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Immunohistochemical expression of CEA in Head and Neck Squamous and Adenosquamous cell carcinoma

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Abstract

Background: Squamous cell carcinoma (SCC) is an epithelial malignancy involving many anatomical sites and is the most common cancer capable of metastatic spread. Development of early diagnosis methods and novel therapeutics are important for prevention and mortality reduction.

Aim of the present study was to evaluate the expression of CEA in Head and Neck Squamous cell carcinoma and Adenosquamous carcinoma with the progression of malignant disease.

Materials and Methods: Sections of formalin-fixed paraffin embedded blocks specimens of (28)Squamous cell carcinoma and (5) Adenosquamous carcinoma were immunostained to assess the expression of CEA in Head and Neck Squamous cell carcinoma cases.

Results: CEA expression was sometimes associated with cytoplasmic staining. The intensity of staining was correlated with the grade of differentiation: grade I was poorly positive staining, while grade III was generally deeply positive staining

Conclusions: The immunohistochemical expression of CEA is useful, in addition to other diagnostic criteria, for establishing a differential diagnosis in the case of primary and metastatic cancer.

Key words: Squamous cell carcinoma (SCC), Immunohistochemistry, Carcimoemryonic antigen (CEA).

Introduction

Large group of diseases with high morbidity may be due to cancer [1]. In the age group below 65year, the cancer is the most common cause of death and the second most common in the age group above 65 [1] . Despite the various and new methods of treatment developed every year, from every three Americans, one contracts cancer of one type or more during his lifetime and only 54% of the patients survive the disease [2] .

Squamous cell carcinoma accounts about 90% of the malignant oral lesions and is the most frequently occurring malignant tumor of the oral structures. Among the continents and within developed and developing countries, epidemiological studies have stated that the incidence of oral cancer is significantly different [3].

The squamous cell carcinoma of head and neck is the sixth most common solid tumor in the world and

the most common malignant tumor of the upper part of aero-digestive tract[4].

Squamous cell carcinomas are reported in many organs of the body including esophagus, bladder, cervix , ovary and teratomas [5] .

Tumor markers

Tumor markers defined as a substances that secreted by the tissue as a response to the tumor or produced by the tumor. In addition, they may be used in the diagnosis, and screening and classification of tumors, as well as in the assessment of prognosis, recurrence and metastasis in cancer cases [6] .

Carcinoembryonic antigen (CEA) has enjoyed its greatest recognition in clinical medicine as a serologic indicator of the growth of colorectal cancer. Immunohistochemically, CEA is strongly expressed in colorectal adenocarcinoma, but it may be found in many other epithelial tumors as well [7] .

Monoclonal antibodies to CEA represent prototypic epitope-specific probes, which recognize a small portion of a large antigen. Different carcinomas express a common portion of the CEA molecule but may also produce mutually exclusive epitopes that are tissue restricted. CEA also continues to be an effective discriminant between metastatic carcinoma in the pleura and malignant mesothelioma [8].

The bulk of the CEA in a healthy individual is produced in colon. There, it is released from the apical surface of mature columnar cells into the gut lumen and disappears with the feces. Thus, only very low levels are normally seen in the blood from healthy individuals. In colon cancer the malignant cells have no basal lamina and are multiplying in the tissue. Moreover, the tumor cells have lost their polarity and CEA is distributed around the cell surface. It is known that components from the plasma membrane are continually exfoliated from the surface as plasma membrane-derived vesicles [9] .

Materials and methods

The Sample of this study included thirty-three formalin-fixed, paraffin embedded tissue blocks, which have been diagnosed as SCC of head and neck were retrieved from the archives of Specialized Surgeries Hospital/ Medical City /Baghdad for the period (2009 – 2013). The diagnosis of all cases were confirmed by examining the Hematoxylin and Eosin (H&E) sections by two specialized pathologists.

The clinical data of each case provided by the surgeon were obtained from the surgical and pathological reports available with the tissue specimens, including patient's age, sex, clinical presentation, site of the tumor, grading and staging. The work was performed in teaching oncological Hospital.

By using immunohistochemistry in each block, one representative section was stained with hematoxylin and eosin for reassessment of histopathological diagnosis and one other section was prepared on adhesive slide for detection of CEA expression.

