

Original Research Article

Expression of CD44 in Saliva among Group of Smoker Patients with Oral Squamous Cell Carcinoma

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Accepted 17 July, 2016

Abstract

The (CD44) biomarker is considered as one candidate molecular marker for detection of head and neck squamous cell carcinoma, it is listed as adhesion molecules. This protein has many functions and considered as multi-structural cell surface protein which involved different pathological events. The over-expression of CD44 was detected in different cancers in human, including oral squamous cell carcinoma (OSCC). Therefore this study designed to evaluate the role of CD44 in OSCC, quantified in stimulated saliva, beside its correlation with smoking. This case-control study composed of 30 patients with oral squamous cell carcinoma who were smokers, and other 60 control persons for comparison: 30 subjects of them were smoking and others were free of smoking. CD44 was assessed by ELISA technique, in stimulated saliva collected from all groups. The result of this study shows that salivary CD44 protein level significantly higher in smoker patients with oral squamous cell carcinoma in comparison to smoker and non-smoker control healthy persons, thus the CD44 protein has an important role, as a non-invasive way, in the early diagnosis and monitoring of oral squamous cell carcinoma patients.

Key Words: OSCC, CD44, saliva, smoking

الخلاصة:

أجريت هذه الدراسة على مجموعة من المرضى في مستشفى الحلة التعليمي / شعبة جراحة الوجه والفكين في بابل لغرض أخذ عينات من لعاب المرضى المصابين بسرطان الفم الحارشي ولغرض تقييم التغيير الجيني للبروتين CD44 ضمن الأشخاص المصابين. وكانت النتائج وجود فرق معنوي واضح لدى الأشخاص المصابين بهذا المرض عن الأشخاص السليمين وبالتالي يعتبر هذا البروتين كطريقة غير عداية للكشف المبكر عن سرطان الفم ومتابعة الأشخاص الحاملين لهذا المرض.

Introduction

The CD44 protein is considered as adhesion molecules. This type of protein can be released in soluble form through the proteases and can be evaluated in normal circulation. This protein has many functions including cell proliferation, differentiation and other pathological events. These biological properties are essential to the physiological powerful functions of normal cells, while from the other hand they are associated with the pathogenic actions of tumor cells [1]. The circulating levels of sCD44 can be correlated with metastases in some and it is linked to

intracytoplasmic signaling pathways and various trans membrane such as those involving epidermal growth factor receptor and others. The interaction between the CD44 and these receptor-mediated signaling pathways were revealed to promote various tumor progression activities that including growth, migration, invasion, and metastasis of tumor cell [2]. There is unusual expression of CD44 variants high risk precancerous and cancerous lesions was reported by Goodison and Tarin [3].

The experimental research done in animals showing that the targeting of CD44 by CD44-soluble proteins and antisense

beside antibodies inhibit the malignant activities of many types of tumor and emphasizing the therapeutic way of the anti-protein agent. The production of anti-CD44 tumor specific agents may be a truthful therapeutic approach, this may be related to the alternative splicing and post-translational modifications that releasing different CD44 sequences which including tumorspecific sequence [1].

The expression of this protein in OSCC has demonstrated some analytical influence [4]. The earlier research found that the decreased in the expression of CD44 protein was a significant predictor of poor prognosis in most OSCC studies [5], but many other studies showing that a poor prognosis in OSCC associated with strong expression of this protein [4].

This difference in the results may be due to the absence of standardization in research regarding this agent, particularly in relation to the anatomical region. Kokko *et al* [6] found that there is a significant differences regarding the expression of this protein and its prognostic influence regarding the anatomical positions in the head and neck area. The CD44 protein family consist of a standard member of CD44 (CD44s) and other alternative variants (CD44v), it is currently becoming evident that differences in the oral squamous cell carcinoma ability to recurrence, loco regional metastasis, in addition to radiation resistance of its malignant cells, this may be due to the over expression of a specific CD44 isoform [7].

The decrease expression of this protein on tumor cells was significantly correlated with poor prognosis in squamous cell carcinoma of tongue, from other side there is an absence of protein variant expression was associated with a lowest survival period in squamous cell carcinoma of the lip [8]. There is an increased immune expression of the variant 9 of this protein alternative splice isoform along with a loss of other variant was significantly associated with a poor picture in oral squamous cell carcinoma clinically, while in the other cases the over expression of CD44v 3 and

CD44v 6 appears to show the cellular invasiveness which leads to the elevated aggressiveness of some oral tumors specifically OSCCs invasiveness [9], this given that these different isoforms are associated with lymph node metastasis and resistance to chemotherapy [10].

