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Research Article

Expression of IGF-2 Receptor In Salivary Gland Tumors and Its Correlation with Tumor Relapse

Emamverdizadeh P[1], Vosough Hosseini S[1], Farnia S*[1], Shokouhi Gavgani B[2], Dehghan Manshadi V[3], Lotfi R[4].

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Corresponding Author:

*** Dr. Samira Farnia,**
Department of Oral and Maxillofacial Pathology,
School of Dentistry,
Tabriz University of Medical Sciences, Tabriz,
Iran.
Tel No: 00989120375937,
Fax No: 00984133346977

*Email Id-
dr_samira_farnia@yahool.com

ABSTRACT

Background: Pleomorphic Adenoma (PA) is one of the most common benign salivary gland neoplasms, whereas mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC) are the most common malignant salivary gland tumors (SGT). The expression of insulin-like growth factor receptors (IGF-R) plays an important role in the pathophysiology of different neoplasms. Since there is a lack of sufficient data on the importance of IGF-R in the pathogenesis of SGTs, the present study aimed at evaluating the IGF-2R expression and its correlation with tumor relapse in three common SGT diseases.

Methods: In a laboratory study, 44 paraffin-embedded specimens (15 in PA, 14 in MEC, and 15 in ACC groups) were studied. The samples were stained for IGF-2R using polyclonal antibody. The expression of IGF-2 receptor was studied by two pathologists, and the patients were observed for any evidence of relapse. Data analysis was done by SPSS 18, and $p<0.05$ was considered as statistically significant.

Results: Out of the 44 enrolled patients, 25 (56.8%) were male. The mean±SD age was 38.8±13.72, 47.07±14.95, and 51.26±14.31 years in the PA, MEC, and ACC groups, respectively. No significant difference was observed in the positive cells for IGF-2R between the three groups, whereas the difference for staining intensity and calculated scale was found to be statistically significant ($p=0.02$, $p=0.04$, respectively). Tumor relapse was reported to be higher in ACC compared to the MEC and PA groups ($p<0.01$). The relationship among staining intensity, calculated scale, and the tumor relapse was statistically remarkable ($p=0.02$).

Conclusion: The results of the present study concluded that the relapse rate of tumors is related to IGF-2R expression in tumor cells. The relapse rate was higher and the expression of IGF-2R was lower in ACC when compared to the PA and MEC types of SGTs.

Key-words: Neoplasm, Salivary gland, Insulin-like growth factor receptor, Tumor relapse

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1 Department of Oral and Maxillofacial Pathology, Dental Faculty, Tabriz university of Medical Sciences, Tabriz, Iran.
2 Department of Medical Pathology, Medicine Faculty, Tabriz university of Medical Sciences, Tabriz, Iran.
3 Department of Maxillofacial Surgery, Dental Faculty, Tabriz university of Medical Sciences, Tabriz, Iran.
4 Faculty of Health, Sepc. Hons Kinesiology & Health Sciences, York University, Toronto, ON, Canada.

Introduction

Salivary gland system is mainly structured into three pairs of glands, i.e., parotid, submandibular, and sublingual glands along with other various minor glands located in the oral cavity (1). Although salivary gland tumors (SGT) are regarded as an important disease of the oral cavity (2), they are rare and represent only 3-10% of head and neck neoplasms and only 2% of other neoplasms (3, 4). About 80% of SGTs are benign, whereas the malignant neoplasms in these glands constitute only 0.5% of all malignancies. The annual incidence rate of malignant SGTs is 1 per 100,000 individuals (5). Pleomorphic adenoma (PA), the most common benign salivary gland neoplasm, comprises 85% of all SGTs and 60% of benign parotid gland neoplasia (3). It contains epithelial and myoepithelial cells in a mesenchymal stroma, and it mainly affects women and mostly occurs in the 4th and 5th decades of life (6). Surgical resection (superficial parotidectomy) of the parotid gland is the therapeutic choice in such neoplasms and has a relapse rate of 0.02 (7).

