Cytokeratin 19 significance in normal and HPV infected oral mucosa

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ABSTRACT

Background

The present retrospective study was performed on normal oral mucosa and infected oral mucosa with human papilloma virus (HPV).
With main objective to establish the expression of cytokeratin 19 in normal and HPV infected oral mucosa due to the virus effect.

Materials and Methods

This study involves a total of (34) cases, (24) sections from oral mucosa of 24 females were infected with HPV virus, and (10) sections from normal mucosa. The expression of cytokeratin 19 was carried out on 4µm specimen sections using Immuno histochemical staining of cytokeratin 19 antibody.
The staining demonstrated the expression intensity, percentage and localization.

Results

Results show cytokeratin 19 was expressed in both normal and HPV infected oral mucosa, but the expression in infected mucosa was significantly different from that of normal one, and the intensity of the staining was more in the basal layer of infected mucosa than normal, in addition to that results show both nuclear and cytoplasmic expression and involves the whole layers of the infected mucosa.

Conclusion

HPV can infect oral mucosa and persistent HPV infection in the oral mucosa might increase the risk of developing oral cancer due to its effect on the differentiating host keratinocyte cells, so HPV infection is one of the contributing factors for OSCC.
Key words: Cytokeratin 19, Human papilloma virus (HPV).

INTRODUCTION:

Cytokeratins (CK) are one of the main families of intermediate keratin filament. They make up the cytoskeleton of both normal and malignant cells of epithelial origin. Among them CK19, a 40KDa epithelial cytoskeletal protein, has been used as a marker for cancers of epithelial origin. CK19 is not expressed in normal hematopoietic cell. Detection of CK19 transcript in peripheral blood of a patient with known OSCC should indicate the presence of carcinoma cells[1].

Cytokeratins (CK) are intermediate filaments, mostly expressed by epithelial cells, which includes a wide range of proteins, varying in molecular weight, isoelectric pH values and affinity [2,3,4]. CK varies among different types of epithelia in their different stages of development, and they may be used as an adjunctive tool for epithelial classification and histological diagnosis [2,5].

Changes in the expression of keratins (Ks), indicating disturbed tissue differentiation, is one possible marker of malignant potential in stratified squamous epithelia. The presence of human papilloma viruses (HPVs) in the epithelium of the uterine cervix is increasingly regarded as a marker of risk for cervical cancer. However a similar role in oral cancer, and precancer remains controversial [6].

Human papilloma virus (HPV) is a member of Human papilloma virus family of viruses that capable of infecting humans. like all papilloma viruses, HPVs establish productive infections only in keratinocytes of the skin or mucous membranes. While the majority of the nearly 200 known types of HPV cause no symptoms in most people, some types can cause warts (verrucae), while others can in a minority of cases lead to cancers of the cervix, vulva, vagina and anus in women, or cancers of the anus and penis in men. It can also cause cancer of the head and neck (tongue, tonsils and throat)[7].

Human papillomaviruses (HPVs) are a group of more than 150 related viruses. They are called papilloma viruses, because certain types may cause warts, or papillomas which are benign(noncancerous) tumors[7].

Some HPVs, such as those that cause the common warts that grow on hands and feet, do not spread easily. However, more than 40 HPV types are sexually transmitted, and these HPVs spread very easily through genital contact [7].

Some types of sexually transmitted HPVs cause cervical cancer and other types of cancer. These are called high risk oncogenic or carcinogenic HPVs. Other sexually transmitted types of HPV do not appear to cause cancer and are called low risk HPVs [7].

The human papilloma virus is a double stranded DNA virus that infects the epithelial cells of skin and mucosa. The epithelial surfaces include all areas covered by skin and/or mucosa such as the mouth, throat tongue tonsils, vagina, penis and anus [8].

Transmission of the virus occurs when these areas come into contact of a virus allowing it to transfer between the epithelial cells. Oral HPV infection is transmitted sexually, but also can be transmitted from mouth to mouth or vertically from an infected mother during delivery[8].

One of the most common virus group in the world today affecting the skin and mucosal areas of the body, is human papilloma virus[9].

Genital warts are known technically as condylomata acominatum and are generally associated with two HPV types, numbers 6 and 11 [9].

There are other forms of HPV which are sexually transmitted, and are a serious problem. These are HPV-16, 18, 31, and 45. these cancer associated types of HPVs cause growth that usually appears flat, and are nearly invisible as compared with the warts caused by HPV-16 and HPV-11.Two types of genital tract HPV in particular, HPV-16 and HPV-18, are known to cause up to 95% of cervical cancers, and new studies show that they may be linked to oral cancer as well.