Materials and Methods

The present study included 30 patients having oral squamous cell carcinoma who were smokers and those patients were diagnosed with OSCC and not subjected to any type of treatment regarding this disease. for comparison, 30 apparently healthy smokers and another 30 non-smokers were enrolled in this study. Control subjects were selected in such a way to be matched with age and gender of the patients. Patients age was ranging from 50 to 70 years, included (13) females and (17) males. The study was conducted in AL-Hilla Teaching Hospitals in Babylon city. The study was held from May 2015 through February 2016. A sample of stimulated saliva (800 μ L) was obtained from each patient and control subjects between 8:00 am and 11:00 am. Saliva was stored at -80°C till the time of analysis. The expression of CD44 protein was assessed by ELISA technique. The concentration of protein is measured in pg/ml. This ELISA kit use Sandwich - ELISA. The micro ELISA plate introduced in the kit is precoated with an antibody that specific for this protein.

Statistical analysis was performed using SPSS version 10. Because they are a non-normally distributed variables, the comparison of median of CD44 concentration among groups was done using the non-parametric Kruskal Wallis test and Mann Whitney U test. P-value was considered significant when it was less than or equal to 0.05 and highly significant when it was less than or equal to 0.001.

Results

The median values of sCD44 in all groups are showing in table 1, Figure 1, the highest results were found among

smokers patients with oral squamous cell carcinoma, followed by non-smoker group OSCC while the lowest values was reported by the smoker group without OSCC. The mean and standard deviation with minimum and maximum values is illustrated in the Table 1.

The flour of the mouth was the most common site of the oral squamous cell carcinoma. Figure 1 shows different locations of squamous cell carcinoma in this study.



Figure 1: The different sites of oral squamous cell carcinoma

Table 1: Mean, Median and Standard deviation for all study groups

Groups	N	Median	Mean	SD	Minimum	Maximum
1 Smoker patients with oral squamous cell carcinoma	30	810.16	930.03	855.53	742.12	2851.32
2 Smoker subjects without oral squamous carcinoma	30	97.21	314.26	627.75	65.33	3140.67
3 Non-smoker subjects without oral squamous cell carcinoma	30	110.32	218.15	431.12	51.54	2448.98

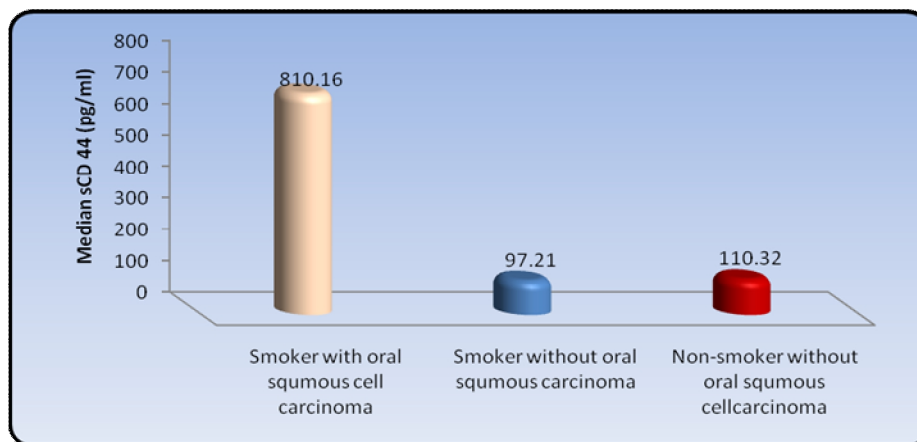


Figure 2: Median values for all study groups

For Statistical point of view, the differences in the median sCD44 level was higher significantly in smoker patients in comparison to other two groups ($P < 0.05$), as shown in Table 2. In addition to that no

significant difference in median values of sCD44 between smoker subjects without OSCC and non-smoker subjects without OSCC was seen ($P > 0.05$), as shown in the Table 2.

Table 2: Statistical differences among the study groups

Groups	Mann Whitney U	DF	P-value
Group 1 vs Group 2	107.00	1	0.009
Group 1 vs Group 3	90.00	1	0.001
Group 2 vs Group 3	190.00	1	0.829
Kruskal Wallis(Among three groups)			
Statistic	12.591		
DF	2		
P-value	0.003		

Performing a regression analysis between sCD44 and smoking, represented by the three groups, revealed a positive significant correlation ($P < 0.05$), as shown in Figure 3. The coefficient of determination R^2 was (0.150) indicating

that the supposed model can predict only 15.0% of exact variation in data. Correlation coefficient r was (0.412) indicating a positive correlation between smoking and sCD44.

