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Research Article

The Association Between Human Cytomegalovirus and Salivary Gland Cancer: An Analytical Study and Literature Review

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Abstract

Background: Human cytomegalovirus (HCMV) is a widespread human pathogen that triggers varying clinical symptoms depending on the host's age and immune status. It appears that HCMV infection plays a role in the development of numerous types of cancer. This study aimed to identify the presence of HCMV in different kinds of malignant salivary gland tumors in Sudanese patients.

Methods: Eighty-four formalin-fixed paraffin-embedded tissues (FFPE) from Sudanese patients previously diagnosed with salivary gland cancer (SGC) between 2014 and 2022 were selected. All cases include normal salivary gland tissue. Immunohistochemical staining for CMV was performed using monoclonal antibodies to detect the presence of the virus among the studied group.

Results: CMV was detected in only 1 out of 84 SGC cases; an adenoid cystic carcinoma. All adjacent normal salivary gland tissues were negative for the virus.

Conclusion: The absence of CMV in the studied cases suggests that the virus was not involved in developing these malignancies.

Keywords:

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1. Introduction

Salivary gland tumors are relatively rare, and their causes, prognostic factors, and risk factors are not well understood. Malignant salivary neoplasms typically appear in individuals in the fifth and sixth decades. They constitute about one-third or a quarter of all salivary gland tumors. The most frequently occurring types are adenoid cystic carcinomas and mucoepidermoid carcinomas (MECs) [1–5].

Human cytomegalovirus (HCMV) also known as human herpesvirus-5 (HHV-5) or salivary gland virus is one member of the *Herpesviridae* family, *Beta-herpesvirinae* subfamily, *Cytomegalovirus* genus. Each mature virion particle is about 200 nm in size and has an outer phospholipid membrane that contains the tegument proteins that are crucial for viral replication [6]. Blood transfusions, allograft transplants, sexual contact, vomit, urine, saliva, and respiratory secretions are all methods of transmission [7, 8].

HCMV is a widespread pathogen, infecting between 45–100% of the global population, with the highest prevalence in South America, Africa, and Asia [9, 10]. In Sudan, CMV was found in 97.3%, 97.5%, and 97.5% of blood donors, nondonors, and pregnant women, respectively [11, 12]. Another study among pregnant women revealed a prevalence of 72.2% [13].

Numerous CMV proteins possess the capability to interfere with the processes that control carcinogenesis, apoptosis, the cell cycle, angiogenesis, cellular invasion, and the host's defensive response [14]. The products of the MIE genes have been shown to have many similarities with other DNA oncogenic viral proteins, particularly those that target members of the Rb and p53 families. As a result, they stimulate the cell cycle, encourage DNA mutations, and hinder apoptotic pathways [15]. By promoting anti-apoptotic pathways, the products of CMV can prevent cell death and may potentially instigate the onset and/or advancement of cancer. It has been discovered that preneoplastic prostatic epithelium expresses high levels of CMV, which fosters the progression of prostatic cancer [14]. Moreover, an *in vivo* study of colorectal cancer and its precancerous lesions found that the CMV IE1-72 protein was consistently present in cancerous tissue. In benign lesions such as adenomas, IE1-72 immunoreactivity was only detected in areas of dysplastic epithelium and was not found in colonic crypts where the cells showed no signs of cellular atypia [16].

Recently, many studies found an association between CMV and malignant tumors from different sites including breast [17–19], gliomas [20, 21], and prostate [22]. Regarding salivary gland tumors, the association with CMV is heavily debated and still a matter of controversy, as many studies were unable to isolate the CMV DNA in salivary gland neoplasm [23–26], while others reported the presence of the virus in salivary gland tumors [27–29].

2. Materials and Methods

Eighty-four patients diagnosed with salivary gland cancer (SGC) at Prof Ahmed Sulieman Oral and Maxillofacial Laboratory, Faculty of Dentistry, University of Khartoum between 2014 and 2022 were selected for the study. The selection criteria were the availability of adequate tissue for the immunohistochemistry (IHC) and the presence of adjacent normal-appearing salivary glands. Patient's clinical data were collected from their files. These data include age, gender, and site of the lesion. Patient's histological slides were retrieved

from laboratory archives and reviewed under light microscopy for histological diagnosis.

2.1. Slide cutting and preparation

From each paraffin block, a 4 microns slide was cut using a Rotary microtome (Leica RM 2125).

2.2. Positive and negative controls

A known positive control slide cut from a previously diagnosed case of vesiculo-pustular skin lesion caused by cytomegalovirus (CMV) was used in this study. The case was previously stained positive for CMV by IHC using monoclonal antibody for CMV. Slide from normal skin was used as a negative control. Positive and negative controls were provided by El Hassan Laboratory, Khartoum, Sudan.