OBJECTIVE:

To investigate cytokeratin 19 expression in normal and HPV infected oral mucosa.

MATERIALS AND METHODS:

-Selection of cases and tissue staining:

A retrospective study was carried out at the Surgical Pathology Center in Davanzo Hospital-Foggia-Italy, between April 2008 - July 2008, involving 24 sections (4µm) from formalin – fixed paraffin embedded blocks were cut from oral mucosa of 24 females were infected with HPV virus, and 10 sections from normal mucosa as control.

Immuno histochemistry staining was then performed on the sections that mounted on poly- L-lysine coated glass slides utilizing mouse monoclonal Cytokeratin – 19 antibody[10].

After antigen retrieval by pressure cooking with ethylene diamen tetra-acetic acid (EDTA) solution as a buffer solution, and quenching in 30% hydrogen peroxide and blocking. The sections were incubated with primary antibody Cytokeratin-19. Then biotinylated anti rabbit immunoglobulin and streptavidin conjugated to horse radish peroxidase (HRP) were subsequently applied. Finally , 3,3- diaminobenzidine was used for color development and haematoxylin was used for counter staining.

The results of Immuno histochemical staining were evaluated by two observers which appear as brown pigmentation.

statistical analysis used are, mean ± S.D. for staining expression percentage of cytokeratin 19.

P – value of < 0.05 was considered significant. By using T- test.

RESULTS:

Staining results show:

-Positive expression of cytokeratin 19 in the whole cases of infected oral mucosa (100%). A mean percentage of expression and standard deviation (SD) of cytokeratin 19 in infected HPV oral mucosa is (85.625 ± 7.116), fig.(1&2), as compared with the expression in non infected mucosa with mean percentage (16.4 ± 5.641), fig.(3), and the difference between two expressions is statistically significant, p-value > 0.05, see table (1).

-Cytokeratin 19 positivity shows cytoplasmic expression and nuclear membrane expression as a result of vacuolization of the nuclei due to the effect of the virus, as seen in fig.(1&2), while normal mucosa expression shows cytoplasmic expression in some areas and nuclear in others, as seen in fig.(3 &4).

- The expression of cytokeratin 19 involves the whole layers of infected mucosa, and more intense in the basal layer, fig.(1&2), while in normal mucosa, are seen in basal and para-basal layers with less intensity, fig. [5].

Table (1): Mean expression of cytokeratin 19 in HPV infected mucosa and normal mucosa

Type	Mean ± SD	*p*- value
Infected mucosa	85.625 ± 7.116	> 0.05
Normal mucosa	16.4 ± 5.641	

DISCUSSION:

HPV can infect oral mucosa. A subgroup of oral cancer clearly is associated with HPV. Oral HPV infection is transmitted sexually but also can be transmitted from mouth to mouth and vertically from an

infected mother during delivery [8].

HPV infection is limited to the basal cells of stratified epithelium, the only tissue in which they replicate [11]. The virus cannot bind to live tissue; instead, it infects epithelial tissues through micro-abrasions or other epithelial trauma that exposes segments of the basement membrane[11].

The infectious process is slow, taking 12–24 hours for initiation of transcription. It is believed that involved antibodies play a major neutralizing role while the virions still reside on the basement membrane and cell surfaces [11].

HPV lesions are thought to arise from the proliferation of infected basal keratinocytes. Infection typically occurs when basal cells in the host are exposed to infectious virus through a disturbed epithelial barrier as would occur during sexual intercourse or after minor skin abrasions. HPV infections have not been shown to be cytolytic; rather, viral particles are released as a result of degeneration of desquamating cells. The HPV virus can survive for many months and at low temperatures without a host; therefore, an individual with plantar warts can spread the virus by walking barefoot [12].

Most HPV infections are cleared rapidly by the immune system and do not progress to cervical cancer. Because the process of transforming normal cervical cells into cancerous ones is slow, cancer occurs in people having been infected with HPV for a long time, usually over a decade or more (persistent infection) [13,14].

Several types of HPV, in particular type 16, have been found to be associated with HPV-positive oropharyngeal cancer (OSCC), a form of head and neck cancer [15,16]. HPV-induced cancers often have viral sequences integrated into the cellular DNA. Some of the HPV "early" genes, such as E6 and E7, are known to act as oncogenes that promote tumor growth and malignant transformation. Oral infection with HPV increased the risk of HPV-positive oropharyngeal cancer independent of tobacco and alcohol use [16]. In the United States, HPV is expected to replace tobacco as the main causative agent for oral cancer[12].