Mucoepidermoid carcinoma (MEC) is the most common malignant tumor of the salivary glands and contains about 30% of all salivary gland malignancies. MEC could either be solid or cystic and consists of mucosal and epidermoid cells. It also affects the patients in their 50s and is more common in females than in males (8). The main therapeutic plan for MEC is surgical excision, but adjuvant chemo-radiation may also be required based on the stage of the tumor (9).

Adenoid cystic carcinoma (ACC), another malignant tumor of the salivary glands, is less common than MEC and has an incidence rate of 50% in major salivary glands and 35% in minor salivary glands. Its incidence is 15% in other sites, such as nasopharynx, oropharynx external ear, lacrimal glands, skin, and female external genitalia (10). Histopathologically, it contains myoepithelial and ductal cells and has regional lymph node involvement in 30% of the cases (11). The main therapeutic intervention in ACC is surgical removal of tumor, and radiation can be prescribed if resection is not possible (12).

Insulin-like growth factors are proteins with structural similarity to insulin and are secreted into the bloodstream from hepatic cells while being stimulated by growth hormone (13). The growth factor is a mainstay of a complex signaling system named IGF Axis, which contains two cell surface receptors (IGF-1R and IGF-2R), two ligands (IGF-1 and IGF-2), and a group of six IGF-binding proteins and some proteinases (14). The axis acts as an essential regulator of the cell growth and development (15).

Several studies have shown that insulin and insulin-like growth factors play an important role in neoplastic transformation and proliferation (13, 16-18).

Lubic et al. (18) concluded that IGF-1 stimulates IGF-1R (insulin receptor) causing cell proliferation and increases androgen levels via de novo steroidogenesis leading to the implementation and progression of prostate cancer.

Tovar et al. (19) studied the role of IGF activation in hepatocellular carcinoma (HCC) and observed IGF axis activation in 21% of early HCC cases. They inferred that insulin receptor or IGF receptor activation has an effective role in neoplastic transformation and cell proliferation in many neoplasms. In an animal-based study, Kalista et al. (20) evaluated the role of follistatin in male mice and found that the protein induced a specific type of muscle IGF-1R leading to muscle fiber hypertrophy. So they concluded that IGF causes muscle cell proliferation via stimulation and activation of their tyrosine kinase receptor on the cell surface.

Fu et al. (21) also studied the clinical importance of IGF-1R in non-small cell lung cancer and found that the tumor tissue has an increased expression of IGF-1R compared to the lung normal tissue. This overexpression is significantly correlated with the disease progression and survival of patients.

Although there are many studies on the role of IGF-R in different malignant cell types and cancer treatment (13, 16, 17), there is a lack of sufficient data on the role of IGF-R in salivary gland tumors. Therefore, the present study was designed to evaluate the role of IGF-R in MEC and ACC and compare them with PA to determine the relationship of IGF-2R expression and tumor relapse in these tumors.

Methods

The archived samples with a diagnosis of PA, MEC or ACC were studied in a census manner in the Pathology Department of Imam Reza Educational Hospital and Oral and Maxillofacial Pathology Department of Dentistry School, Tabriz University of Medical Sciences, Tabriz, Iran. The Hematoxylin & Eosin (H&E) stained slides were observed by using Olympus (U-MDOB) optical microscope (made in Japan). The paraffin embedded samples with definite diagnosis, appropriate fixation, and sufficient size for further staining were included in this study.

The exclusion criteria were neoplasms other than salivary gland tumors, growth disorders, metabolic syndrome, diabetes mellitus, pancreatic disorders, inappropriate staining quality, lack of demographic

characteristics, lack of access to patients, equivocal or ambiguous data about tumor relapse, and lack of patient's consent to participate in the study.

Forty-four samples were finally enrolled into the study and segregated into PA (15), MEC (14), and ACC (15) groups. The immunohistochemical (IHC) staining of IGF-2R was done by using polyclonal antibody. First, 3 μm sections of paraffin embedded blocks were prepared, and the slides were set by covering the sections with 3-aminopropyltriethoxysilane. The samples were then deparaffinized using xylene, rehydrated in ethanol, and then dried at the room temperature.