2.3. Immunohistochemistry (IHC)

Immunohistochemical staining was performed using the following monoclonal antibodies: anti-CMV (clones CCH2 and DDG9, dilution 1:200, Zytomed Systems Germany), which is a cocktail of two antibodies that react with a 76 kDa HCMV early protein and the delayed early DNA-binding protein p52.

2.4. Antigen retrieval

The slides containing both positive and negative controls underwent a process of dewaxing in xylene and dehydration through graded alcohol concentrations to distilled water. Following this, the slides were immersed in preheated buffer (Zytomed HIER T-EDTA Buffer pH 9.0), which was prepared 1 in 10 (v/v) at 96°C in a water bath for 30 mins. Upon completion of this retrieval process, the Coplin jar housing the slides was removed from the water bath and left to cool to room temperature.

2.5. Staining protocol

The staining procedure was carried out by using anti-CMV antibodies [MSK121-05]. The concentrated primary antibody was diluted 1:50 using antibody diluent (ZUC025-100).

Once the slides had cooled to room temperature, they were rinsed in a PBS buffer (Zytomed System Wash Buffer, ZUC02-500) with a pH of 7.6 for 5 mins. Following this, a circle was drawn around each section using an immune pen (Zytomed, Pink PAP Pen Mini, ZUC065). The sections were then treated with 3% hydrogen peroxide (H_2O_2) for 5 mins to inhibit endogenous peroxidase activity. After another rinse in the PBS buffer for 5 mins, the sections were incubated with a primary antibody for CMV for 60 mins, as per the manufacturer's instructions. The sections were then rinsed again in PBS for 5 mins, then the secondary antibody (Zytomed, One-Step Super Sensitive^{TM} Polymer-HRP IHC Detection System/DABQD620-XAKE) was applied for 40 mins. Slides were then washed in PBS buffer for 10 mins. Following the previous steps, the DAB chromogen was then applied to the slides, using a mixture of 1 ml substrate buffer and 1 drop of DAB chromogen. The sections were then rinsed with distilled water and counterstained with Mayer's Hematoxylin for 1 min. After another rinse with distilled water, the slides were left to air dry for 5 mins. The final steps involved clearing the slides in xylene and mounting them with a cover glass using DPX mounting media.

2.6. Immunohistochemical scoring

Sections that were immuno-stained were examined by two histopathologists. In instances of disagreement, they engaged in a discussion using a multiheaded microscope until a consensus was reached. The presence of the HCMV protein was determined by the brown staining in the nucleus with or without a diffuse pattern.

3. Results

Pathological examinations were performed on 84 patients with SGC; distributed evenly between males and females (male: female = 1:1). The mean age was 51.3 ± 17.1 years ranging from 19 to 90 years. Regarding the tumor locations, most of the lesions occurred in minor salivary glands with the highest frequency of tumors occurring in the palate, which accounted for 38.09% and stands out significantly compared to others. This was followed by the parotid representing 19% (Figure **1**). Salivary gland carcinomas exhibit diverse histological types. Among the diagnosed cases, MEC was the most prevalent (31%), followed by adenoid cystic carcinoma (23.8%); the prevalence of all diagnosed histological types of SGC is presented in Table 1.

In this study, CMV IHC using a monoclonal antibody detected CMV-positive expression in only one case: an adenoid cystic carcinoma. The patient, a 63-year-old male, presented with a parotid area swelling extending to the submandibular area. The macroscopic examination revealed the following: in Bottle 1, there was unoriented salivary gland tissue measuring 6×4.5×2 cm; Bottle 2 contained the parotid gland with the tumor and a cervical lymph node measuring 9×5×4 cm. The cut section of the tumor appeared lobular, soft, and white. Additionally, an unoriented muscle fragment measuring 2.5×2.7×0.4 cm was present. The histopathological assessment revealed sheets of malignant blue cells with scant cytoplasm and hyperchromatic nuclei. The presence of basement membrane material was indicated by periodic acid-Schiff (PAS) positivity. Notably, all lymph nodes were involved in this case.

The adjacent normal salivary gland tissues were negative for the virus in all cases including the positive case.

Table 1: Clinical and pathological characterization of all diagnosed salivary gland carcinomas.