E6 and E7 are the HPV proteins associated with cancer. The HPV genome is composed of six early (E1, E2, E3, E4, E6, and E7) and two late (L1 and L2) proteins.(16.19). After the host cell is infected E1 and E2 are expressed first. High E2 levels repress expression of the E6 and E7 proteins. When the host and HPV genomes integrate, E2 function is disrupted, preventing repression of E6/E7 [17].

The p53 protein prevents cell growth and stimulates apoptosis in the presence of DNA damage. The p53 also upregulates the p21 protein, which blocks the formation of the Cyclin D/Cdk4 complex, thereby preventing the phosphorylation of RB and, in turn, halting cell cycle progression by preventing the activation of E2F. In short, p53 is a tumor suppressor gene that arrests the cell cycle when there is DNA damage [18].

The E6/E7 proteins inactivate two tumor suppressor proteins, p53 (inactivated by E6) and pRb (inactivated by E7) [18].

The viral oncogenes E6 and E7 are thought to modify the cell cycle so as to retain the differentiating host keratinocyte in a state that is favorable to the amplification of viral genome replication and consequent late gene expression. E6 in association with host E6-associated protein, which has ubiquitin ligase activity, acts to ubiquitinate p53, leading to its proteosomal degradation. E7 (in oncogenic HPVs) acts as the primary transforming protein. E7 competes for retinoblastoma protein (pRb) binding, freeing the transcription factor E2F to transactivate its targets, thus pushing the cell cycle forward. All HPV can induce transient proliferation, but only strains 16 and 18 can immortalize cell lines in vitro [19].

Kellokoski et al., in 2006 found that in HPV DNA- positive biopsies in the basal cell layer was more intense than in HPV DNA- negative biopsies, and the more efficient expression of Ck 19 in HPV DNA-positive samples suggests that viral infection might accelerate the production of low molecular weight cytoskeletal protein. This could be interpreted as evidence that HPV might disturb the keratinocyte differentiation in the basal cells [20]. In this study same findings are found, in addition to that we found that the expression involves the whole layers of infected mucosa.

Association of high risk human papilloma virus (HR-HPV) with oral cancer has been established recently, detecting these viruses in oral cavity is important to prevent oral lesion related to them. Saini et al., in 2010 studied (105) oral squamous cell carcinomas (OSCC) affecting Malaysian population, found HPV to be significantly associated with OSCC [21].

Oncogenic HPVs have been detected in OSCC. HPV16 is the most frequently detected type of HPVs in oral SCC and is present in up to 22% of cases, either alone or in combination with other HPV types. HPV 18 is present in up to 14% of cases. HPV 16 and HPV 18 are present together in approximately 6% of cases. However, HPV 16 and 18 are also detected in normal oral mucosae (10% and 11%) respectively. These data suggest that high risk HPV infection may be a co-factor in oral carcinogenesis and that latent HPV infection of the oral mucosa is common. A role of HPV infection in oral carcinogenesis is supported by the ability of high risk HPVs to immortalize oral keratinocytes in vitro. Immortalization may involve (i) deactivation of pre-formed tumor suppressor proteins by viral oncoproteins, (ii) blocking of tumor suppressor gene transcription as a result of HPV oncogene insertion or (iii) stimulation of cellular oncogene transcription by the upstream insertion of HPV- derived transcription activating sequences. Hence, infection of oral keratinocytes with high risk HPV may be involved in the pathogenesis of some oral SCCs [22].

The detection of oral HPV infection is done by southern blotting hybridization (SBH) and polymerase chain reaction (PCR) [22].

This study proved the infection of oral mucosa with HPV which characterized by the expression of cytokeratin 19 as compared with normal mucosa specially in the basal layer of keratinocyte cell in which they replicate and result in infected differentiated keratinocyte cells, so involve the whole mucosal layers with long standing infection, these findings were in agreement with many other studies mentioned here.

CONCLUSION:

Cytokeratin 19 was expressed in both normal and HPV infected mucosa, but the expression in infected mucosa is significantly different from normal mucosa and with different distribution throughout the mucosa.

So we conclude that HPV can infect oral mucosa and persistent HPV infection in the oral mucosa might increase the risk of developing oral cancer due to its effect on the differentiating host keratinocyte cells, so HPV infection is one of the contributing factors for OSCC.

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