Original Study

EVALUATION OF SERUM LEVELS OF CD 109 IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Aim: Squamous-cell carcinoma (SCC) of Oral cavity is a common malignant tumor of the mouth that typically affects elderly men and women. CD109 a glycosilphosphatidylinositol-anchored cell surface glycoprotein, is expressed at high levels in some human tumors including SCCs. We aimed to evaluate the serum level of CD109 in patients with Oral-SCC (OSCC) compared with normal individuals and consider its clinical significance.

Materials and Method: In this case-control study, 60 serum samples from OSCC patients admitted to the ENT department of Khalili hospital, affiliated to Shiraz University of Medical Science in Southern Iran, (42 males, 18 females with mean age of 62.68) and 28 serum samples from healthy individuals(19 males, 9 females with mean age of 53.75) were collected. CD109 concentration was measured using the ELISA method.

Results: The serum CD109 level was significantly lower in patients with OSCC compared with the healthy controls (6.34±4.09 vs. 13.59±7.85 ngr/ml, P<0.001). The same result was also achieved for each sex group (for males P<0.001, for females P=0.003). For patients, there was no significant difference between males and females (P-value=0.6). However, mean CD109 for males was significantly higher than females in control group (P-value=0.021). T-size and involved lymph node was not related to CD109. The relationship between age and CD109 was significant in male control group (r = 0.522, P-value = 0.022). Also, there was no significant difference between groups of t-site, levels of his-grade, T, N and M.

Conclusions: Serum CD109 can not be used as a serological marker for the diagnosis of OSCC.

Key Words: CD109, Oral Squamous Cell Carcinoma, Serum.

Introduction

Oral cancer consistently ranks as one of the top ten cancers worldwide, with broad differences in geographic distribution. They represent approximately 5% of cancers in men and 2% in women.[1]Approximately 90% of oral cancers are squamous cell carcinoma (SCC) which often develops after the age of 50, with a highest peak in the sixth decade of life.[1,2]

The etiology of SCC appears to be multifactorial and strongly related to lifestyle, mostly habits and diet (particularly tobacco alone or in betel, and alcohol use). [2]

Oral SCC typically presents as a persistent mass, nodule, or indurate ulcer which is seen typically on the lip, lateral part of the tongue or floor of the mouth usually as a lump or ulcer that is white, red, or mixed white and red.[1,2]

Oral lesions are asymptomatic initially, highlighting the need for oral screening and they are often indurated and firm with a rolled border. As the lesions increase in size, pain, dysarthria, and dysphagia may result.[3]

Tumor size and nodal status are the most significant prognostic factors.[1]

At the time of diagnosis, the majority of patients with SCC present advanced disease (stage III-IV), and approximately one third of them show lymph node metastasis which decreases survival rate by about half. Metastases reach the regional lymph nodes first and later the lungs.[1,3]

They can develop from precancerous lesions, such as leukoplakia and erythroplakia, or apparently normal epithelium. Histopathologically, they can be categorized into three degrees of differentiation: [1]

- well differentiated disease shows greater than 75% keratinization.
- Moderately differentiated disease contributes to the bulk of SCC and is characterized by 25% to 75% keratinization.
- Poorly differentiated disease demonstrates less than 25% keratinization.

The degree of differentiation may vary from one part of the tumor to another.[1] Histological grade correlates poorly with patient outcome and thus has limited value for prognostication. Tumor size and nodal status are the most significant prognostic factors. At the time of diagnosis, the majority of patients with SCC present advanced disease (stage III-IV), and approximately one third of them show lymph node metastasis. After curative treatment, about 50% of the patients suffer recurrences; 80% within 2 years and the remaining within 4 years. The major cause of death is loco-regional failure.[1]

Oral SCC (OSCC) is particularly common in the developing world, mostly in older males. There is concern about an ongoing increase in younger patients and in women, as well as in the oropharynx. Other factors such as infective agents may also be implicated, particularly in oropharyngeal cancer. Immune defects or immunosuppression, defects of carcinogen metabolism, or defects in DNA-repair enzymes underlie some cases of SCC. Sunlight exposure predisposes to lip cancer.[2]

Findings from the history and clinical examination by a trained diagnostician are the primary indicators of OSCC, but the diagnosis must always be confirmed histologically with repeated biopsies if the clinical picture is consistent with SCC[2]

CD109 is a glycosylphosphatidylinositol (GPI) anchored cell surface glycoprotein and is a member of the a2-macroglobulin-C3, C4, and C5 family of thioester-containing proteins that is expressed in some tumor cell lines.[4]

This is a cell surface antigen expressed by CD34+ acute myeloid leukemia cell lines, T-cell lines, activated T lymphoblasts, endothelial cells, and activated platelets.[5]

Furthermore, it has been shown that the GOVa/b alloantigen epitopes, which are implicated in refractoriness to platelet transfusion, post-transfusion purpura and neonatal alloimmune thrombocytopenia, are localized to the CD109 protein on platelets, and that a Tyr703Ser polymorphism of CD109 defines GOVa/b alloantigen phenotypes.[6-11]