SGC histological type	Prevalence	Percentage (%)
Mucoepidermoid carcinoma	26	31
Adenoid cystic carcinoma	20	23.8
Polymorphous adenocarcinoma	10	11.9
Salivary duct carcinoma	10	11.9
Myoepithelial carcinoma	5	6
Acinic cell adenocarcinoma	5	6
Epithelial myoepithelial carcinoma	4	4.8
Carcinoma ex pleomorphic adenoma	2	2.4
Basal cell adenocarcinoma	2	2.4
Total	84	100

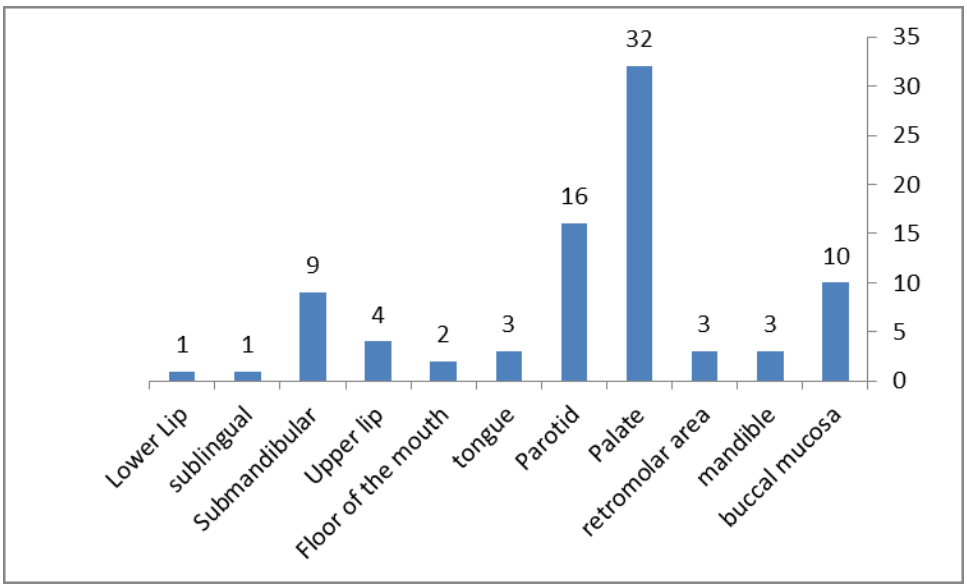

Figure 1: The site distribution of salivary gland cancer lesions.

4. Discussion

Viruses have long been identified as an etiologic factor in cancer development. This association extends back to Rous' almost a century-ago observation that viruses can cause sarcomas [30]. Since then, cancer biology has continued to be concerned about viral tumorigenesis. In 2014, the WHO National Cancer Research Agency report stated that viruses are responsible for 17.8% of human cancer cases [31].

Although CMV has not been definitively linked to human cancer, recent evidence suggests that active CMV infection may be associated with various types of malignancies, such as cancers of the breast [17–19], gliomas [20, 21], and prostate [22]. In a recent Sudan-based study, researchers investigated tissue blocks from 150 patients previously diagnosed with nasopharyngeal cancer (NPC). They found that CMV was identified in 53 out of 150 samples (35.3%). This discovery highlights a substantial association between CMV and NPC [32]. Another study was conducted to determine the effect of CMV infection on the mortality rate in patients with head and neck cancers who underwent radiation or radio-chemotherapy. It was claimed that identifying and treating these viral infections is essential for effective cancer treatment since plasma CMV-positive patients were thought to have a significantly higher mortality risk than those with no CMV detected in plasma [33].

A review of the existing literature revealed a scarcity of research exploring the impact of viruses on the formation of salivary gland tumors. Considering the exposure of salivary glands to numerous infectious agents, and the frequent presence of CMV in the ductal epithelium of these glands [34], along with the known carcinogenic properties of other herpesviruses such as EBV and HHV8, it is postulated that CMV could have a substantial influence on the onset of SGC.

Despite the prevalence of CMV infection among the Sudanese population [11–13], this study found no presence of the virus in healthy salivary gland tissue. Moreover, the virus was detected in just one case of adenoid cystic carcinoma. This finding aligns with numerous other studies that have found no correlation between salivary gland tumors and CMV.

