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Study the Oncomodulation Potential of Human Cytomegalovirus and its Correlation with TGF-β1 in a Group of Iraqi Patients with OSCC

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Abstract: ***Background: Within the oral cavity, Oral squamous cell carcinoma(OSCC) is the commonest malignant tumor which represents more than 90% of these malignancies. Human cytomegalovirus (HCMV), a member of the Beta herpesvirinae subfamily, is a ubiquitous herpes virus that leads to a life-long persistence.The role of the virus involvement in the cancer development is summarized in the concept of "oncomodulation". Transforming growth factor beta1 (TGF-β1) play an important role during cancer cell proliferation when the signaling pathway. Objectives: 1. Study the oncomodulation of CMV in a group of Iraqi patients with OSCC. 2. Study the correlation between the CMV and TGF-β in those patients. Methods: A total of (42) formalin-fixed, paraffin embedded oral tissue blocks were enrolled in this retrospective research during the period from 1987 till 2014.These samples were divided in to two groups : a study group of (25) blocks of OSCC and a control group of (17) blocks obtained from apparently healthy individuals. The expression of HCMV pp65 and TGFb1 were investigated using immuohistochemistry application. Results: The expression of the HCMVpp65 were found in 84% (21 out of 25) of the OSCC cases. Immunohistochemical staining showed positive results for the expression of TGFb1 in 88% (22 out of 25) of the study group. There is no statistical correlation between the expression of HCMV and TGFb1 in the patients with OSCC. Conclusion: There is an increase findings that suggest the possible involvement of HCMV as anoncomodulatory virus during the development of the OSCC. However, the definite role of HCMV needs to be further investigated using other factors and cellular signal pathways that correlate with cancer progression like smoking , tobacco, Cyclogenase -2 pathway , angiogenesis, MMPs and Apotosis pathways which provide a promising insight for the researchers to develop effective strategies for cancer therapy.***

Keywords: Human cytomegalovirus pp65 , Oral squamous cell carcinoma , Transforming growth factor-1

1. Introduction

Within the oral cavity, Oral squamous cell carcinoma (OSCC) is the commonest malignant tumor which represents more than 90% of these malignancies. In Iraq, oral cancer remains a highly lethal and disfiguring disease. Different environmental factors, viral infections and genetic variations have a reciprocal affect on the initiation of the Oral squamous cell carcinoma (OSCC) (Basim*et al;*2017,Shirin*et al;*2015).

Human cytomegalovirus (HCMV), a member of the Betaherpesvirinae subfamily, is a ubiquitous herpes virus that leads to a life-long persistence. HCMV is widespread in the general adult population with a range from 50% to 100%. (Meike*et al;*2009). HCMV may cause serious *in utero* infections as well as acute and chronic complications in immunocompromised individual. The involvement of HCMV in late inflammatory complications underscores its possible role in inflammatory diseases and cancer .(Martin*et al;*2009).

The virus can infect most organs of the human body including blood, brain, breast, colon, eye, kid- ney, liver, and lung. Therefore, HCMV exhibits broader tropism. (Georges and Kumar, 2014). It's also present in the gingival sulcus fluid (GSF) in many healthy people (Foglio*et al;*2010).

The phosphoprotein pp65 (ppUL83) is an abundant components of the HCMV tegument (Roby & Gibson, 1986) which attracted considerable attention because of its role in different stages of the virus replication cycle (Sabine *et al;*2010)
Kalejta (2008) reviewed that during HCMV infections ,pp65 counteracts both innate and adaptive immune responses and so it is responsible for modulating/evading the host cell immune response during HCMV infections (Kalejta,2008)

Although human cytomegalovirus (HCMV) is generally not regarded to be an oncogenic virus, HCMV infection has been implicated in malignant diseases from different cancer entities. Some studies use the concept of "oncomodulation" to better explain the role of HCMV in cancer. Oncomodulation means that HCMV infects tumor cells and increases their malignancy.(Martin*et al;*2009)

Transforming growth factor beta1 (TGF-β1) is a member the transforming growth factor superfamily produced by every leukocyte lineage, including lymphocytes, macrophages, and dendritic cells. In normal cells, TGF-β, acting through its signaling pathway, stops the cell cycle at the G1 stage to stop proliferation, induce differentiation, or promote apoptosis. In many cancer cells, parts of the TGF-β signaling pathway are mutated, and TGF-β no longer controls the cell. These cancer cells proliferate.(Blobeet al;2000 ;Letterio& Roberts 1998).

