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ASSOCIATION OF CXCR1 EXPRESSION WITH HISTOLOGICAL GRADE AND CLINICAL STAGE OF ORAL SQUAMOUS CELL CARCINOMA.

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ABSTRACT

Background:. Interleukin-8 (IL-8) is a pro-inflammatory cytokine which exerts its effects via binding to CXCR1 and is known to promote angiogenesis. CXCR1 represent potential prognostic biomarkers and therapeutic target.

Aim of the study: To evaluate the expression of CXCR1 in OSCC and control tissue as well as correlate the expression of the CXCR1to the clinicopathologic parameters of OSCC .

Material and Methods: Immunohistochemical analysis of 22 cases OSCC and 7 control cases sections stained by anti-CXCR1antibody. Immunohistochemical staining will be performed using a Labeled Strept-Avidin Biotin complex method (LSAB).

Results: Expression was recognized in all cases of OSCC and normal control cases and the pattern of expressed in both the cytoplasm and nucleus of the malignant epithelial cells (total cell reactivity), as well as high expression was noticed in low histological grade and clinical stage malignancy, whereas decrease of expression was detected in high grade and stage malignancy.

Conclusions: the results show an association between CXCR1and the clinicopathologic parameters, and considered as prognostic marker in OSCC.

KEYWORDS : Oral squamous cell carcinoma, interleukin-8 receptors1, histological grade , clinical stage, immunohistochemistry.

Introduction

IL-8 is a pro-inflammatory chemokine, which exerts its effects when it binds to the chemokine receptors CXCR1 [1]. CXCR1 is G protein-coupled receptor which when activated initiate various signalling cascades resulting in angiogenesis, and mitogenesis[2,3].IL-8 expressed from the tumor cells binds to CXCR1 on the same tumor cells setting up an autocrine loop. Additionally, over expression of IL-8 by tumor cells attracts large numbers of leukocytes to the site of the tumor by chemotaxis. These leukokytes secrete growth factors and further promote tumor proliferation and growth, initiating a paracrine loop[1.4]. The CXCR1 are present on neutrophils, monocytes, keratinocytes, melanocytes, endothelial cells, fibroblasts, and smooth muscle cells. In addition, the receptors have been identified in the pathologic cells of the following disease states: melanoma, psoriasis, leukemia, breast cancer ,OSCC[5],ovarian cancer[6], pancreatic cancer[7], pilomatricoma[8], endometrial carcinoma[3] ,prostat cancer [9] ,gastric cancer[10],and nasopharyngeal carcinoma[11].

In the literature few workers have emphasized a possible role of CXCR1in the pathogenesis of OSCC. in this repot we used immunhistochemical to evaluate the expression of CXCR1 in OSCC and control tissue as well as to correlate the expression of the CXCR1 to the clinicopathologic parameters.

Materials and method

Twenty (22) specimens obtained from cases diagnosed with different histological grades and clinical stages of OSCC, The cases were collected from the Departments of Cranio-Maxillofacial and Plastic Surgery, Faculty of Dentistry, Alexandria University. Seven specimens of normal tissue was used as control case.The specimens were fixed in 10% neutral buffered formalin, processed and embedded in paraffin wax using the conventional procedures. Serial sections of 3-4 µm thick were placed on glass slides and stained using hematoxylin and eosin (H&E), and Immunohistochemical (IHC) staining was performed using the Labeled Strept-avidin biotin complex method (LSAB) Then, the sections were examined by the image analyzer computer system using the software Leica Qwin 500 . The device includes a light microscope cabled to a microcomputer that performs high speed digital imag processing .

Statistical Analysis

The difference in the mean area percent and mean optical density of expression and the clinicopathologic parameters were estimated using analysis of variance (ANOVA) test..A p value less than 0.01 was considered highly significant.

Immunohistochemical result

All control cases showed positive immunoreactivity. It appeared as total cell reactivity in the form of diffuse brownish nuclear and cytoplasmic reactions in epithelial cell layers(fig 1).All cases showed positive immunoreactivity with uneven intensities. The reaction was localized in the cytoplasm and nucleusof malignant epithelial cells.

The well differentiated OSCC cases revealed diffuse positive cytoplasmic immunoreactivity. It was concentrated in malignant epithelial cells forming the keratin pearls while few cells showed total cell reactivity (Figure 2). ***The moderately differentiated OSCC cases*** showed intense immunopositivity. The reaction was detected in the cytoplasm and nucleus of almost all malignant epithelial cell nests .Only one case showed intense brownish immune reaction at the peripheral malignant epithelial cells forming epithelial pearls and nest while the central cells and keratin totally were free from any reaction, (Figures 3). ***The poorly differentiated OSCC cases*** exhibited positive immunostaning in both the cytoplasm and nucleus of malignant epithelial cells (Figure 4).

