

Detection of Viruses in Dentin Caries and Necrotic Pulp Caries Using ELISA Technique in Mosul, Iraq

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Abstract

Background: Dental caries is a multifactorial disease that can affect human teeth, and viruses play a hidden role in forming caries cavities and dental plaque. **Objectives:** This study attempted to assess the detection of the role of some viruses such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus type1 (HSV-1), and human papillomavirus (HPV) using the ELISA technique to detect the presence of viral antigens from three samples (dentine caries, necrotic pulp, and saliva) and to assess the relation between the presence of viruses and gender and age. **Materials and Methods:** From March 2022 to October 2022, samples were collected from 90 patients which were divided into three age groups, such as >20 years, 21–40 years, and <41 years, at the Dental Teaching Hospital of the University of Mosul. These patients represented both genders (45 men and 45 women). **Results:** The results indicated that the prevalence of HSV-1 was 66.6%, CMV was 68.8%, EBV was 86.6%, and HPV was 16.0% out of 90. The percentages of these viruses HSV-1, CMV, and EBV were higher in females than in males at 60.2%, 58.1%, and 43.4%, respectively, especially in the age group 21–40 years, with dominant of HSV-1 at 50%. Many studies focus on the role of bacteria in different types of dental caries, while the hidden role of viruses is excluded. Viruses play an important role in forming dental biofilm, and failed therapy may be due to viral infection. **Conclusion:** This study concludes that viruses in this study were detected in higher numbers in dentine caries, necrotic pulp, and saliva by ELISA technique, except human papillomavirus 16, which did not give any result.

Keywords: CMV, ELISA technique, Epstein-Barr virus, HSV-1, Human papillomavirus, oral cavity

INTRODUCTION

The majority of oral disorders, including dental caries, affect 2.5 billion people globally, including 573 million children. It is a complicated condition affected by environmental, genetic, behavioral, and microbiological variables, all of which are important in the emergence of caries.^[1] A sophisticated ecosystem exists within the mouth, diverse in its niches inhabited by Archaea, fungi, and viruses in addition to bacteria. A few viruses and various bacteriophages have been found in oral cavities, and the role of oral viruses is little understood, despite the importance of bacterial and viral interactions with the entire oral microbiome.^[2] Viruses may be a part of dental biofilm formation and may be associated as a cause and progression of dental caries or necrotic pulp, without focusing on bacteria as the main cause only.^[3]

In dental practice, viral infections of the oral tissues are frequently seen; nevertheless, diagnosing difficulties and management receive little attention. Tumor development is linked to specific viral infections.^[4] The human herpesvirus (HHV) family is made up of a wide range of DNA viruses with the ability to live a lifetime latently after a first infection, latent HHVs can reactivate, especially in patients with immunosuppressive or incapacitating diseases, and either cause an open clinical infection or a subclinical infection with a low rate of viral replication.^[5] HSV types 1 and 2 are members of

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the HHV family but only share roughly 50% of their genomic sequences. The EBV infects B lymphocytes and oropharyngeal epithelial cells, and the CMV and HHV-5 establish latency in CD4 + T lymphocytes and establish latent infection in salivary gland cells, endothelial cells, macrophages, lymphocytes, and possibly in hematopoietic progenitor cells and others. Any HHV can cause asymptomatic subclinical or clinical infection with or without oral manifestations.^[6] HHVs are easily transmitted during active productive infection because, depending on the site of the virus's reproduction, viral particles are shed in saliva, vaginal fluids, urine, tears, and respiratory secretions. Following an initial oral HSV infection, the viruses ascend to the trigeminal ganglia, where they become latent, from peripheral nerve terminals close to the infected epithelium. When viruses are reactivated, they move into sensory neurons in the trigeminal ganglia and are discharged there. From there, they move into the mouth epithelium and proliferate, causing an infection that frequently progresses into viral lesions.^[7,8] One of the most prevalent viruses that affect oral mucosa and nearby skin is the HPV-16, a member of the Papillomaviridae family. Nevertheless, there is disagreement in the published reports regarding the incidence of different viruses, in particular, HPV-16, in various dental illnesses, which raises significant concerns about their existence and function in the oral cavity. In oral epithelium that has not undergone pathological alteration, the prevalence of HPV can range from 0.6% to 81%. The manner of collecting the specimens and the DNA detection techniques used is to blame for this disparity.^[9]

Even 40 years after its inception in the diagnosis of diseases, ELISA is still the most popular method with a great affinity for the amount of disclosure of Abs and all antigenically lively molecules. Around the world, ELISA is recognized as the technique that is used the most frequently in clinics and hospitals. Additionally, it has been frequently employed for the precise detection and measurement of biological substances.^[10]