The positive control were obtained according to antibodies manufacturer's data sheet. Slides were prepared from blocks of patient having tissue known to contain the target antigen against which the primary antibody used in this study was reactive.

The negative control slides were prepared from test tissue processed in a manner identical to the test section with omitting the primary antibody and instead put 20ml of phosphate buffer saline (PBS).

Principle of test:

The labeled streptavidin-biotin (LSAB) method utilized abiotinylated secondary antibody that links primary antibody streptavidin-peroxidase conjugate and by adding the chromogen substrate, a colorimetric reaction will form at the antigen binding site.

DAB (3- diaminobenzidine tetra hydrochloride) substrate offers the greatest sensitivity in the horse – radish peroxidase enzyme system as a colorimetric chromogen; a brown precipitate will form at the antigen – binding site.

CEA evaluation:

Quantitative assessment of immune histochemical expression of CEA antibody used was performed according to Staining intensity such as weak, moderate and strong staining.

Statistical Analysis:

The analysis of data was aided by computer. An expert statistical advice was sought for. Statistical analyses were done using SPSS version 14- computer software (Statistical Package for Social Sciences).

Frequency distributions for selected variables were done first. P value less than 0.05 was considered statistically significant.

The studied parameters were scored and considered as categorical data thus they presented as count and percentage.

Results

In this study, the mean age of patients with head and neck squamous cell carcinoma was 58 years. Among 33 patients, 17(51.5%) of them were males and 16(48.5%) were females. Regarding the variable of gender; the highest frequency was among 50- 59 years old 6(35.3%) cases for males and 8(50%) cases for females as shown in (Table 1) .

The studied specimens of the HNSCC, 29 cases (87.9%) showed positive CEA staining and 4 cases (12.1%) showed negative CEA staining. There was no association between gender and mean CEA labeling index (Table 2).

Histological analysis of each slides were indicated, 13 cases (39.4%) were well differentiated SCC, 16(48.5%) moderately differentiated SCC and 4(12.1%) poorly differentiated grade. CEA expression was sometimes associated with cytoplasmic staining. The intensity of staining was correlated with the grade of differentiation: grade I was poorly positive staining, while grade III was generally deeply positive staining (Figure 1).

While it is apparent that there is a parallel between rising in CEA expression and progression of malignant disease, it is important to determine if there is sufficient correlation between these two factors to be able to use CEA marker to detect or anticipate progressive disease before it becomes clinically evident (Figure 2).

In conclusion, the immunohistochemical expression of CEA is useful, in addition to other diagnostic criteria, for establishing a differential diagnosis in the case of primary and metastatic cancer. In addition, carcinomasadenosquamous carcinoma represents a challenge in diagnostic routine for its rarity, diverse range of clinical presentations and histological features, since the adenocarcinomatous component may be, at times, difficult to identify.

Discussion

Carcino embrionic antigen was first discovered in extracts of colon cancer. It is a tumor marker that has been used for colorectal cancer, ovarian cancer, renal cancer and breast cancer. It was

thought that a tumor specific marker had been found, but some `studies later discovered that not all colon tumors produced this type of marker. This is because of the composition of tumors are very heterogeneous. Similarly, in heavy smoker's elevated blood CEA has been observed who were without tumor [10].

The present study showed rare positive expression in the squamous component and intense positivity in the adenocarcinomatous component, contrasting with other study reported by Abdelsayed et al. in which only the adenocarcinomatous component was strongly positive to this marker, CEA is a marker largely used for the final diagnosis of adenosquamous carcinoma [11]. Also other study by Sheahan et al. agrees with this study that not observe any positivity to carcinoembrionic antigen in their adenosquamous carcinoma cases[12].

The purpose of a tumor marker is a non-invasive test to track a patient's health after recovering from cancer. Researchers are always seeking noninvasive tests like tumor markers to make early diagnosis and track a patient's recovery. This is a strong point for the tumor marker because it has the ability to track cancer formation in different areas of the body in different organs. The CEA subgroup members are cell membrane associated and show a complex expression pattern in normal and cancerous tissues [13].

