RESEARCH ARTICLE

Comparison of SKA1 serum levels in oral potentially malignant disorders and oral squamous cell carcinoma

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Abstract

Objective: To compare serum levels of spindle and kinetochore-associated complex subunit 1 in oral potentially malignant disorders and oral squamous cell carcinoma.

Method: The analytical, cross-sectional study was conducted from July 2022 to April 2023 at the Department of Otolaryngology of Dr Ruth Pfau Civil Hospital, Karachi, Dr Ishrat-ul-Ebad Khan Institute of Oral Health Sciences, Karachi, and the histopathological section of the Dow Diagnostic Reference and Research Laboratory at the Dow University of Health Sciences, Karachi, and comprised serum samples of patients with oral squamous cell carcinoma and oral potentially malignant disorder. The concentrations of serum spindle and kinetochore-associated complex subunit 1 were determined using the enzyme-linked immunosorbent assay method. Data was analysed using SPSS 21.

Results: Of the 90 patients with age ranging 30-90 years, 45(50%) had oral squamous cell carcinoma; 34(75.6%) males and 11(24.4%) females. There were 45(50%) patients with oral potentially malignant disorders; 35(77.8%) males and 10(22.2%) females ($p>0.05$). The median serum spindle and kinetochore associated complex subunit 1 levels were significantly elevated in patients with oral squamous cell carcinoma compared to those with oral potentially malignant disorders ($p<0.05$).

Conclusion: Spindle and kinetochore-associated complex subunit 1 could be a promising non-invasive serum marker for the early detection of malignant transformation in oral potentially malignant disorders and for prediction of prognosis in oral squamous cell carcinoma cases.

Key Words: Spindle and kinetochore-associated complex subunit 1, Oral squamous cell carcinoma, Oral potentially malignant disorder.

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Introduction

Oral squamous cell carcinoma (OSCC) is a highly malignant tumour of the oral cavity. Worldwide, 90% of oral cancer cases are confirmed as OSCC on histopathological analysis.[1] The Pakistan Atomic Energy Commission Cancer Registry (PAECR) estimated that in Pakistan, oral cavity tumours are the second most common tumours diagnosed in 2018-19.[2] In our setting, OSCC patients usually present at stages III/IV compared to stages I/II and patients who are diagnosed with early-stage cancer have a good prognosis compared to the late stages. As tissue biopsy may not be convenient in all suspected cases of OSCC, there is a need of early diagnostic markers that can detect malignant transformation of oral dysplasia into OSCC.[3,4]

The development of oral cancer is the consequence of a complex process that is defined by a wide range of unique genetic and epigenetic changes. Certain carcinogens, such as alcohol, smoking, human papilloma virus (HPV) infections, etc., lead to genetic mutations, chromosomal abnormalities and epigenetic modification that eventually promote oral carcinogenesis. These ongoing molecular events result in the transformation of normal mucosa to dysplastic mucosa, eventually ending up as invasive carcinoma.[5] The cell cycle is a highly ordered programme in the eukaryotic cell and it is controlled by various intracellular checkpoints. However, because of genetic mutations or structural deformity of chromosomes, functions of these checkpoints are lost, which leads to abnormal cell proliferation and, hence, tumour development.[6] Human spindle kinetochore-associated complex subunit 1 (SKA1), previously named as chromosomes 18 open reading frame 24, is a cell cycle regulatory gene, mostly controlling cell cycle during the second growth phase to the mitotic (G2/M) phase.[7] Over-expression of SKA1 has been associated with the

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pathogenesis of various solid tumours, including OSCC.[6,9-11]

To the best of our knowledge, there is a lack of data available that highlights the role of SKA1 in the malignant transformation of oral dysplasia into OSCC.

Patients and Methods

The analytical, cross-sectional study was conducted from July 2022 to April 2023 at the Dow University of Health Sciences (DUHS), Karachi. The demographic data was taken from an earlier study[12] After approval from the institutional ethics review board, the sample size was calculated using PASS version 11[13] based on the chi-square test for association with 95% confidence interval (CI). 80% power of the test, effect size reference between Tumour-Node-Metastasis (TNM) stage and SKA1 0.345 and degree of freedom 1.[6] Those included were patients aged >20 years who histopathologically confirmed to have OSCC and oral dysplasia. Patients with prior chemotherapy and radiotherapy were excluded. A non-probability purposive sampling and simple random sampling techniques was used. After taking informed written consent from the patients, the samples were taken from Otolaryngology ward and outpatient department (OPD) of Dr Ruth Pfau Civil Hospital, Karachi, Dr Ishrat-ul-Ebad Khan Institute of Oral Health Sciences (DIKIOHS) and the histopathology section of Dow Diagnostic Reference and Research Laboratory (DDRRL). Group A consisted of histopathologically confirmed OSCC cases and group B consisted of histopathologically confirmed oral dysplasia cases.