Aim of the study

Many studies focus on the role of bacteria in different types of dental caries, while the hidden role of viruses was excluded. Viruses play an important role in forming dental biofilm, and failed therapy may be due to viral infection.^[11,12]

MATERIALS AND METHODS

Collection of samples

Ninety (90) samples were collected (30 dentine caries, 30 necrotic pulp, and 30 saliva) from the period from the beginning of March 2022 to the end of October 2022. The samples from dentine caries were taken by dental probe and by a paper point from the necrotic pulp, while the

sample of saliva was taking by spitting the patient in sterile container, and then, all three samples transferred to the viral transport media. Samples were taken in both genders (45 males and 45 females), and their age groups were as follows: >20, 21–40, and < 41 years, who have attended the Dental Teaching Hospital at the University of Mosul, in Mosul, Iraq.

ELISA-based viral detection

The detection of the four viruses, such as EBV, CMV, HSV-1, and HPV-16, using the ELISA technique was done in the Biology Department, College of Science, University of Mosul's Advanced Immunology Laboratory. The test was conducted in accordance with the manufacturer's guidelines. The ELISA kit uses the Sandwich-ELISA method, the run utilizing SunLong Biotech Co., LTD, China for ELISA Kit of EBV, CMV, HSV-1, and HPV-16.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patient's verbal and analytical approval before the sample was taken. The study protocol and the subject information and the consent form were reviewed and approved by a local ethics committee according to document number 4189 on February 2, 2022.

RESULTS

The present study was designed to evaluate the role of the four viruses such as HSV-1, CMV, HPV-16, and EBV in dentine caries (cariou lesion) and the necrotic pulp in Mosul City. The result of ELISA showed the prevalence of four viruses. All disorder samples are shown in Table 1.

The prevalence of each virus in different places, such as dental caries, necrotic pulp, and saliva, out of 30 samples for each virus is shown in Table 2.

Regarding the distribution of each of the three viruses, EBV, CMV, and HSV-1, in dentine caries, necrotic pulp, and saliva, HPV-16 was not present in all studied cases. The viruses EBV, CMV, and HSV-1 gave positive concentrations in dentine caries, necrotic pulp, and saliva

Table 1: Percentage of four viruses in oral samples

Viruses	Positive	Negative	%
EBV	78	12	86.6
CMV	62	28	68.8
HSV-1	60	30	66.6
HPV-16	0	0	0

that related to the factors of gender and age. The patient's age is divided into three groups as follows: the first group was under 20 years old, the second group was from 21 to 40 years old, and the third group was above 41 years old. In the current study, the percentage of the three viruses EBV, CMV, and HSV-1 were higher in females than in males (60.2%, 58.1%, and 43.4%, respectively), as shown in Tables 3–5. The prevalence of CMV, HHV-1, and EBV ranged from 60% to 100% of the world's population, with significant differences between wealthy and developing nations.

DISCUSSION

This study used the ELISA technique for the first time to detect the prevalence of viruses in dental caries, including dentin caries (cariou lesion) and pulpitis (necrotic pulp). There are no references on this topic that

can be compared to the findings under investigation, while in their studies, the researchers used molecular methods to detect different viruses in saliva or measured the antibody titers in saliva in several countries such as Iran and Turkey.^[13,14]

Li^[15] found that EBV DNA was substantially more prevalent in endodontic pathogens (43.9%), the percentage of measurable numbers of HCMV DNA in endodontic pulpitis patients was (15.9%), and the HSV-1 DNA was found to the low percentage in endodontic patients (13.4%). This result agrees with the current study in the detection of viruses in endodontic and necrotic pulp, and also results showed that ELISA results have a detection percentage which indicates that confirmed the ELISA is considered the most sensitive immunoassays available test, but the molecular detection by PCR is more accurate. The HHV-1, EBV, and CMV are between 60% and 100% of people worldwide, with considerable variances between developed and developing countries. South America, Asia, and Africa frequently have the lowest rates, followed by Western Europe and the United States.^[16,17] It is possible to deduce that there is a connection between HPV-16 and the incidence of periapical infection.^[18] While there was no association between high-risk oral HPV and self-reported periodontitis among Indigenous South Australians

Table 2: The prevalence of each virus in the cases of dentine caries, necrotic pulp, and saliva out of 30 samples

Virus diseases	EBV%	CMV%	HSV-1%	HPV-16%
Dentine caries	60	66.6	56.6	0
Necrotic pulp	100	76.6	73.3	0
Saliva sample	100	63.3	70	0

Table 3: Number and percentage of CMV presence and relationship with age and gender in different caries samples