Although its physiological functions remain largely unknown,[12] CD109 overexpression in cells accelerated cell proliferation and impaired an antiproliferative effect mediated by TGF-β1 in vitro,[4] actually CD109 suppresses transforming growth factor (TGF)-β signaling in human keratinocytes via binding to TGF-β receptor I.[12] These findings suggest that CD109 expression plays a role in the development of oral cancer.[4]

Materials and Methods

This study is a cross-sectional study, that we used serum samples from 60 patients (42 males, 18 females with mean age of 62.68) with Oral Squamous Cell Carcinoma (OSCC), whom reffered to the ENT department of Khalili hospital, affiliated to Shiraz University of Medical Science in Southern Iran, and 28 healthy individuals (19 males,9 females with mean age of 53.75).

The blood samples were prepared in the day before the surgical operation. The final diagnosis of OSCC was confirmed by histopathological examination of biopsied specimens. The healthy control group was enrolled the volunteer individuals without any positive history of known inflammatory or infectious diseases. Also, OSCC patients with a past history of chemotherapy, radiotherapy or surgery, as well as other malignancies or mentioned diseases above, were excluded. The Ethical Sciences Committee of the Shiraz University of Medical Sciences approved the study and all the participants were informed about the nature of the study and agreed to participate by signing an informed consent form.

The serum samples were centrifuged at 4°c and the sera were stored at -70°c until the time of analysis. To measure the serum density of CD109 we used an ELISA Kit (Blue Gene company) with sandwich ELISA method, according to the manufacturer's instructions.

Data were analyzed by SPSS v.18.0 (SPSS Inc, Chicago, IL, USA), using Student's t, Kruskal-wallis H, and Mann-Whitney U tests as well as Pearson's correlation coefficient test. p-value < 0.05 was considered as significant.

Results

In order to compare the CD109 between controls and patients or among male and female the independent t-test was used. The result indicated that the mean CD109 of patients was significantly lower than controls (p-value <0.001). For each sex group, it was also demonstrated that the mean CD109 of patients was significantly lower than controls (for males p<0.001 and for females p=0.003). [Table 1]

	Sex		p - Value	Total	p – Value
Group	Male	Female			
Patient	6.52 ± 4.78	5.91 ± 1.65	0.006	6.34 ± 4.09	< 0.001
Control	15.38 ± 8.84	9.82 ± 2.90	0.021	13.59 ± 7.85	
p - Value	< 0.001	0.003			
Total	9.28 ± 7.5	7.21 ± 8.81			*p – Value: 0.013

**P-value for comparison between two sex groups, in total.*

Table 1: Comparison of Mean CD109 between case and control groups and two sexes

In total, the mean CD109 of males was higher than females (p-value=0.013). For patients, there was no significant difference between males and females (p-value=0.006). However, mean CD109 for males was significantly higher than females in control group (p-value=0.021). [Table 2]

Correlation Coefficient test was used to investigate the relationship of CD109 with age, T-size and involved lymphnodes. T-size and CD109 had no significant relationship (p-value = 0.633) and also invl.lymphnode was not related to CD109 (p-value = 0.912). [Table 3]

The relationship between age and CD109 was significant just in control group (r = 0.461, p-value = 0.014) that it means people with higher age have higher CD109 serum level. This significance association was only in male subgroup (r = 0.522, p-value = 0.022). [Table 4]

Kruskal Wallis test was used to compare the CD109 in different groups of t-site and his-grade. The results showed that there was no statistically significant difference between groups of t-site (p-value=0.239), and also different levels of his-grade were not different in CD109, (p-value=0.508). [Table 5]

Kruskal-Wallis test was used to compare the relationship between CD109, tumor size and the number of involved lymph nodes. The results showed no statistically significant relationship among them (p-values are 0.258 and 0.056 respectively).

In order to compare the CD109 between those with & without Metastasis (M), Mann-Whitney test was used, the

results indicated that there was no difference between levels of M. (p-value=0.100).

Sex	Group			CD109
Male	Control	Age	Correlation Coefficient	0.522*
			p-value	0.022
	Patient	Age	Correlation Coefficient	0.142
			p-value	0.368
Female	Control	Age	Correlation Coefficient	0.118
			p-value	0.761
	Patient	Age	Correlation Coefficient	-0.313
			p-value	0.207
	Male	Age	Correlation Coefficient	0.005
			p-value	0.967
	Female	Age	Correlation Coefficient	-0.289
			p-value	0.144
	Control	Age	Correlation Coefficient	0.461*
			p-value	0.014
	Patient	Age	Correlation Coefficient	0.081
			p-value	0.539
		t.Size	Correlation Coefficient	-0.063
			p-value	0.633
		invl.lymph node	Correlation Coefficient	-0.015
			p-value	0.912