Objectives

1) Study the oncomodulation of CMV in a group of Iraqi patients with OSCC.
2) Study the correlation between the CMV and TGF-β in those patients.

2. Materials and Methods

Study Groups

A total of (42) formalin-fixed, paraffin embedded oral tissue blocks were enrolled in this retrospective research during the period from 1987 till 2014 These archival tissue blocks were obtained from College of Dentistry/Baghdad University/ Departments of Oral Diagnosis.

These samples were divided in to two groups : a study group of (25) blocks of OSCC and a control group of (17) blocks obtained from apparently healthy individuals.

Each block was cut with 4 µm thickness and sticked on two types of slides .The first tissue section was mounted on ordinary slide and specified to be used for Hematoxyline and Eosin staining. In addition , the two sections were mounted on a charged slides for immunohistochemistry stainig of CMV and TGFb1.

Anti-Cytomegalovirus pp65 antibody [2 and 6] and Anti-TGF beta 1 antibody [2Ar2] from ABCAM company/ United Kingdom were used in this study.

According to manufacturer's protocol. The slides were deparaffinized and rehydrated by xylene and serially graded alcohol for 5 minutes each and then distill water. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 10 minutes. Slides were washed in phosphate-buffered saline. Then treated with protein block, incubated at 37°C for 5 minutes and washed with PBS. Primary antibody was applied to cover slides and incubated for 1 hours in humidity chamber at 37°C (Primary Antibody was prepared at dilution 1/200 for pp65 and 1/100 for TGFb1). Slides were rinsed gently in PBS. The secondary antibody was added for 10 minutes at room temperature, followed by the addition of Streptavidine-HRP antibodies for 10 minutes at 37˚C. After washing, samples were stained with diluted liquid DAB for 15-45 minutes at room temperature. Slides were counterstained with hematoxylin for 30 second and washed well in running tap water, then dehydrated and mounting with permanent-mounting medium (DPX), examined under light microscope first at 10 then at 40 magnification was finally done.(Areej*et al*;2013).

Statistical Analysis:
Statistical analysis was done using SPSS (Statistical package for social sciences), Social Science Statistic (http://www.socscistatistics.com) and Excel application. Inferential statistics: (Chi square test) was performed to find out the relation between each marker with the OSCC and Normal groups, as well as the relation between both markers. P value (<0.05) was considered statistically significant, and (< 0.000) as highly significant.

3. Results

A total of sixty (25) Iraqi patients with oral squamous cell carcinoma(OSCC) were enrolled in this study. The mean age of the patients was 54 years in which 68% of the patients were above the age of 50 (17 out of 25) and 32% under the age of 50 (8 out of 25). The majority of the patients were females 72% (18 out of 25) whereas males represent 28% (7out of 25) of the total cases. According to tumor grade , the well differentiated OSCC represent the predominant grade followed by moderate differentiated OSCC then poorly differentiated OSCC (68%, 20% and 12%) respectively. Figure (1).

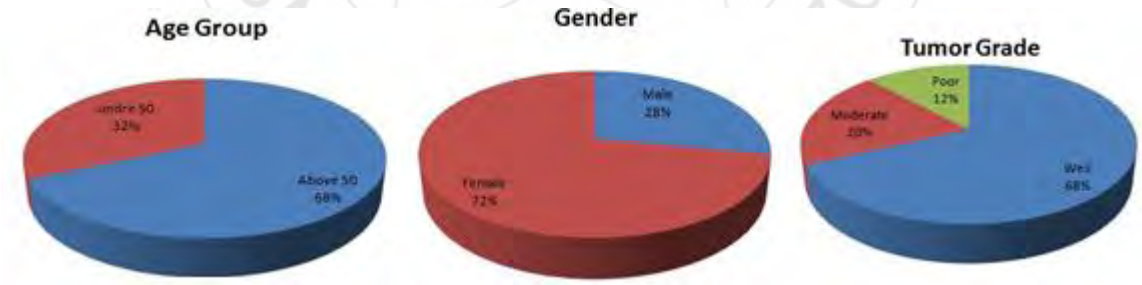

Figure 1: Sample Description

Immunostaining results demonstrated that there is significant correlation of the expression of CMV in the patient with OSCC (p<0.05) when compared with the Apparently healthy individuals Table (1) & figure (2)

Table 1: Immunohistochemical expression of HCMV pp65 protein

Case	CMV positive	CMV negative	Marginal row totals	Chi-square
OSCC	21	4	25	8.3506
Normal	7	10	17	P= 0.0038
Marginal column totals	28	14	42	Significant

Regarding the expression of TGFb1 we found highly significant correlation of the protein in the OSCC cases (P< 0.000) table (2) and figure (3).