Some authors have indicated that poorly differentiated SCCs tend to be negative for positivity of CEA and therefore, this marker may not be useful in confirming the diagnosis of SCCs [14]. However, in this study, nearly 88% of all well, moderately and poorly differentiated SCCs, exhibited positive CEA expression. While 12% of all cases exhibited negative CEA expression that different from other studies of CEA expression in other organs[14,15,16].

Many studies have been performed on the prognostic value of parameters such as histologic type and grade of the tumor, radial surgical margin and the spreading of tumor. Most of those parameters have been shown to have a prognostic value, while studies on some are yet to be completed[17]. Nonetheless, pathologic stage is the most important prognostic indicator of colorectal cancer. In the present study, we evaluated the positivity of CEA expression along with disease stages in head and neck SCCs patients.

In (2001) Zheng showed that the prognostic value of CEA in colorectal cancer patients by evaluating Dukes stages and tumor marker values, and stated that patients with advanced stage had significantly increased levels of CEA[18] . In addition to other studies that compared preoperative CEA values and Dukes stages in colorectal cancer patients and determined an association between tumor marker values and disease stage[18]. In present study 27.3% of the head and neck cancer patients had strong CEA expressions, however, no significance was found in term of the disease stages.

Basbug evaluated the prognostic value of CEA in colorectal cancer patients and found a statistically significant relationship between positivity of tumor marker and advanced TNM stage[19]. Similarly, Xue-Qin Yang investigated the prognostic importance of preoperative CEA values in colorectal cancer patients and found a correlation between increased preoperative values of these parameters and advanced stage[20].

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Table (1): Age incidence of Head and Neck squamous Cell Carcinoma and Adenosquamous carcinoma in both gender in percent.

Age group	Number of Male	%	Number of Female	%	Total	%
(10 - 19)	1	5.9	–	–	1	3.0
(20 - 29)	–	–	–	–	–	–
(30 - 39)	2	11.8	–	–	2	6.1
(40 - 49)	–	–	1	6.2	1	3.0
(50 - 59)	6	35.3	8	50	14	42.4
(60 - 69)	5	29.4	3	18.8	8	24.2
(70 - 79)	2	11.8	3	18.8	5	15.2
(80 - 89)	1	5.9	1	6.2	2	6.1
Total	17	100	16	100	33	100

Table 2: The difference in median CEA expression scores by gender among cases with Head and Neck Squamous cell carcinoma and Adenosquamous carcinoma .

		Gender		
Parameters	**Female**		**Male**	
CEA	**N**	**%**	**N**	**%**
Negative	2	12.5	2	11.8
Weak positive	8	50	3	17.6
Moderate positive	3	18.75	6	35.3
Strong positive	3	18.75	6	35.3
Total	16	100	17	100
Median	Weak positive		Moderate and Strong positive	

