Immunohistochemical Study of CK18 Expression In Head and Neck Squamous Cell Carcinoma

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Abstract

Background: CK18 is an epithelial-specific cytokeratin that undergoes cleavage by caspases during apoptosis. In a variety of organs, the expression of distinct intermediary filaments is associated with patient prognosis. Intermediary filaments are involved in cell motility and cancer progression.

Objective: To evaluate the expression of cytokeratin18 in head and neck Squamous cell carcinoma and to correlate the expression of Ck18 with different parameters such as age , gender and grading of tumor.

Materials and Methods: Thirty patients with histopathological proven and surgically treated head and neck squamous cell carcinomas were investigated for the immunohistochemical expression of Ck18. Correlations between clinicopathological features and the expression of Ck18 were evaluated statistically by Chi-square test and the level of significance was 0.05 (two-sided) in all statistical testing.

Results: Among patients 16 were males, 14 were female's patient, aged between 14 and 80 years (mean age 56 years) were evaluated. Out of the 30 specimens of the Head and Neck Squamous Cell Carcinoma studied, 23 cases (76.7%) showed positive CK 18 staining and 7 cases (23.3%) showed negative CK18 staining. Regarding to gender, in males 6 (37.5%) sections with weak positive expression, 1 (6.25%) sections with moderate positive expression, 3 (18.75%) sections with strong positive expression and 6 (37.5%) section with negative expression, While in females 7(50%) sections with weak positive expression, 3 (21.43%) sections with moderate and strong positive expression and only one (7.14%) section with negative expression.

Conclusion: CK 18 showed higher expression levels on moderately and poorly differentiated squamous Cell Carcinoma cases than well differentiated SCC cases. The correlation between CK-18 staining and grade level was non-significant that seemed to be going together with the pleomorphism and the number of mitosis.

Key worlds:CK18, head and neck squamous cell carcinoma, immunohistochemistry.

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Introduction

In the world, the sixth most common solid tumor is the squamous cell carcinoma of head and neck and the most common malignant tumor of the upper aero-digestive tract. The therapeutic options are limited and around half of persons have squamous cell carcinoma of the head and neck die within 5 years in spite of these treatments[1], and to date ,Sobin LH [2] showed that; according to the TNM classification of the tumor they

have only been able to predict the prognosis of these patients.

The intermediate filaments (IFs) of epithelial cells formed in various combinations ofcytokeratins expressed depending on the epithelial type and the degree of differentiation. In immunohistochemistry, the most commonly studied is the class of IFs. To excludes the possibility of malignant lymphoma, sarcoma or melanoma and the diagnosis of carcinoma can be typically helped by Cytokeratin positivity [3].

Dinsdale *et al* who explained that, in many types of carcinomas such as breast, lung, colon, liver and prostate,it is expressed, whereas CK18 is not present in neuronal and lymphoid cells and tissues. During apoptosis after initiation of effector caspases 3, 6, 7 and 9 the proteolytic fragments are yield from CK18 that liberating neo-epitopes (NE) at the cleavage sites. Cytokeratin 18 is the major component of glandular epithelial cells and single layer and the type I intermediate filament protein [4].

Walker *et al* who mentioned that CK18 can be specifically recognized by M30 CytoDEATH™ monoclonal antibody and can be cleaved by capases, to liberate a neo-epitope (M30). Specific M30 CytoDEATH™ antibody not detect viable or necrotic cells but it detects only apoptotic. The apoptosis index by TUNEL is associated with reactivity of M30 CytoDEATH™ antibody in immunohistochemistry and when DNA double-strand breaks occur independent of apoptosis shows superior reliability in conditions [5].

Cohen *et al* who described that the apoptosis can be initiated by specific signals that activate specific caspases and it is a biochemically and morphologically definitive form of programmed cell death [6]. As a result, the nuclear condensation results from intrinsic suicide programme so, the cell will be eliminated, cytoskeletal reorganization, plasma membrane blebbing and loss of cell adhesion [7].

The aim of this study is to evaluate the staining of Cytokeratin18 Squamous cell carcinoma of head and neck and to correlate the staining of Ck18 with various parameters such as age, gender and grading of tumor.