Table 2: Correlation between age, serum variants and CD109

T Site	N	Mean	Median	Minimum	Maximum
Tongue	21	7.51 ± 6.54	6.09	3.3	34.95
Larynx	32	5.91 ± 1.36	5.63	3.66	9.07
Oral Cavity	7	4.80 ± 2.05	4.57	2.44	7.06

Table 3: serum CD109 level in sites of OSCC

Histological Grade	N	Mean	Median	Minimum	Maximum
Well Differentiated	21	6.04 ± 1.64	6.08	2.68	9.07
Moderately Differentiated	22	5.55 ± 1.67	5.18	2.44	9.12
Poorly Differentiated	9	5.83 ± 1.63	5.39	3.78	8.91
Unreported	8	9.88 ± 10.26	6.42	4.55	34.95
Total	60	6.34 ± 4.10	5.83	2.44	34.95

Table 4: Serum CD109 level in grades of OSCC

		N	Mean ± SD	Median	p - Value
T	1	10 (16.7)	6.25 ± 1.51	5.96	0.258
	2	29 (48.3)	619 ± 1.67	6.09	
	3	19 (31.7)	6.82 ± 6.98	5.39	
	4	2 (3.3	4.39 ± 0.87	4.39	
Total		60 (100)	6.34 ± 4.09	5.84	
N	0	41 (68.3)	5.81 ± 1.75	5.86	0.056
	1	11 (18.3)	9.34 ± 8.59	7.06	
	2	7 (11.7)	4.89 ± 0.51	4.81	
	3	1 (1.7)	5.36	5.36	
Total		60 (100)	6.34 ± 4.09	5.84	
M No		59 (98.3)	6.29 ± 4.11	5.81	0.100
YES		1 (1.7)	9.461	9.461	
Total		60 (100)	6.34 ± 4.09	5.84	

Table 5: Clinicopathological profile of 60 OSCC patients and correlation of it with CD109

Discussion

Oral Cavity SCC (OC-SCC) is the most common cancer of the head and neck (with the exception of nonmelanoma skin cancer) and is the life threatening oral disease that recent study shown a rise in the incidence of this malignancy.[13,14] In oral cavity cancer, most cancer patients present with advanced-stage Cancer. Finding new premalignant tumor marker could help physcicien to do better treat and management of this malignancy. CD109 is a glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein, that over expressed on SCC tumor cell surface including pre malignant tumors.[4] Recently, Sakakura and collegues found an soluble form of CD 109 in serum of CD109 Transgenic and Tumor Xenografted Mice,[15] but detailed study of CD109 concentration in serum of normal population and cancerous patients is necessary for application of CD109 as a tumor marker.

In previous studies, CD 109 has been identified as a biomarker that can be used in the diagnosis of some cancers. It can also reveal the prognosis and stage of tumors. The essential role of the expression of CD109 in some cancers, including mammary, salivary, and lacrimal glands; and in prostate basal cells, and urothelial carcinoma, has been established.[16,17] Sakakura *et al.* showed that soluble form of serum levels of CD109 rise in serum of CD109 transgenic mice with CD109 over expression.[15]

In the present study, we found a significant difference in CD109 serum levels between patient and control group. Unexpectedly, CD109 serum level in control group was more than it in patient group.

Separately, the female and male patients compare with female and male control group had less serum level of CD109, significantly.

Our study showed no significant difference in CD109 serum levels between later and earlier TNM stages in

patients with SCC (p=0.5), although the serum level of CD109 was lower in the later TNM stage.

Hagiwara *et al*. by immunohistochemical analysis showed that CD109 expression was higher in low-grade tumors than in high-grade tumors. This result was well-matched with there previous study using oral cavity SCC, in which CD109 expression is higher in well- and moderately-differentiated SCC than in poorly differentiated SCC.[4,16]

In the present study, there was no significant correlation between serum levels of CD109 in patients and tumor size and lymph node involved malignant cancers.

In the present study, decrease in serum levels of CD109 was seen in patient cases compared with the control group, and the difference was statistically significant.

The study sample size of control group is low, and this point may have caused the smaller difference. Serum levels of CD109 were reduced in patients with OC-SCC compared with the healthy population; however, the differences between the clinicopathological characteristics of malignant tumors were not statistically significant. Down regulation of serum CD109 in patients with oral cavity SCC may cause lower serum level of CD109 compare with normal individuals. The authors suggest another study be performed with a larger control sample size for better statistical evaluation.

Conclusion

By exploring the presence of biomarkers those are secreted by cancer into the blood flow will also be a candidate for population based screening. In accordance with this study, the levels of serum CD109 can not be used as a serological marker for the diagnosis of OC-SCC. However, prospective studies on a larger sample size are needed to validate our findings before confirm conclusions on the utility of these markers drift in the diagnosis of oral SCC.

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Conflict of Interest Statement

The authors have no financial relationships to disclose that may pose a conflict of interest. No financial support received for this study.

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