The staining process against IGF-2R was done using rabbit polyclonal antibody in concentration of 200 μg/ml (made by USCN, Cloud-Clone Corp, China) according to the manufacturer's protocol.

The stained slides were reviewed by two pathologists using the same x400 microscope. The percentage of positive cells was recorded for each specimen under the classifications in Table 1 according to the study by Glas et al. (7). The staining intensity for IGF-2R was also recorded. Subsequently, a scale was calculated for each sample by multiplying the "positive cell percentage group number" with the "staining intensity group number."

The demographic characteristics of all the patients were recorded from their medical records and documents, and they were invited for an evaluation of any relapse of primary tumors. Further medical information of each patient was recorded as per the need of the study. Although there was no intervention in this observational study, informed consent was taken, and all rights of the patients were reserved. The study proposal was approved by the Ethical Committee of Tabriz University of Medical Sciences, code: TBZMED.REC.1394.1067.

The recorded data was analyzed using the SPSS analytical software (Version 18). Mean±SD and frequency-percentage were used for quantitative and qualitative statistics, respectively. One-way ANOVA test for parametric and Kruskal-Walis test for nonparametric samples were used for comparison among the three SGT groups, whereas Independent Sample's T and Mann-Whitney U tests were used for comparisons between two groups. Chi-Squared test was used for determining the relationships, and $p<0.05$ was considered as statistically significant in all tests.

Results

Forty-four patients, including 25 (5.8%) males, were enrolled in the study. The mean±SD age of the patients was 45.68±14.95 years (range, 21 to 74 years). Detailed demographic characteristics of all the patients are available in Table 1. While the mean age of the ACC patients was higher than that of the MEC and PA groups, the difference was not statistically significant. The sex distribution of patients was equivocal, i.e., the PA group was female-dominant while the ACC and MEC groups were male-dominant without any remarkable difference. Since the samples were stained for IGF-2R using polyclonal Ab, the slides were evaluated by two pathologists using optical microscope. The classified percentage of positive cells for IGF-2R (Table 2) was the highest in the MEC group and the lowest in the ACC group; however, this discrepancy was not significant. There was no significant difference in gender-based positive cells percentage. The staining intensity was also evaluated using the same method for positive cells percentage, following the same grading (Table 2). Here, the difference was statistically significant ($p=0.02$); most of the ACC samples had weak or negative intensity, whereas the samples of PA and MEC groups had moderate to high intensity for IGF-2R staining. Gender-based difference was not significant.

Table 1: Demographic Characteristics of Patients

	Number of patients	Age (Mean±SD)	Sex		Age Range	
			Male n (%)	Female n (%)	Youngest (year)	Oldest (year)
Pleomorphic Adenoma	15	38.8±13.72	6 (40)	9 (60)	21	65
Mucoepidermoid Carcinoma	14	47.07±14.95	9 (64.3)	5 (36.7)	25	74
Adenoid Cystic Carcinoma	15	51.26±14.31	10 (66.7)	5 (33.3)	21	72
Total	44	45.68±14.95	25 (56.8)	19 (43.2)	21	74

SD: Standard deviation

Table 2: IGF-2R staining Results

	Grade	Definition	Tumor Type			
			PA	MEC	ACC	Total n (%)
Positive Cells Percentage	1	0%	1	3	6	10 (22.7)
	2	1-4%	1	0	1	2 (4.5)
	3	5-19%	4	3	4	11 (25)
	4	20-39%	1	0	1	2 (4.5)
	5	40-59%	2	0	0	2 (4.5)
	6	60-79%	3	2	2	7 (15.9)
	7	80-100%	3	6	1	10 (22.7)
Staining Intensity	0	Negative	1	3	6	10 (22.7)
	1	Weakly Positive	3	4	5	12 (27.3)
	2	Moderate	6	2	3	11 (25)
	3	Highly Positive	5	5	1	11 (25)
Calculated Scale (Positive cells grade* Staining intensity grade)	Mean± SD		10.33±6.92	10.28±8.87	4.46±6.17	8.31±7.72
	Median		10	9.5	3	6.5