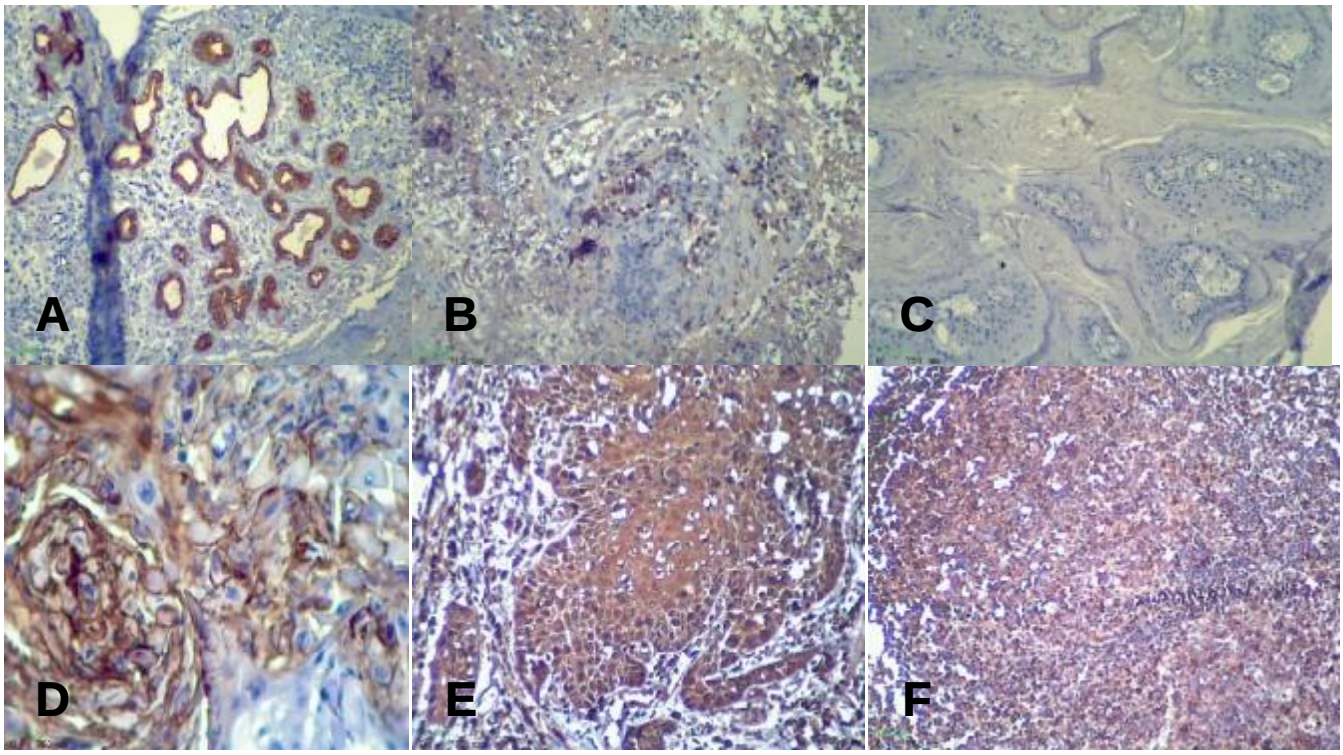

Figure1: (A) Strong positive CEA immunohistochemical expression in Squamous cell carcinoma and Adenosquamous carcinoma with moderate differentiated (X20),(B) Weak positive CEA immunohistochemical expression in Squamous cell carcinoma with well differentiated (X20), (C) Negative CEA immunohistochemical expression in well differentiated squamous cell carcinoma(X20), (D) Moderate positive CEA immunohistochemical expression in moderately differentiated squamous cell carcinoma(X20),(E) Strong positive CEA immunohistochemical expression in poorly differentiated squamous cell carcinoma(X20),(F)Moderate positive CEA immunohistochemical expression in well differentiated squamous cell carcinoma(X20).

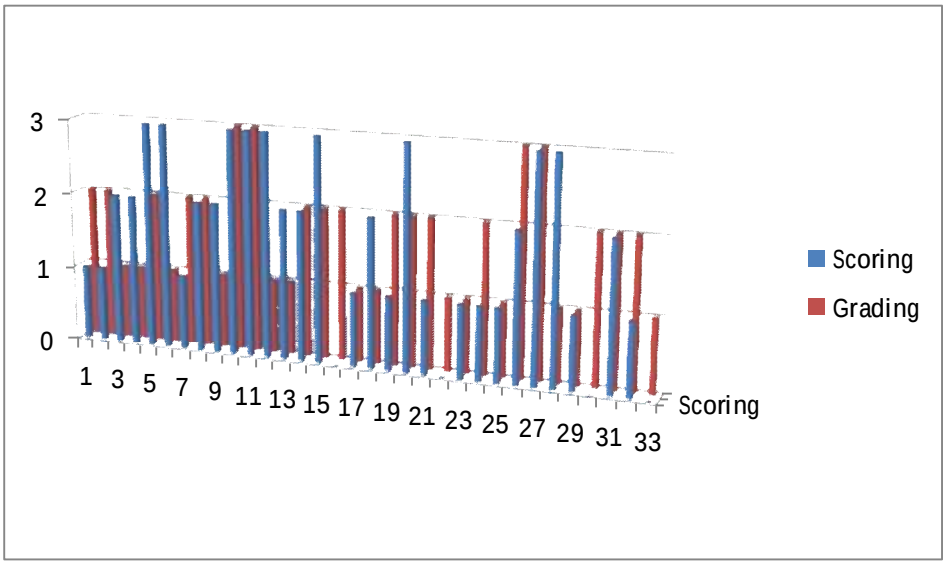

Figure2 : Frequency distribution between the grading of tumor and the scoring of CEA expression scores.