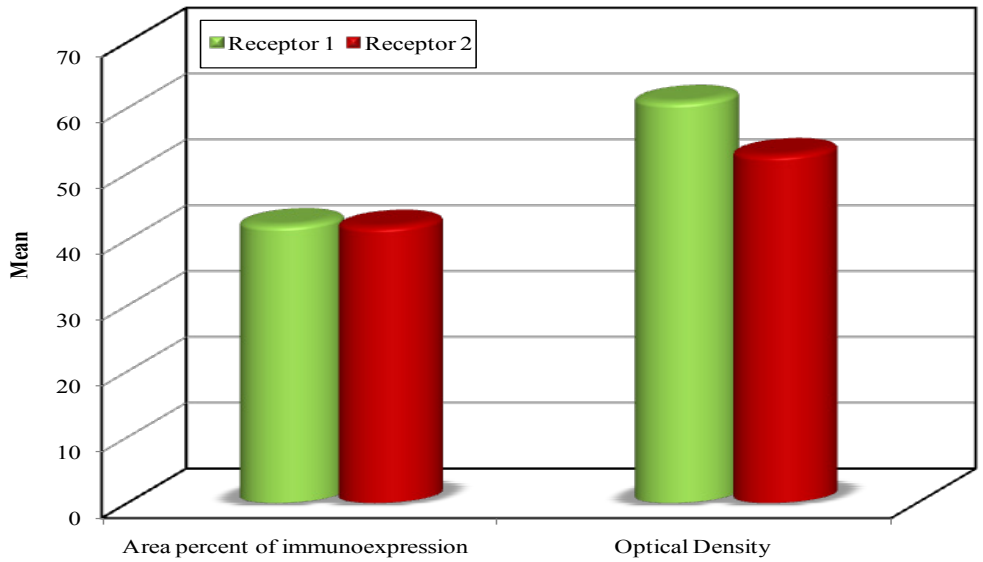

Figure 5. Comparison between CXCR1& CXCR2 in OSCC cases

4. Discussion

Angiogenesis is an indispensable requisite for tumor growth, infiltration, and metastasis. Although early-stage tumors are avascular, the cells in tumors 1 to 2 mm or larger or infiltrated fibroblasts around tumor cells secrete substances that stimulate angiogenesis to proliferate new micro-vessels [32].

In this work, both CXCR1 and CXCR2 were expressed in all cases of OSCC. Other workers found also the same result on oral cancer cell lines [33], and many types of tumors [34,35,36,37]. While Richards et al [38] and Kuwada et al [39] showed the expression of both receptors in HNSCC sections (97%) and human pancreatic cancer (78.9%) respectively. In this work, the difference was statistically significant between CXCR1 and different histological grades. This is in accordance with the results of Richards et al [38] on HNSC and Li et al [37] on gastric cancer tissues. But disagree with the results of Ewington et al [35] on endometrial carcinoma and Chen et al [41] on human pancreatic cancer cases.

Many factors are involved in angiogenesis. VEGF is secreted by diverse cells and has specificity to vascular endothelial cells. VEGF receptors such as VEGF-1 and VEGF-2 are known to play a role in this specificity. These factors are located in the cell membranes of endothelial cells and are activated after binding to other factors in the extracellular matrix. They are known to promote cell nucleus division and contribute to angiogenesis through extracellular matrix dissolution and endothelial cell movement [40].

According to the present results of VEGF immunohistochemical tests, increased VEGF expression was observed in the cytoplasm of invasive tumor cells in all cases of oral squamous cell carcinoma.

This is consistent with the results of other studies indicating that, when VEGF is normal, its expression will be limited in endothelial cells [41,42]. In addition, based

on histopathological findings, little VEGF expression was observed in well-differentiated and less-invasive intraepithelial carcinoma tissues or highly differentiated oral squamous cell carcinoma than in normal cells. On the other hand, strong VEGF expression was observed in less-differentiated invasive oral squamous cell carcinoma. These results suggest that the degree of VEGF expression is correlated with the degree of differentiation or invasiveness of carcinoma; this was supported by the statistical analysis conducted with VEGF expression levels based on the results of immunohistochemical staining and clinical and histological profiles of carcinomas. The correlation coefficient r of the Pearson correlation analysis, which was the statistical method used to that end, can be interpreted as indicating a very high correlation when its value is 0.5 or higher, moderate correlation when its value is between 0.4 and 0.5, and very low correlation when its value is lower than 0.4. Consequently, in the present study, the correlation between the degree of histological differentiation and VEGF expression was significant, as was the correlation between classification according to tumor size and VEGF expression [43].

In conclusion, IL-8 receptors were expressed in all cases of OSCC being detected with variable intensity in both the cytoplasm and nucleus of the malignant cells. Expression was correlated to histologic grades and clinical staging of malignancy. VEGF gene expression was highly increased in progressed oral squamous cell carcinoma. These results lead to the inference that VEGF expression in carcinomas is involved in angiogenesis and progression and may affect the prognosis. However, studies are required to clarify the correlation between IL-8 receptors and VEGF expression in oral squamous cell carcinoma.

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