PA: Pleomorphic Adenoma; MEC: Mucoepidermoid Carcinoma; ACC: Adenoid Cystic Carcinoma; SD: Standard deviation

A scale was calculated for each stained sample by multiplying the grade for positive cell percentage with the staining intensity. The range for the scale was 0 to 21, where zero means the weakest and 21 denotes the highest expression for IGF-2R. While the calculated scale is a discrete quantitative scale, both the mean±SD and median were reported for each group in Table 2. The results showed a statistically significant relationship between the tumor type and the calculated scale ($p=0.04$), although there was a significant correlation between positive cells percentage and staining intensity based on Spearman's ($p<0.01$ & $r=0.87$).

The patients were scheduled for follow-up visits for any relevant relapse in their salivary gland tumors. Twenty-two patients (50%) had a relapse with 2 (13.3%) patients in PA group, 6 (42.9%) in MEC group, and 14 (93.3%) in ACC group. The difference in relapse rate was significant among all the groups ($p<0.01$).

The correlation between relapse rate and positive cells percentage was not remarkable, but it was significant between the relapse rate with staining intensity and the calculated scale in all the groups ($p=0.02$ and $p<0.01$, respectively).

Our results showed that the lower the IGF-2R expression, the greater was the relapse rate. Nevertheless, the IGF-2R expression was the lowest in ACC patients, but the relapse rate was as high as 93%. Figure 1 shows the summarized results for scale and relapse rates of all the three groups.

Discussion

SGTs are less common in comparison to other tumors, but their annual incidence rate is about 1-6 patients per 100 individuals. SGTs include a wide spectrum of benign and malignant neoplasms, such as hemangioma, lymphoma, and the metastatic tumors. Out of these, PA is considered as the most common benign tumor, whereas MEC and ACC are considered as the most common malignant tumors of these glands. Many studies have examined these three SGT types because of their higher prevalence rate (22). Biological recognition of these SGTS can lead to better diagnostic, therapeutic, and prognostic approaches in their treatment. Hence, the role of different proteins in these tumors was studied (23). Eslami et al. (24) designed a study to evaluate the progesterone receptor expression in PA and ACC and observed that the progesterone receptor was not expressed in these tumors. The expression of insulin hormone, insulin-like growth factors 1 and 2 along with their receptors and binding proteins have been observed in neoplastic cells. The role of IGF-1 and IGF-2 and their receptors have also been studied in HCC, head and neck squamous cell carcinoma, and malignant tumors of pancreas, prostate, and breast (25-28).