Table 2: Immunohistochemical expression of TGFb1

Case	TGFb1 positive	TGFb1 negative	Marginal row totals	Chi-square
OSCC	22	3	25	15.1288
Normal	5	12	17	P= 0.0001
Marginal column totals	27	15	42	High Significant

Regarding the correlation of the expression of CMV and TGFb1 in the OSCC patients and although there is a high

expression of both in the patients with OSCC, we found no statistical correlation between them table (3).

Table 3: Correlation between the expression of HCMV and TGFb1 in the OSCC tissues

Target	positive	negative	Marginal row totals	Chi-square
CMV pp65	21	4	25	0.1661
TGFb1	22	3	25	P= 0.683
Marginal column totals	43	7	50	Non-Significant

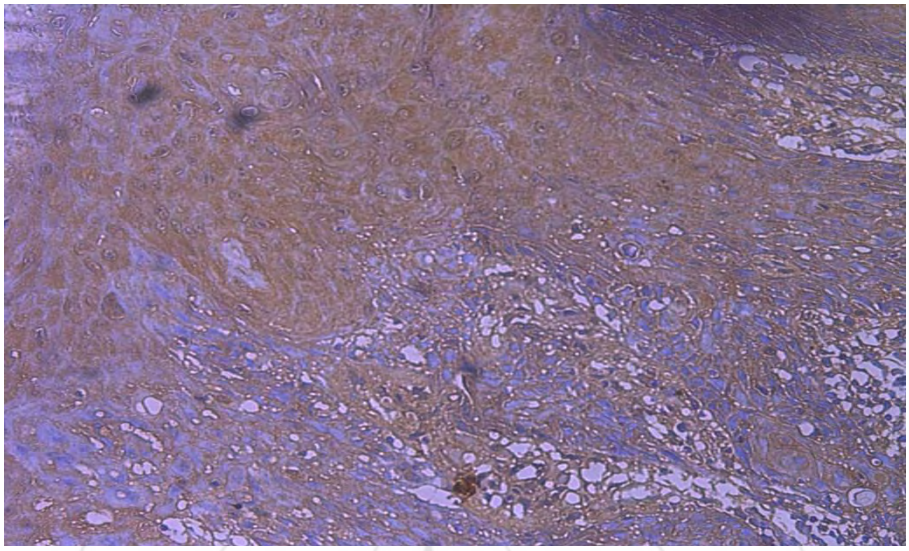

Figure 2: Immunohistochemical expression of Human Cytomegalovirus pp65in oral squamous cell carcinoma(Original magnification 200x.)