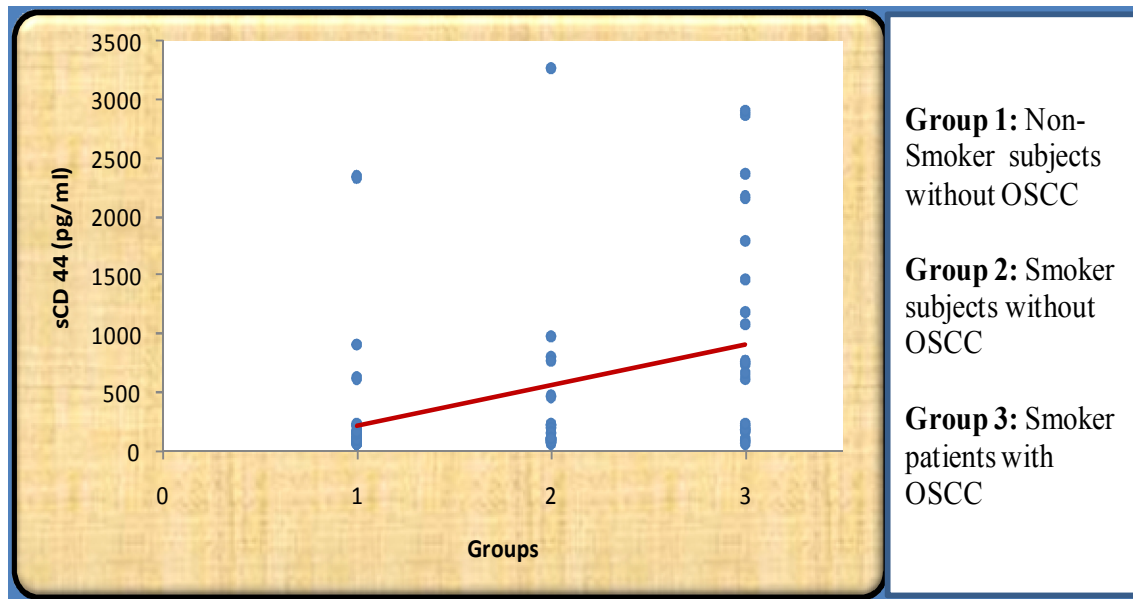


Figure 3: Regression analysis between sCD44 and smoking

Discussion

The present study showed a statistically significantly higher expression of salivary CD44 in patients with OSCC than non-smoker control subjects. In accordance with present study Dalia *et al* [11] stated that there was a highly significant difference in the mean value of salivary sCD44 level between control group and the oral squamous cell carcinoma groups [12].

The smoking increases salivary protein level in the saliva, this was showing through single linear regression analysis test, between smoking and salivary CD44 protein.

The participation of CD44 in the pathogenesis of OSCC may be explained by that this protein is a complex family composed of molecules, associated with aggressive malignancies and cancer stem cells. In another word, the role of the protein variants in tumor progression and treatment resistance is not clear [12], but this protein has been reported to be involved with tumor growth and metastasis and has implicated as a marker oral squamous cell carcinoma. However, the prognostic value of CD44 still remains controversial. Expression of CD44, a transmembrane hyaluronan-binding glyco-

protein is variably considered to have prognostic significance for different oral cancers, including oral squamous cell carcinoma [13]. But on other side Jianqian *et al* [14] stated that no clear association was revealed between protein expression and oral squamous cell carcinoma. On the contrary Performing single linear regression analysis in the present study between sCD44 and smoking revealed a positive significant correlation which is in agreement with Ghallaband Shaker [15] who investigated salivary CD44 level in smoker and non-smoker subjects, with no carcinoma, and concluded a significantly higher level among smokers, in accordance with the results of the present study.

The increasing the CD44 level in the saliva of patients with OSCC can be utilized as a non-invasive alternative for early diagnosis of suspicious oral lesions in smoker patients, beside that a target therapy, as stated by Spiegelberg *et al* [12], can be investigated as a form of treatment to stop tumor progression.

Conclusion

Salivary sCD44 protein can serve as a non-invasive way and can be used as an additive way to the biopsy method that aid

in the diagnosis of oral squamous cell carcinoma patient and used in follow up monitoring for those patients.

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