A study conducted by Jayaraj *et al.* to explore the potential role of CMV in the development of MEC [25]. They analyzed four cases of MEC and normal salivary gland tissue. Interestingly, the pp65 antigen expression was found to be negative in all studied MEC cases. In addition, Atula *et al.* conducted a study to explore the possible role of CMV in salivary gland tumors. They analyzed fresh tissue samples from 19 pleomorphic adenomas and 19 malignant salivary gland tumors; however, they did not find any CMV DNA in these tumor samples [23]. Furthermore, Kärjä *et al.* used *in situ* hybridization to examine 106 malignant and 113 benign salivary gland tumors, and found no CMV DNA in any of the cases [24]. Similarly, Senft *et al.* analyzed 51 salivary gland carcinomas and 26 benign tumors using IHC but found no CMV positivity in any of the cases, and concluded that CMV infection is not a significant pathogenic factor in SGC [26]. In a more recent study, 25 pleomorphic adenomas, 31 recurrent adenomas, and 12 carcinoma ex-pleomorphic adenomas were examined for the presence of herpes viruses. However, all samples from all tumor types tested negative for CMV DNA [35]. Conversely, numerous studies have reported a significant presence of CMV in salivary gland tumors. Radunovic *et al.* utilized CMV immediate-early (IE) and early gene products to investigate the prevalence of CMV in various histological types of SGC; they analyzed 20 samples of normal salivary gland tissue, which were collected during autopsies, and 92 cases of different histological types of SGC [27]. They found that a substantial proportion of SGC (70.6%) showed positive CMV antigen expression, while the healthy acinar tissue of salivary glands and the salivary gland tissue surrounding tumors did not express CMV antigen [27]. They also observed a higher expression of IL-6 in SGC (70.7%) compared to normal tissue (20%) and noted an association between the presence of CMV and the presence and intensity of IL-6. They concluded that the positive expression of CMV antigens in SGC cells suggests that it could play a significant role in carcinogenesis by stimulating the production of IL-6 and inhibiting apoptosis and tumor growth. Furthermore, another study detected CMV DNA in three out of five samples of SGC (28).

On the other hand, Melnick *et al.* studied 39 human MEC specimens that were classified by oral and maxillofacial pathologists using the modified Healey system [29]. The cases were examined through IHC using antibodies against the hCMV proteins IE1-72 and pp65. Out of these lesions, 38 showed reactivity to IE1-72 in the nucleus and/or cytoplasm. It was observed that higher tumor grades were associated with increased levels of IE1-72 reactivity. Both the cytoplasm and nuclei of tumor cells, along with inflammatory cells in the tumor stroma, showed reactivity to pp65. However, neither antibody reacted with the surrounding normal salivary gland tissue. Considering that hCMV was found in most cases of MEC, and only the cancerous tissue contained the infectious agent, and given that hCMV-specific gene expression was demonstrated at the cellular level and was positively correlated with the grade of MEC, the authors concluded that their findings satisfied the causal criteria for HCMV being the cause of MEC.

In the studies conducted, two primary methods were employed for virus detection: polymerase chain reaction (PCR) and IHC. PCR, known for its high sensitivity, can detect even minute amounts of viral genetic material, making it accurate for early detection when viral loads are low [36]. However, IHC, which identifies specific tumor antigens in tissue sections, is less sensitive and specific than

PCR [37]. Of note, all PCR-based studies yielded negative results, while one IHC study showed a positive outcome.

The absence of CMV expression in this study might be due to the fact that in most cancers caused by viral infections, the viral DNA is typically present in extremely small quantities, often less than one DNA copy per ten tumor cells [38]. As a result, a highly sensitive and accurate method is needed to detect the likely low levels of CMV within the tumor. Another potential explanation for the negative results could be that while CMV might contribute to the development of SGC, the viral proteins may no longer be identifiable within the tissue. The conclusions of this study are somewhat constrained by the relatively small sample size assessed for each tumor type. Additionally, the storage conditions for paraffin-embedded blocks warrant consideration. These blocks were stored under hot conditions, and in some instances, the paraffin partially melted. Given their prolonged storage duration, there's a potential risk of gradual antigen degradation, which could have impacted antigen detection in this study.

5. Conclusion

The results of this study showed that several histologic types of SGC possess negative CMV protein. The absence of CMV in these cases suggests that the virus has no role in the pathogenesis of salivary glands tumors.

6. Recommendation

To address the limitations posed by small sample sizes, future studies with a collaboration with multiple research centers or institutions are recommended to pool data. This collaborative approach would allow for a larger and more diverse sample and include more cases from the rare tumor types.

Declarations

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Ethical Considerations

The study was approved by the Ethical Committee Unit at the Faculty of Medicine, University of Khartoum. Patient's data were treated with confidentiality, and cases were referred to by their slide number.

Competing Interests

None.

Availability of Data and Material

All Data is presented in this article shall be available on request.

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Abbreviations and Symbols

CMV: Cytomegalovirus

EBV: Epstein-Barr virus

HCMV: Human cytomegalovirus

FFPE: Formalin-fixed paraffin-embedded tissues

SGC: Salivary gland cancer

HHV-5: Human herpesvirus-5

IHC: Immunohistochemistry

HPF: High power field

WHO: World Health Organization

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