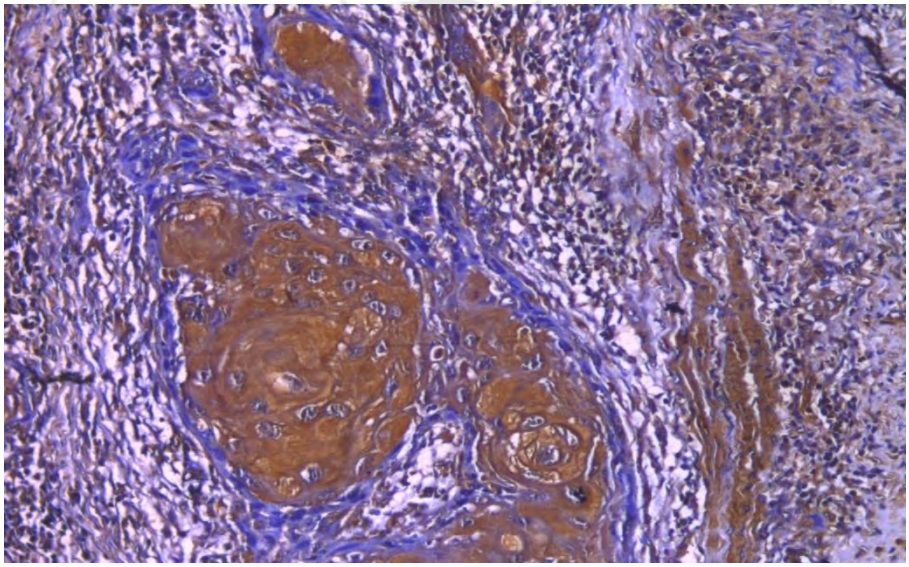

Figure 3: Immunohistochemical expression of Transforming growth factor-1 TGFb1 in oral squamous cell carcinoma (Original magnification 200x.)

4. Discussion

Oral Scc is the commonest malignant tumor of the oral cavity, accounting for more than 90% of these malignancies. (Saad*et al;2011*).

Although CMV infection can cause serious, life threatening conditions in individuals with impaired or underdeveloped immune systems, Cytomegalovirus (CMV) infections are endemic worldwide with asymptomatic signs in healthy individuals (Redwan*et al;*2011) ,this outputs coincides with our results in a healthy group which shows (41%) positive results of the healthy individuals Table (1) . This is because cytomegalovirus (CMV), like other members of Herpesvirus family can establishe latent infection and reactivation may occur at any time during the life of the human host, it is also notable that the consumption of glucocorticoid increased risk of CMV.(Ghandi and Khanna,2004 ; Ko et al;2015)

In the present study , there is a significant correlation of the expression of CMV in the OSCC when compared with healthy individuals (p value=0.0038)(table1). Our result is in agreement with Wei who found high prevalence of HCMV in OSCC tissues (Wei and Xiaoming ,1996).

Because of variety of genetic, environmental, or viral agents ,It is notable that the incidence of herpesviruses is diverse in different geographic areas, this explains the wide range of HCMV expression in OSCC between different researchers (Shirin*et al*;2015 ,Delavarian*et al*;. 2010, Yang et al; 2004, Wei and Xiaoming ,1996, Saad et al; 2013, Sugiura et al; 2006)

From The above results both in healthy and oscc cases, we found an important role for the HCMV tegument protein pp65 in the immune evasion during the viral infection and prevent infected cells from being destroyed by the immune system. Furthermore, it can protect infected cells from the immune response by inhibiting the components of the immune system through binding to them and inhibit their activation .(John ,2012 ; Arnon*er al;* 2006)

In this paper, we aimed to study the oncomodulatoryrole of HCMV in OSCC by study the ability of HCMV to induce changes in the tumor microenvironment using TGFb1 as a parameter.our theory is that the OSCC may escape from the immune monitoring by creating a highly suppressive environment around the tumor.

In the present study, the expression TGFb1is significantly increased in the OSCC patients (p-value=0.0001) (table 2).Joseph et al found un important role for the TGFb1signals in the development of OSCC (Joseph *et al;* 2007).
The overexpression of TGF-β1 affects the surrounding stromal cells, immune cells, and endothelial cells, leading to immuno- suppression, angiogenesis, and tumor invasion.(Chan *et al* ;2012) and since TGF-β1 can induce immunosuppression; therefore, its overexpression in the OSCC may refer to its participation in the creating of an immune- suppressive microenvironment and play important role in it.

In regarding to our objective, the most interesting theory is that the HCMV may influence individual infected cells, surrounding tissues, and/or immune reactions through TGF-β1 production and/or activation. This may promote virus replication and interfere with host immune responses against tumor cells.

In table (3) we found non-significant correlation between the expression of CMV and TGFb1 in the OSCC, in addition the evidences in table 1 and 2 increase the suggestion of independent role of each parameters in the development of OSCC and the virus plays an important role in the oncomodulatory effect of this virus on OSCC.

5. Conclusion

There is an increase findings that suggest the possible involvement of HCMV as anoncomodulatory virus during the development of the OSCC. However, the definite role of HCMV needs to be further investigated using other factors and cellular signal pathways that correlate with cancer progression like smoking , tobacco, Cyclogenase -2 pathway, angiogenesis, MMPs and Apotosis pathways which provide a promising insight for the researchers to develop effective strategies for cancer therapy.

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