Materials and Methods

The samples of this retrospective study was include of thirty tissue blocks fixed with formalin and embedded with paraffin, which have been diagnosed as HNSCC ,which was dated from (2009 till 2013). The study samples were obtained from ghazy al-hariri hospital for surgical specialist ,medical city,Baghdad. Each case was confirmed by diagnosing the Hematoxylin and Eosin sections by two pathologists. The clinical and demographic data were obtained from the surgical and pathological reports with the tissue specimens and these supplied by the surgeon, including patient's age, gender, clinical presentation and grading of tumor[2].

Immunohistochemistry

Each paraffin-embedded block was cut to One 4-µm-thick section and deparaffinized at 37°C for 24 hours. Through graded concentrations of alcohol, the slides were rehydrated to retrieve surface of the antigens. Then, during 5 minutes they were put in a microwave oven in boiling citrate buffer and floated in phosphate-buffered saline (PBS) (pH=6). Then, the steps of immunohistochemical staining were followed sequentially: by using 11.11% hydrogen peroxide for 5 minutes blocking the endogenous peroxides activity; then, immersion in PBS; by using of the monoclonal antibody of Ck18 (Dako, Denmark A/S; Lot no: 00049086) for 30 minutes; immersion in PBS; and use of the envision solution for 15 minutes with dilution 0.5%.

The samples were stained with hematoxylin and eosin and diagnosed at high

magnification (40x) under a light microscope.

The estimated staining intensity is represented by four intensity score such as(1, no staining; 2, weak; 3, moderate; 4, strong). The proportion score represented the estimated fraction of positively stained tumor cells (1 <10%; 2 = 10 to 50%; 3 = 50 to 80%; 4 ≥80%).

Statistical analysis

By using the computer in Data analysis and using SPSS version 14 computer software (Statistical Package for Social Sciences) to statistical analyses. The categorical data represent the scoring of studied parameters thus they presented as percentage and count. Frequency distributions for selected variables were done first. The relationship between categories was evaluated by Chi-square test. P value was less than 0.05 considered statistically significant.

Results

In this study, according to gender 16 were male and 14 were female patients, and the age group between 14 and 80 years. The mean age is 56 years and were evaluated as shown in table (1) .

Table (1): Age incidence of Squamous cell carcinoma of head and neck in both gender in percent.

Age group	Number of Male	%	Number of Female	%	Total	%
(10 - 19)	1	6.25	–	–	1	3.3
(20 - 29)	–	–	–	–	–	–
(30 - 39)	1	6.25	1	7.14	2	6.7
(40 - 49)	–	–	2	14.28	2	6.7
(50 - 59)	6	37.5	7	50	13	43.3
(60 - 69)	5	31.25	2	14.28	7	23.4
(70 - 79)	3	18.75	1	7.14	4	13.3
(80 - 89)	–	–	1	7.14	1	3.3
Total	16	100	14	100	30	100

Non-significant

There was no association between mean CK 18 labeling index and the gender. Out of the 30 specimens of the HNSCC studied, 23 cases (76.7%) showed positive CK 18 staining and 7 cases (23.3%) showed negative CK18 staining (Table 2).

According to gender in male 6 (37.5%) sections with weak positive expression, 1(6.25%) sections with moderate positive expression, 3 (18.75%) sections with strong positive expression and 6 (37.5%)section with negative expression, While in female 7(50%) sections with weak positive expression,3 (21.43%) sections with moderate and strong positive expression and only one (7.14%) section with negative expression with non-significant correlation (Table 2).

Table (2): The difference in median CK18 staining scores by gender among cases with head and neck squamous cell carcinoma.

parameters	Female	Gender	Male	
Ck18	N	%	N	%
Negative	1	7.14	6	37.5
Weak positive	7	50	6	37.5
Moderate positive	3	21.43	1	6.25
Strong positive	3	21.43	3	18.75
Total	14	100	16	100
Median	Weak positi		Negative and Weal positive	

Under high magnification, the tumor fields can be evaluated by differentiation scores, revealed that at well differentiated tumors , the single cell keratinization rich tumors and keratin pearl are mostly seen . However this feature was seen together with poorly differentiated tumors with high mitoses,pleomorphism of cell and infiltrating the border of tumor (Figure 1).

