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CORRELATION BETWEEN CYTOTOXIC T-LYMPHOCYTES CD8 WITH SQUAMOUS CELL CARCINOMA OF GINGIVAL ORAL PATIENTS BY USING IMMUNOHISTOCHEMICAL ASSAY

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ABSTRACT

Squamous cell carcinoma occurance and mortality from the gingival have increased during the recent years. while, the current study was designed to determined the correlation of tumor infiltrating cytotoxic T-lymphocyte CD8 in some Iraqi patients with gingival squamous cell carcinoma by using immunohistochemical technique. Thirty specimens were collected during the period between September 2015 until march 2016. The histological types included 19 well differentiated GSCC, 3 moderately differentiated and 8 poorly differentiated GSCC. Cytotoxic T-lymphocytes was detected immunohistochemicaly in 12 out of 30, positive results of cytotoxic T-lymphocytes was related in highly significant with each of age, gender, stage and grade of the tumor. It could be concluded from our study that immunohistochemical assay for detection of CD8 T-lymphocytes was useful in the clinical evaluation of patients with gingival squamous cell carcinoma, also it is seemed to be an additional prognostic marker that was associated with well, moderate and poor differentiated of the tumor.

KEYWORDS: Squamous cell carcinoma, T-lymphocytes.

INTRODUCTION

Oral cancer consistently ranks as one of the top ten cancers worldwide, more than 90% of oral cancer occurs in people older than 45 years. Lesions of gingival account for approximately 10% of the oral squamous cell carcinomas, males are affected more by this form of carcinoma than females.[1,2] Tumor infiltrating lymphocytes (TILs) are the main effector cells in anti tumor cell immunity.[3,4] They are activated by specific antigens that

stimulate them to exert their helper , effector or regulatory functions to mount and orchestrate an efficient immune response.[5] Recent studies show that the several kinds of tumor infiltrating lymphocytes (TIL) associated with better disease comes from various cancers of human.[6,7] The effect of particular immune response determined by the balance between various type of T-cell. Previously it has been shown that the tumor infiltrating lymphocytes CD8 promotes survival in oral cancer patients.[3,4] CD8 T cells mostly play a role in killing antigen bearing cancerous cells.[5]

MATERIALS AND METHODS

The present study involved of thirty patients with gingiva cancer with mean age 50 years ranged between (28-87) years , male to female ratio was 2:1 with 21 male and 9 female , the total of 30 formalin fixed paraffin embedded tissue block were collected randomly from histopathology department of dentistry college / university of Baghdad ,when already diagnosed as gingival squamous cell carcinoma by specialist , compared with 10 apparently healthy control were their age and sex matched to our patients group.

Cytotoxic T-lymphocytes CD8 were determined in specimens by using immunohistochemical assay, performed as recommended in leaflet with kits

- Detection system for p53 protien.

Universal Dakocytomation labeled streptavidin- Biotin 2 system, Horseradish peroxidase (LSAB-2 system . HRP).ready to use detection system, code no. K0673 (CA.USA)for (IHC).

- Ready to use N-series primary antibody (Monoclonal mouse Anti Human p53 protien clone: DO7. Code N 1581).Dako. Denmark.

Immunohistochemical assay

- Paraffin embedded block tissue were sectioned in 4µm thikness by using microtome.All these sections were deparaffinized and dehydrated, these blocks were dewaxed in xylene , aseries of (100,90,70%) ethanol and D.W. respectively, then placed in an endogenous peroxidase block for 25 min., added p53 as a primary antibody in each slides of samples for 90 min., washing with PBS, secondary Ab was added and incubated for I hr. in humid chamber, streptavidin for 30 min. counterstained by Mayer's hematoxyline, dehydration by using serial of ethanol (70,90,100%) and xylene.

Statistical analysis

- The statistical analysis system-SAS[8] was used to effect of differences factors in study parameters. The chi-square x^2 test at the comparative between percentage in this study.

RESULTS

The thirty formalin fixed paraffin embedded tissue blocks of gingival squamous cell carcinoma were immunohistochemically studied by using monoclonal antibody to detection for cytotocic T-lyphocytes (CD8).

The mean age of our studied patients was 50 years ranged between (28-87)years male to female ratio was 2:1 with 21 men and 9 women . gingival squamous cell carcinoma as any oral carcinomas increased with age, 17 out of 30 cases were older than 50 years and 13 of them falling under 50 years, as shown in table (1) there were highly significant association between cytotoxic CD8 with GSCC patients age ($p = 0.0017$), histological grade ($p= 0.0002$), gender ($p=0.0001$) and the stage of the tumor ($p= 0.0003$) respectively at ($p< 0.01$). as seen in figure (1) the positive rate of CD8 was detected in 12 out of 30 (40%) for gingival sqaumous cell carcinoma patients by using immunohistochemical assay (IHC).

