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Possible role of human cytomegalovirus in the pathosis of ameloblastoma: A QRT-PCR study

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Abstract

Background: Odontogenic tumors and cysts are believed to originate from the epithelial rests of the odontogenic apparatus. However, the cause behind the activation, proliferation of these rests and the pathosis of the odontogenic lesions is not known. The aim of the present study was to investigate the presence of Human Cytomegalovirus in Ameloblastoma. Materials and Methods: This study included 10 Formalin-fixed Paraffin- embedded blocks of Ameloblastoma that were retrieved from the archive. The DNA was extracted from all the tissues and subjected to qRT-PCR using the HCMV primer. Result: HCMV-DNA was detected in 6 out of 10 samples of Ameloblastoma. Conclusion: The presence of HCMV in 60% samples of Ameloblastoma cannot be incidental. Although this study has not proved

that CMV has a role in pathogenesis of Ameloblastoma, such a possibility cannot be precluded.

Key words: Ameloblastoma, HCMV, Odontogenic lesions, QRT-PCR.

Introduction

Ameloblastoma is classified as a benign neoplasm of the enamel organ. Researchers believe that it is of varied origin including the activation of the remnants of the odontogenic apparatus, mutation in the HOMEO BOX genes, from the lining of a odontogenic epithelium among others.[1] Although, the stimulus instigating the proliferation of the cells still remains a mystery.[2]

Human Cytomegalovirus (HCMV) belongs to the Herpesviridae family. It has a prevalence of 55-100% within the human population.[3]

Due to its high cellular tropism, HCMV has the propensity to infect various cells like neutrophils, monocytes, lymphocytes, fibroblasts, endothelial and epithelial cells.[4]. The replication of virus takes place inside the host cell's nucleus. It can be appreciated as intranuclear inclusion bodies also called as owl's eye nucleus but it is not visible in routine H and E sections. Immunohistochemistry (IHC) and in situ hybridization is employed to give a definitive viral diagnosis.[5]

Many earlier studies have evinced the presence of HCMV in different tumours such as renal carcinomas, colon and breast neoplasms.[6] As a matter of fact, HCMV is believed to promote tumorigenesis, causes immunosuppressive effects in the tumour environment and displays immunity evasiveness. HCMV also has a role in hijacking proangiogenic mechanisms and exhibits anti-apoptotic properties.[7]

Various studies have used IHC as well as PCR and have proved the presence of HCMV in apical periodontitis, radicular cyst and odontogenic keratocyst. Until now, one study has detected HCMV in Ameloblastoma. Hence the current study aims to detect the presence of HCMV in Ameloblastoma and to correlate its presence with the pathogenesis of the tumour. To the best of our knowledge, this is the first study to be conducted in Indian subcontinent to unravel the HCMV's presence in Ameloblastoma.

Materials and methods

The retrospective study included 10 formalin fixed paraffin embedded (FFPE) blocks of Ameloblastoma. All the cases were retrieved from the archive of Department of Oral Pathology, GDCRI, Bengaluru.

The H&E slides and pathology reports of all the cases were reviewed and confirmed.

10µm thick sections were taken and transferred to 2ml Eppendorf tubes.

Figure 1: 10µm thick sections taken in soft tissue microtome.

Deparaffinization and rehydration was done using xylene and different grades of alcohol respectively.

DNA was extracted from the tissue using the manufacturer's protocol (Hi Per® Bacterial Genomic DNA Extraction – Hi Media).

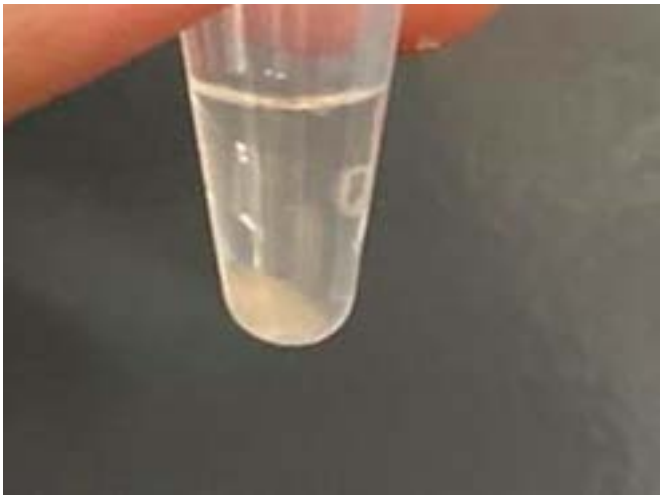

Figure 2: The isolated DNA solution

The primer for HCMV was resourced from Juniper Life Sciences with the following sequence;

Forward:5′-CCACCCGTGGTGCCAGCTCC-3′

Reverse: 5′CCCGCTCCTCCTGAGCACCC-3′

A working solution was prepared by adding forward and reverse primers along with nuclease free water (NFW).

Quantitative RT-PCR reaction was performed in a final reaction volume of 10µL containing 1µL of the isolated DNA solution, 1µL of working solution of the primer, 5µL of master mix(SYBR™ Green PCR Master Mix - Thermo Fisher Scientific) and NFW was added to make the solution 10µL.