Peripheral blood 3ml was collected from each patient one hour before surgery by using ethylenediaminetetraacetic acid (EDTA)-containing yellow tube. The blood samples were stored in an icebox and transferred to the laboratory for further processing. In laboratory, the serum was separated from venous blood by centrifugation of tube for 20 minutes at 2000 revolutions per minute (rpm). After the separation of serum, it was pipetted into 1ml eppendorf tubes and stored at -80°C.

Before starting the procedure, all reagents and serum samples were brought to room temperature. Measurement was made within three hours after the first freeze-thaw cycle. The estimation of serum SKA1 was done by using human SKA1 Sandwich enzyme-linked immunosorbent assay (ELISA) kit (Catalog # E7579Hu) manufactured by Bioassay Technology Laboratory (BT lab). The procedure was done as per the guidelines provided by the manufacturer. The protein concentration was measured by using an ELISA reader (Model 3020-1311, Thermo Fisher Scientific, Finland).

Data was analysed using SPSS 21. Data normality was checked using the Shipro-Wilk test. The difference between the serum SKA1 levels in OSCC and OPMD cases was assessed using independent t test. The value of SKA1 in both groups was expressed as median with interquartile range (IQR). Spearman's correlation coefficient was used to analyse the relationship between serum SKA1 levels and the ages of the patients. For the analysis of association of serum SKA1 with various clinic-pathological parameters in OSCC and OPMD cases, Mann-Witney and Kruskal Wallis tests were used. P<0.05 was taken as significant.

Results

Of the 90 patients with age ranging 30-90 years, 45(50%) were in group A; 34(75.6%) males and 11(24.4%) females. There were 45(50%) patients in group B; 35(77.8%) males and 10(22.2%) females (p>0.05). In group A, 26(57.8%) patients belonged had low socioeconomic status (SES) compared to 25(55.6%) in group B. In group A, 33(73.3%) cases consumed smokeless tobacco (SLT) compared to 37(82.2%) in group B (Table 1).

Table-1: Demographic features of OSCC and OPMD cases..

Category	OSCC n%	OPMD n%	p-Value
Age			
30—40 years	13 (54.2%)	11 (45.8%)	0.918*
41—50 years	11 (45.8%)	13 (54.2%)	
51—60 years	11 (50.0%)	11 (50.0%)	
61—70 years	10 (55.6%)	8 (44.4%)	
71—80 years	0 (0.0%)	1 (100.0%)	
81—90 years	0 (0.0%)	1 (100.0%)	
Gender			
Male	34 (75.6%)	35 (77.8%)	0.803*
Female	11 (24.4 %)	10 (22.2 %)	
Socioeconomic status			
Upper middle class	6 (13.3%)	0	
Middle class	13 (28.9%)	20 (44.4%)	
Lower middle class	26 (57.8%)	25 (55.6%)	
Oral habits			
Tobacco (smoking)	5(11.1%)	1 (2.2%)	
Tobacco (SLT)	33(73.3%)	37 (82.2%)	
Both	7(15.6%)	7 (15.6%)	

OSCC: Oral squamous cell carcinoma, OPMD: Oral potentially malignant disorders, SLT: Smokeless tobacco.

Table-2: Serum SKA1 median levels in OSCC and OPMD cases.

Groups	Median	1st and 3rd quartile	p-Value
OSCC	12.74	9.5—15.9	<.00*
OPMDs	2.61	1.1—3.9	

*Independent t test p<0.05, OSCC: Oral squamous cell carcinoma, OPMD: Oral potentially malignant disorders,

Table-3: Comparison of serum SKA1 levels with respect to clinico-pathological features of oral squamous cell carcinoma (OSCC).

Category	n=45	Median (IQR)	p-Value
Gender			
Male	34	12.8 (6.82)	.751*
Female	11	12.7 (5.5)	
Risk factor			
Tobacco (SLT)	33	12.95(6.36)	0.894**
Tobacco (smoking)	5	11.31(6.76)	
Both	7	13.01(14.04)	
Tumour grades			
GI=Well differentiated	3	16.63	.279**
GII=Moderately differentiated	38	12.44 (6.19)	
GIII=Poorly differentiated	4	16.43(7.4)	
TNM stages			
Stage I	12	8.4 (3.9)	p=0.002
Stage II	3	14.45	
Stage III	9	12.6 (8.5)	
Stage IV	21	15.4 (6.5)	

*Mann-Whitney U, **Kruskal Wallis Test, p<0.05.
SKA1: Spindle and kinetochore-associated complex subunit 1, SLT: Smokeless tobacco. TNM: Tumour-Node-Metastasis.

Table-4: Comparison of serum SKA1 levels with clinic-pathological features of oral potentially malignant disorders (OPMDs).