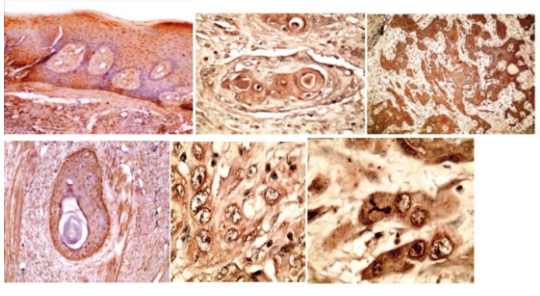

Fig 1-normal tissue showing immunopositivity (x100). Fig 2- Well differentiated OSCC showing a strong cytoplasmic immunoreactivity in the malignant epithelial cells. Note the few cells showing total cell reactivity (x400). Fig 3-Moderately differentiated OSCC a) showing total immunoreactivity in all malignant epithelial cells nests (x200). b- OSSC exhibiting intense immunoreactivity. The reaction is concentrated in the peripheral malignant cells forming the epithelial pearl while the keratin was free from any reaction (x200). **Fig4-(A), (B)** Poor differentiated OSCC revealing intense cytoplasmic and nucleus immunoreaction. (x1000).

II-Statistic results

Both area percent and the optical density were greater in well differentiated OSCC, with the least mean value recorded in poorly differentiated OSCC. Analysis of variance (ANOVA) test revealed that the difference was statistically significant (Table 8, Graph 10)……

The area percent and the optical density tended to decrease with increase in stages of OSCC. ANOVA test revealed that this decrease was statistically significant (Table 9, Graph11)…….

DISCUSSION

In the current study, the positive immunoreactivity was observed in all the control sections .This is consistent with the findings reported in previous studies[3,5, 9, 12], They showed that positive immunoreactivity in all normal tissues that used as control sections. In contrast, **Kuwada et al**[13] found that negative normal control pancreatic tissues.

In this work, CXCR1 was expressed in all cases of OSCC. Other worker found also the same result on oral cancer cell lines[12] ,endometrial carcinoma[3] , prostate cancer [9], benign, borderline and malignant ovarian epithelial tumors\ [6] , and nasopharyngeal carcinoma [11] . while **Richards et al**[5] **, Kuwada et al**[13],and **Li et al**[10] showed the expression of CXCR1 receptors in HNSCC sections (97%) ,human pancreatic cancer(55%) , and (61.0%)gastric cancer tissues respectively.

In this research, the immunoreaction was seen in the both cytoplasm and nucleus (total cell reactivity) of the malignant epithelial cells. This is in accordance with the results of **Ewington et al**[3] on endometrial carcinoma .In contrast, **Murphy et al**[9] found expression more localized to the cytoplasm of prostate cancer cells

In this work, the difference was statistically significant between CXCR1 and different histological grades. The well differentiated type has the highest in MOD% and MA%, while the poorly differentiated type was the lowest in MOD% and MA%. This is in accordance with the results of **Richards et al**[5] on HNSCC,. But disagree with the results of **Ewington et al**[3] on endometrial carcinoma ,and **Chen et al**[7] on human pancreatic cancer cases.

Richards et al[5] found minimal reaction of CXCR1 in the basal layer and higher in superficial layer in the well differentiated . In this work, the immunoreaction of the well differentiated OSCC was concentrated mainly in the keratin pearl. The immunoreactivity of moderately differentiated OSCC was concentrated in almost all malignant epithelial cells forming epithelial pearl and cell nests, while one of the cases showed intense brownish immune reaction at the peripheral malignant cells of the nest with the central cells and keratin was totally free from any reaction. In poorly differentiated OSCC the immunostaning was mainly in all anaplastic malignant epithelial cells.

In the present research, the overexpression was in accordance with stage I. While low expression was in accordance stage IV. the difference in expression between different stages appeared to be statistically significant.This finding is not accepted by study of **Ewington et al**[3] in their study on endometrial carcinoma and,**Horikawa et al**[11] on nasopharyngeal carcinoma, and Murphy **et al**[9] on prostatic cancer .

Conclusions: According to our immunohistochemical results of the present study, the following can be concluded: expression in OSCC and control group , as well as the results show an association between CXCR1and the clinicopathologic parameters, and considered as prognostic marker in OSCC

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