Type of samples	Gender			Age			
	No (%)			No (%)			
	Female	Male	Total	>20	21–40	<41	Total
Dentine caries	11 (36.6)	9 (30)	20 (66.6)	7 (23.3)	8 (26.6)	4 (13.3)	19 (63.2)
No (%)							
Necrotic pulp	13 (43.3)	10 (33.3)	23 (76.6)	6 (20)	14 (46.6)	3 (10)	23 (76.6)
No (%)							
Saliva sample	12 (40)	7 (23.3)	19 (63.3)	7 (23.3)	6 (20)	6 (20)	20 (63.3)
No (%)							
%	58.1	41.9	–	33	46	21	–
Total positive		62			62		

Table 4: Number and percentage of HSV-1 presence and relationship with age and gender in different caries samples

Type of samples	Gender			Age			
	No (%)			No (%)			
	Female	Male	Total	>20	21–40	<41	Total
Dentine caries	10 (33.3)	7 (23.3)	17 (56.6)	3 (10)	8 (26.6)	2 (6.6)	13 (43.2)
No (%)							
Necrotic pulp	14 (46.6)	10 (33.3)	24 (79.9)	4 (13.3)	15 (50)	3 (10)	26 (73.3)
No (%)							
Saliva sample	12 (40)	7 (23.3)	19 (63.3)	9 (30)	7 (23.3)	5 (16.6)	21 (69.9)
No (%)							
%	43.4	40	–	26.6	50	16.6	–
Total positive		60			60		

Table 5: Number and percentage of EBV presence and relationship with age and gender in different caries samples

Type of sample type	Gender			Age			
	No (%)			No (%)			
	Female	Male	Total	Under 20	21–40	Above 41	Total
Dentine caries No (%)	11 (36.6)	8 (26.6)	17 (63.2)	4 (13.3)	7 (23.3)	7 (23.3)	18 (59.9)
Necrotic pulp No (%)	18 (60)	11 (36.6)	29 (96.6)	6 (20)	19 (64)	5 (16)	30 (100)
Saliva sample No (%)	18 (60)	12 (40)	30 (100)	16 (53.4)	8 (26.6)	6 (20)	30 (100)
Total (%)	60.2	39.7	–	33.3	43.5	23.1	–
Total		78				78	

according to Ali *et al.*^[19] The higher percentage according to age was to the group of middle age 21–40 years, HHV-1 was the higher one at 50%, and the incidence of primary HHV-1 infections usually occurs at an early age. The majority of HHV-1 infections occur during childhood and frequently cause itchy skin lesions or mucosal lesions, though they can also go undetected. HSV multiplies at the point of introduction, frequently in the oral or vaginal mucosa, infecting sensory nerve ends. HSV travels to the regional ganglia after entering the sensory nerve ends and develops latency there.^[20] The second percentage is in cytomegalovirus at 46%, and the last one was Epstein-Barr virus at 43.5%.

A recent study showed a higher percentage of herpes viruses in the oral cavity of pregnant or non-pregnant women. The high percentage in females in this study may be according to the OWH Trusted Source. Herpes can be spread through vaginal, oral, or anal gender, touching infected genitalia, or breastfeeding. If a baby comes into contact with an infected mother's sore childbirth, the mother may pass the virus to the unborn child due to hormonal changes in women or contamination with infected saliva.^[21]

Yodgorova *et al.*^[22] mentioned that HHV-1, which was identified as having higher percentages, is estimated to millions of persons with HHV type 1 infection at any place, translating to a global prevalence of 66.6% in 0–49 year old. van Boven *et al.*^[23] demonstrated successful outcomes with anti-EBV immunoglobulins. Children under the age of 10 had the highest proportion (34.0%), which was followed by people in the 21–40 age group. Although this outcome somewhat concurs with our study, it was based on a different sample type. Additionally, predicted reactivation rates rise from low levels in toddlers to 2%–4% annually in women over the age of 50 years,^[24] while^[25] the study of the immune response to EBV demonstrates how each response to initial exposure during early childhood and throughout life persists as a result of ongoing latent virus activation, which may lead to reactivation of latent EBV and an

increase in titer in patients as young as 6–7 years old as well as at age 17 years or possibly in older individuals. These results match the description provided.^[20,25] Thus, dental caries depend on the most popular diseases and open many lines to detect the microorganisms (either viruses or bacteria) that cause different types of tooth decay.^[26,27]

CONCLUSION

The current study concludes that viruses were detected in higher numbers of HHV-1, HCMV, and EBV in dentine caries, necrotic pulp, and saliva cases by the ELISA technique in Mosul, Iraq, while HPV was not detected in any site of the sample collection.

Conflict of interest

There are no conflicts of interest.

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