Category	n=45	Median (IQR)	p-Value
Gender			
Male	35	2.61 (2.91)	.548*
Female	10	2.68 (3.16)	
Risk factor			
Tobacco (SLT)	37	2.25 (3.19)	.49**
Tobacco (Smoking)	1	0.99	
Both	7	2.67 (1.66)	
Clinical Presentation			
Leukoplakia	34	2.07 (2.91)	0.04**
Erythroplakia	7	4.11 (1. 69)	
Oral Submucous Fibrosis	4	2.08 (2.26)	
Dysplasia Grading			
Grade I	29	1.97 (2.78)	.28**
Grade II	6	3.94 (0.98)	
Grade III	10	2.86 (2.07)	

*Mann-Whitney Test, **Kruskal-Wallis Test, p<0.05. SLT: Smokeless tobacco.
SKA1: Spindle and kinetochore-associated complex subunit 1, SLT: Smokeless tobacco, IQR: Interquartile range.

The median serum SKA1 levels were significantly elevated in group A patients compared to those in group B (p<0.05) (Table 2).

Serum SKA1 levels were significantly associated with TNM stages (p=0.002). Specifically, patients with stages III/IV disease exhibited higher serum SKA1 levels compared to those in stages I/II (Table 3).

In group B, serum SKA1 levels were significant different among the different clinical presentations (p=0.04). Notably, erythroplakia cases exhibited higher median serum SKA1 levels compared to leukoplakia and oral submucous fibrosis (Table 4).

Discussion

The current study compared the serum levels of SKA1 in OPMDs and OSCCs, which makes it a unique study because this element, to our knowledge, has not been discussed in literature. The chances for the development of head and neck squamous cell carcinoma significantly increases in the presence of oral dysplasia. Hence, it is essential to identify molecular biomarkers that can effectively differentiate oral lesions with a higher risk of progression to invasive cancer. SKA1 is a novel gene involved in the carcinogenesis of oral cancer. The current study compared serum levels of the SKA1 in OPMD and OSCC cases to evaluate the prospects of further studies to validate this gene as a biomarker for the prediction of oral cancer in OPMDs.

The role of SKA1 in the malignant transformation of various solid tumours has been studied worldwide. The gene is mainly involved in the progression of tumour by dysregulating the cell cycle.[6,9,10] The present study found a median SKA1 serum level of 12.74ng/ml in OSCC cases, which was significantly higher compared to OPMD cases having serum levels of 2.61ng/ml, higher making it a potential marker for malignant transformation in precancerous lesions in oral mucosa.

The present study found no significant association between serum SKA1 levels and various clinic-pathological parameters of oral cancers. However, an increased median level of this gene was observed in the advanced stages of oral cancer compared to the early stages. The intergroup comparison showed that the mean SKA1 value was different in early stage I than in stage III and IV tumours. The findings, although based on serum samples, were consistent with a study done in 2020, which reported increased tissue expression of SKA1 through immunohistochemistry in advanced cases of oral cancer compared to early-stage tumours.[6] Another study on oesophageal squamous cell carcinoma suggested that the gene acts as an oncogene and might be involved in the proliferation and migration of cancer cells.[11]

More recent research documented an increased susceptibility of malignant transformation of oral erythroplakia into OSCC with a transformation rate of 19.9% to 45%.[14] In the present series, an intergroup comparison between different clinical presentations of OPMD cases showed that the mean SKA1 value was higher in erythroplakia cases compared to leukoplakia cases.

The role of SKA1 in the tumour progression, differentiation and migration has been established in literature.[6,15-17] The exact mechanisms behind the raised serum levels in oral cancer patients and oral dysplasia patients, however, are unclear. One possible hypothesis might be the involvement of SKA1 in the tumour microenvironment. Recent research utilising bioinformatics techniques concluded that the increased expression of SKA1 in tumour cells inhibited lymphocytes and macrophages, eventually resulting in the inhibition of immune cells, such as natural killer cells, cluster of differentiation 4 (CD4+T) and CD8+T cells18. It has been also found that SKA1 expression positively correlates with the expression levels of anti-phagocytic genes, such as beta-2 microglobulin (B2M), anti-programmed cell death ligand 1 (PDL1) and CD47, and it has been suggested that this gene can be employed as a biomarker for diagnosing pancreatic ductal adenocarcinoma.[18] In this scenario the current results may contribute to the existing body of evidence, and, hence, may provide direction for further research.

The current study has several limitations. First, the sample size was relatively small, which may have limited the statistical power and generalisability of the findings. Second, healthy control subjects were not enrolled. Further studies with larger sample sizes and broader molecular analyses are needed to determine the role of SKA1 in malignant transformation of OPMDs.

Conclusion

Serum SKA1 levels were significantly higher in OSCC cases compared to OPMD cases, suggesting that it can be a potential biomarker for early malignant change in OPMDs with dysplasia. Higher serum levels of SKA1 were also observed in advanced stages of OSCCs, highlighting its role in predicting prognosis for these cancers.

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SK: Concept, writing, data collection and statistical analysis.
MMA: Supervision, histopathological reporting, critical revision, editing and final approval.
UB: Co-supervision, histopathological reporting and revision.
MSF: Clinically supervised the research, helps in clinical assessment and reads the final version.