The well differentiated rate is 19 out of 30 (63.3%)for all these cases , whereas, the positive count was 7 out of 12 (58.8%), the positive moderate count 3 out of 12 (25%), and the poor differentiated positive count was (16.6%) 2 out of 12 , twenty GSCC patients out of 30 (66.6%) were falling in stage I and II and the rest 10 out of 30 were falling in the stage III and IV of the tumor.

Table (1): Distribution patent of sqaumous cell carcinoma of oral gingival patients according to their age, gender, histological grade and stage of the tumor in relation with CD8 by Immunohistochemical assay.

Factor	CD8 positive No. 12	CD8 negative No. 18	P – value X^2 test
Age **50 >** **50 <**	 7(58.8%) 5(41.1%)	 6(33.3%) 12(66.6%)	 P = 0.0017 X^2 = 8.44
Grade **Well** **Moderate** **Poor**	 7(58.8%) 3(25%) 2(16.6%)	 12(66.6%) 0(0.0%) 6(33.3%)	 P= 0.0002 X^2=9.82
Gender **Male**	 9(75%)	 12(66.6%)	 P = 0.0001

Female	3(25%)	6(33.3%)	$X^2 = 10.39$
Stage **I II** **III IV**	 8(66.6%) 4(33.3%)	 12(66.6%) 6(33.3%)	 P = 0.0003 $X^2 = 7.69$

All these results at $p < 0.01$

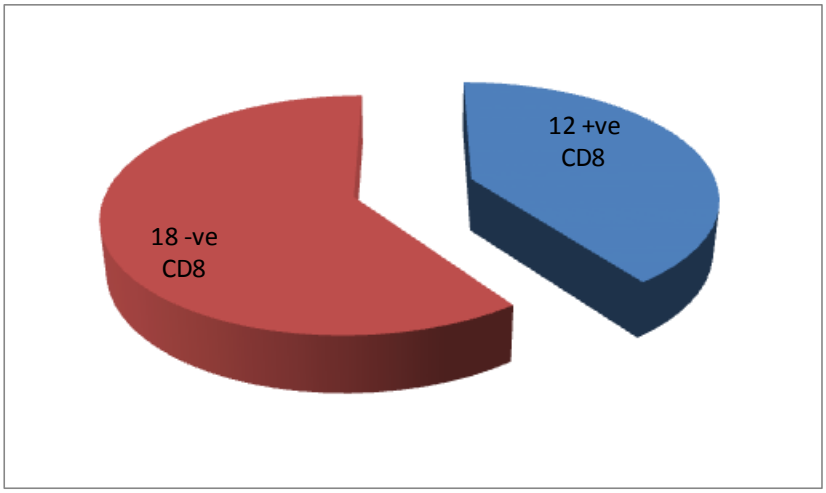

Figure (1): the positive rate of CD8 correlate with gingival sqaumous cell carcinoma patients by using immunohistochemical assay (IHC).

DISCUSSION

Sqaumous cell carcinoma defined by a malignant epithelial neoplasm exhibiting squamous differentiation which is characterized by the formation of keratin or presence of intercellular bridges.[9]

The results shows that the mean age for our patients was 50 years ranged between 28-87 years, our results were consistent with previous study who reported that more than 90% of gingival oral cancer occurs in people older than 45 years, and also confirmed with other study which their patients mean age ranged between 36-65 years.[10] Male to female ratio in this study was 2:1 with 21 man and 9 woman, this study compatible to (2) their males are affected more by gingival SCC than females.

In the present study there was highly significant and highly percentage in the tumor occurrence in stage I,II, GSCC is often a symptomatic , with initial symptoms are usually an intraoral mass or swelling, ulceration, pain, mobility of teeth and unhealed extraction wounds.[11] The occurrence early stages is often the lesion closely simulates advanced periodontitis. with minimal pain and lead to delay of diagnostic.[12]

The current study shows highly significant correlation between CD8 cytotoxic lymphocytes with histological grade of the tumor at p <0.01 with 19 well differentiated, 3 moderately and 8 poor differentiated carcinomas, this results approach to other studies[13] who reported there was a trend for adverse prognosis to accumulate in patients with moderate and poor differentiated carcinoma.

It could be concluded from the study and from the highly significant relation between CD8 and, age, gender, histological grade and stage of GSCC that it was useful in clinical evaluation of patients with gingival squamous cell carcinoma , tumor removed may improve the cellular immunity of patients.

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