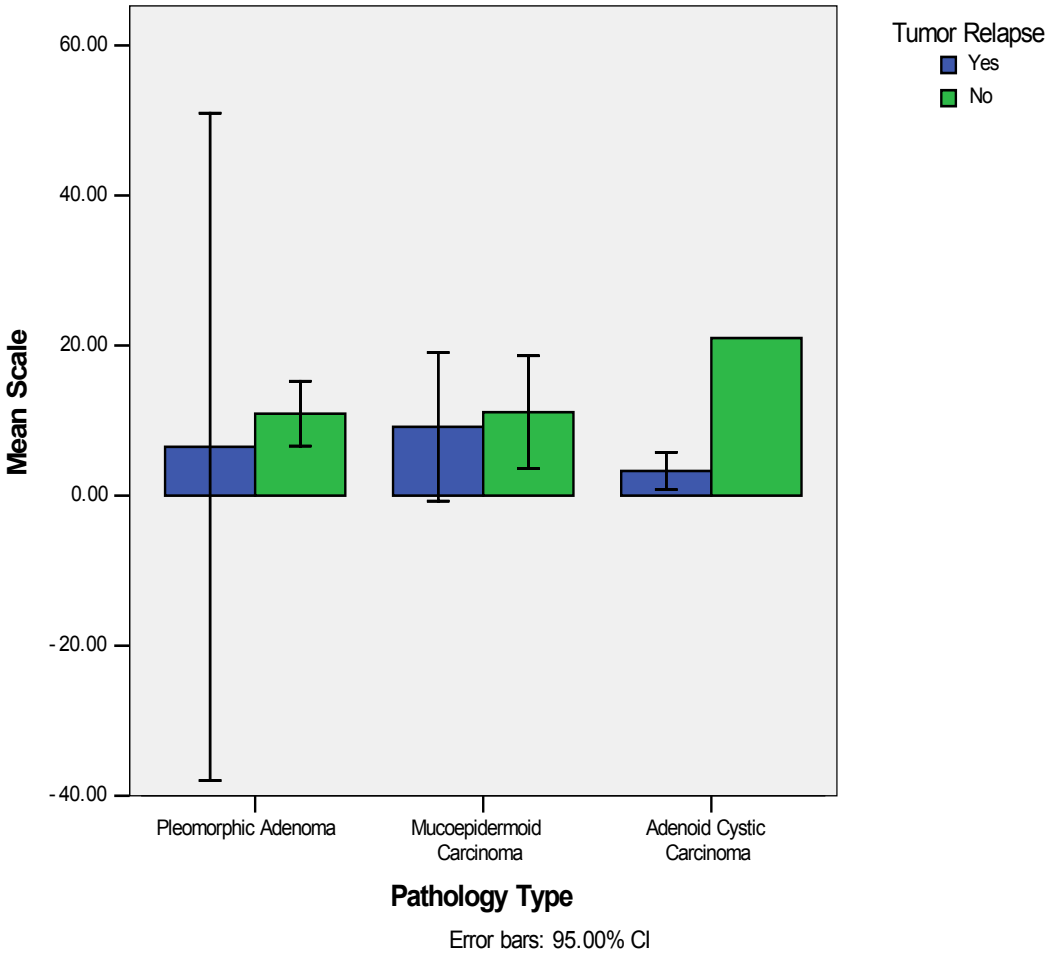

Figure 1: Relapse rate and Calculated Scale based on Tumor Type

The role of IGF axis has been studied in several tumors but not in malignant tumors of salivary glands. Therefore, Glas et al. (7) studied the expression of estrogen, progesterone, and IGF-1R in recurrent PA of the parotid gland and concluded that the expression of estrogen receptor is low. The progesterone receptor expression is high in recurrent PA but low in simple PA. The IGF-1R expression is more extensive in both simple and recurrent PA compared to the normal parotid gland tissue (7). In the present study, 56% of the total number of included patients was male, which is in contrast with the result of studies that reported a higher prevalence of SGTs in women than in men, although the sex distribution for PA group is parallel to the previous studies (8-11). The mean age of all the patients was between their 4th and 5th decades of life, which was similar to previous studies (6, 8, 11).

The final results of IHC study for IGF-2R expression showed that the percentage of positive cells for the receptor is lower in the ACC group compared to the PA and MEC groups. Staining intensity for IGF-2R showed that the difference is again remarkably lower in ACC group. The calculated scale differed significantly among the three groups. Since there was no study on the expression of IGF-2R in SGTs, we failed to compare this finding. However, the relapse rate was higher in the ACC group (93%), which was reported to be as low as 30% by Ko and colleagues (11). On the other hand, we found a relapse rate of 13% in the PA group, which matches with the findings of Enescu et al. (3).

The results of our study revealed that the lower the expression of IGF-2R, the higher was the relapse rate. We also found that the relapse rate was the highest and the IGF-2R expression was the lowest in the ACC group.

Conclusion

Nevertheless, different studies have reported the higher expression of IGF-2R in different neoplastic tissues and the use of IGF-2R as a target for therapy in neoplastic cells. In addition, the role of IGF and its receptors is undeniable in cell stimulation and proliferation. However, our study revealed an inverse correlation between the expression of IGF-2R and tumor relapse rate. Therefore, further studies are needed to determine the role of IGF axis in SGTs.

Author's Note

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