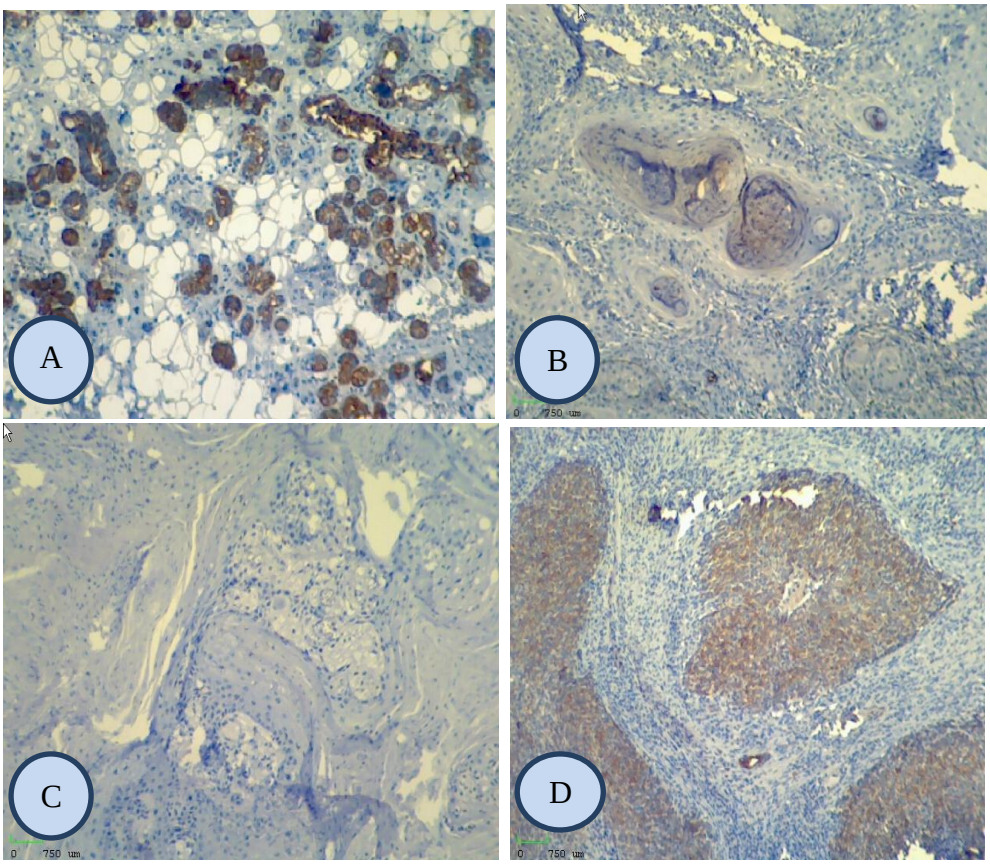

Figure 1: (A) Strong positive CK18 immunohistochemical expression in Squamous cell carcinoma with moderate differentiated (X20), (B) Weak positive CK18 immunohistochemical expression in Squamous cell carcinoma with well differentiated (X20), (C) Negative CK18 immunohistochemical expression in Poorly differentiated squamous cell carcinoma(X20), (D) Moderate positive CK18 immunohistochemical expression in moderately differentiated squamous cell carcinoma(X20).

The interpretation of the results by correlating and cross tabulating with the clinical and histopathologicalcatogries.

The higher expression of ck18 staining levels on moderately and poorly differentiated SCC cases than well differentiated SCC cases .

The correlation between CK18 staining and grade level (P= 0.24) was non significant that seemed to be going together with the pleomorphism and the number of mitosis (Figure 2).

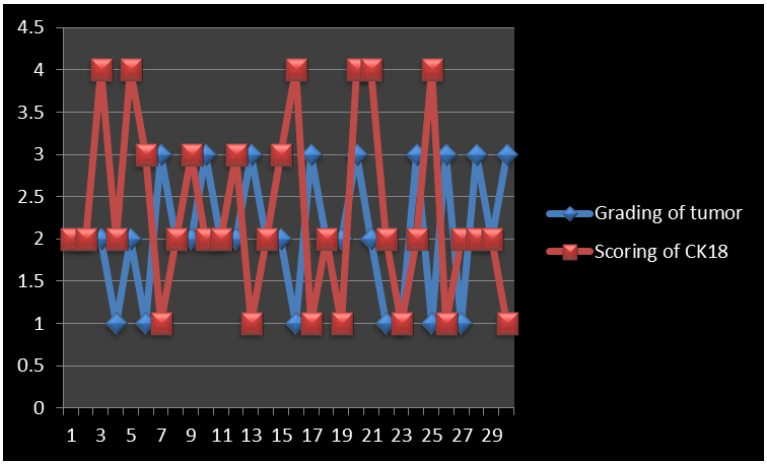

Figure (2): Frequency distribution between the grading of tumor and the scoring of CK18 expression