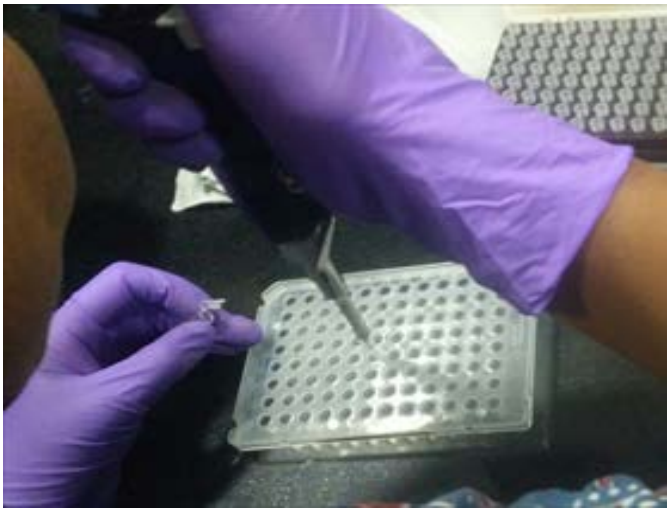

Figure 3: Loading the final reaction volume in the PCR plate wells.

The quantitative polymerized chain reaction machine was set up after placing the sample loaded plate. Following the completion of the reaction, graphs and cycle threshold (Ct) values were obtained.

Results

Out of 10 cases included in the study, HCMV-DNA was detected in 6 cases with mean CT value of 35.88+1.039.

	Number of cases	Mean+SD
Ct value	4	UNDETERMINED
	6	35.88+1.039

Table 1: HCMV-DNA determination

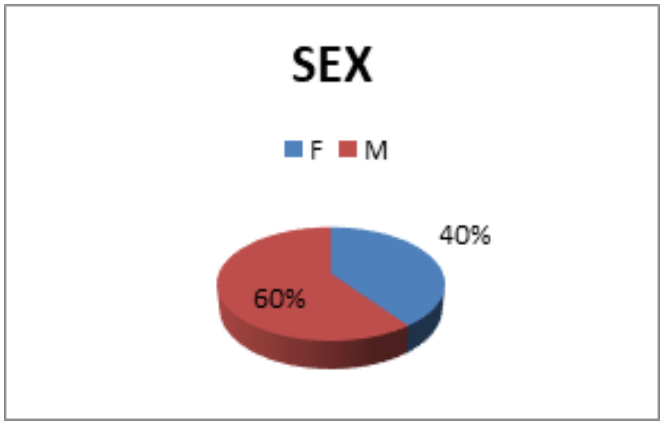

Figure 4: Sex distribution

The male to female ratio was 6:4 (Figure 4) with posterior maxilla being the most involved site. (Figure 5)

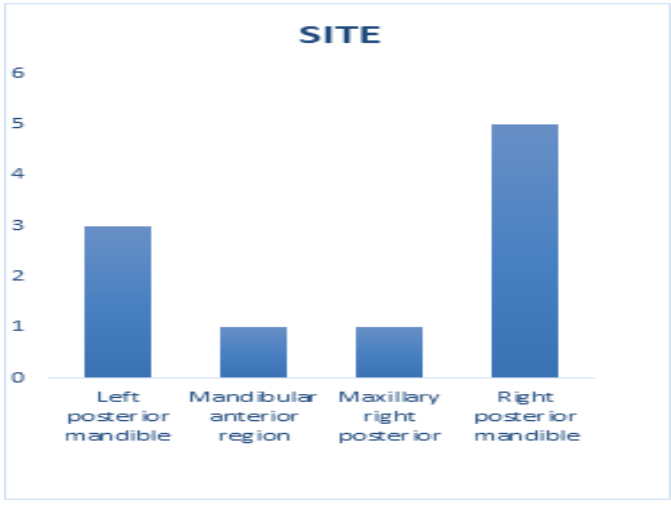

Figure 5: Site distribution.

There was moderate negative correlation (r = -0.429) between age and Ct value and the correlation was found to be statistically insignificant (p=0.397).

Discussion

This study has shown the presence of HCMV in the Ameloblastoma samples. Previous studies have proved the presence of HCMV in periapical lesions, radicular cysts and periodontitis cases.

Researchers have confirmed that macrophages and peripheral monocytes are the major sites where the HCMV resides.[8] As macrophages constitute a major part of the inflammatory cells' infiltrate in the inflammatory periapical lesions,[9] it might be conjectured that the presence of HCMV in the cyst wall is a result of consequence of infiltration by the infected macrophages. However, in a study by Andric et al. in 2007, it was found that HCMV was also present in the developmental cysts like odontogenic keratocyst (OKC), which was unexpected because these lesions did not show any signs of inflammation.[10]

Mohammed Amjed et al. in 2021 demonstrated that the presence of HCMV viral protein in Ameloblastoma (10 out of 16 samples) was more than in dentigerous cyst and OKC.[11]

The detection of the virus in the epithelial lining of ameloblastoma can possibly be explained by two hypotheses;

1)HCMV infected macrophages and monocytes may invade the tumour leading to the PCR recognition.[12]

2)The higher prevalence of HCMV in Ameloblastoma could indicate a possible role of the virus in the etio-pathogenesis.

HCMV's finding in the odontogenic epithelium can indicate an active virus involvement rather than just incidental detection. Evidences have conveyed that HCMV has role in tumorigenesis and on comodulation of tumours, it helps by inhibiting apoptosis, further promoting the survival of its host cells through encrypting different viral proteins.[13]Therefore we cannot disbar the role of HCMV and the fact that it helps the odontogenic epithelial cells of Ameloblastoma to survive and escape the apoptosis.

Conclusion

Although the incidental finding of HCMV cannot be excluded, but 60% of samples showing prevalence in the odontogenic epithelium of Ameloblastoma could indicate a possible role of the virus in the pathogenesis and progression of the tumour. More investigations with larger samples are needed to explore the role of HCMV in the pathogenesis of various odontogenic lesions.

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