Discussion

In the this study (25) (83.3%) cases of HNSCC cases were above 50 years old with an age ranged (14-80) years and mean age (56 years).These results were agree with other studies that the association of aging with oral cancer development could be explained by the prolonged exposure to different environmental carcinogenesis such as viruses,chemicals and radiation [8,9].

Furthermore among known risk factors, aging appears to have a great association with carcinogenesis since it causes cellular dysregulation through the alteration in suppressor genes and cell growth [9].

CK18 are markers that their staining are not expressed in an early molecular finding in malignant transformation and normal laryngeal epithelium [10]. Seven (23.3%) cases were negative immunohistochemical expression of CK18 and the rest 23(76.7%) cases are positive expression in squamous cell carcinoma. Result of Suo *et al* [11] agree with this study which showed few cases of positive CK18 immunoreactivity in squamous cell carcinoma of oral cavity in spite of almost a hundred percent positivity in squamous cell carcinoma of the larynx and hypopharynx.

The staining of distinct intermediate filament proteins is tissue-specific and highly conserved during carcinogenesis.The intermediate filaments represent the central components of the intracellular skeleton. The intermediate filaments interpretation as mere bystanders and markers of clear6distinct intracellular, cell-specific regulation mechanisms.Several other studies associated an altered cellular behavior with changes in intermediate filament expression[12].

During neoplastic transformation ,there is alterations in CK expression have been detected and these alterations may be associated with invasion, tumorigenesis and metastasis[13,14]. Because of keratin downregulation, impairment of desmosome may lead to loss of adhesion and increase the migratory propensity[15]. The Stem cells of stratified epithelium is the explanation of the major cellular targets for malignancy causing

changes and so, might give in a long term rise to the development of SCC's. In the presenting literature, the context seems logical that Squamous cell carcinomas stay and are pheno- expression of glandular cytokeratins such as Ck 8 pinpoints a significant subgroup of SCC's. The poor prognosis were associated with carcinomas, independently of the staging of cancers. It is important to stress that this held also true in multi parameter analysis[16]. On the one hand be due to the lower significant numbers of investigated tumour cases, and on the other hand caused by different techniques for the determination for the expression of cytokeratins 18 on the protein and RNA level. Also, similar results to our findings have also been reached for skin carcinomas. Nevertheless, others tudies with chemical carcinogens strongly support our results and showed an important role of CK 18 in various steps of the progression and pathogenesis of SCC's[17].

The changes in substantial cellular properties result from change in the intermediate filament expression pattern. In other carcinomas, Cytokeratin expression patterns are Strong homologies and highly-conserved . With different approaches In primary breast cancer, this could be done. In breast cancer poor prognosis and increased metastatic properties are associated with Down-regulated expression of Ck 8/18 [18]. Van der Velden *et al* [10] showed that in 29 LC specimens, the expression of ck18 and, contrary to our results, found increase in the tumor grade and increase in staining intensity. Expression was showed to be especially abundant in tumors with a basaloid cell phenotype, and in ten cases staining increased to the tumor margins. As a result, CK-18 was found to have increased advance stage of disease with expression that reached statistical significance with CK18. Upon comparison with similar other studies, we realized that staging, grading and immune reactivity scoring systems in those studies were not unique and studies that used CK cocktail antibodies [19]. Mmay have various results with the studies that monoclonal antibodies are used [20]. In other non-epithelial cells and fibroblasts ,the production of CK18 has been reported in small amounts [21]. Recently, the observation of CK filaments aggregation rapidly in apoptotic cells [22,23].

In conclusion, CK 18 showed higher expression levels on moderately and poorly differentiated squamous Cell Carcinoma cases than well differentiated SCC cases. The correlation between CK-18 staining and grade level was non-significant that seemed to be going together with the pleomorphism and the number of mitosis. The resuts of this study should becorrelated with results of other tumor markers of other studies and should use large number of samples.

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