

Effect of periodontal surgery on the salivary Herpes simplex virus-1 levels-a pilot study

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ABSTRACT

Background and aim: Herpes simplex viruses (HSV) are one of the most important groups of human pathogenic viruses. The prominent characteristic of this virus is latency and the ability to reactivate. One of the possible factors for reactivation of this virus is dental procedures. The aim of this study was to evaluate the salivary level of Herpes simplex viruses, before and after periodontal (crown lengthening) surgery and its relation with age and sex.

Materials and methods: 30 HSV seropositive patients, who needed the crown lengthening surgery and accepted to cooperate in this research, were included as experimental group of this study. Unstimulated Saliva samples of the patients were collected in 1.5 ml micro-tubes, before and 24 h after the surgery, and were analyzed by Premix EX taq probe qpcr, using PCR real-time method.

Results: No significant statistical differences were observed in the salivary level of HSV before and after crown lengthening procedure ($p = 0.18$). However, the level of HSV in saliva after surgery was significantly higher than its level before surgery in women as compared to men ($p = 0.003$). The differences in virus level did not have any significant relationship with patients' age ($p = 0.9$).

Conclusion: It seems that periodontal (crown lengthening) surgery does not affect the level of HSV in saliva yet, but it could be one of the stimulators of increased HSV level after surgery in women as compared to men; but age does not play an important role in changes of level of virus before and after the surgery.

1. Introduction

Herpes simplex 1 and 2 (HSV-1 and HSV-2) are among the most important human pathogenic viruses.¹ HSV-1 is an alpha-herpes virus that is found almost everywhere. Serologic test of this virus is positive in 65% of adults over 70. HSV-1 causes oral and HSV-2 causes genital herpetic lesions.² These viruses are very common and contagious. HSV-1 is transmitted by infected saliva while HSV-2 is transmitted through sexual intercourse; however, it is possible for HSV-2 cultivation to be positive from oral lesions as a result of changes in the pattern of sexual activities. This virus can also be transmitted through skin scratches, but a healthy skin prevents it from infecting the body.^{3,4}

In most cases HSV-1 primary infection is presented sub-clinically and appears mostly in children and adolescent. The prodromal period

consists of fever, loss of appetite, weakness and myalgia. Oral pain leads to a decrease in food intake and the patient might be hospitalized due to dehydration.⁵ These viruses embed in neurons and go through latent phase, stimulants like stress and emotional pressure can reactivate the virus. It reaches the skin through neuron axons. The proliferation of the virus causes herpetic lesions. Herpes simplex virus can infect different parts of the body such as oral and pharyngeal lesions, inflammation of cornea and conjunctiva (keratoconjunctivitis), skin herpetic lesions, encephalitis and etc.⁶ Oral lesions appear as erythema, groups of vesicles or ulcers on keratinized mucosa of hard palate, attached gingiva and dorsal surface of tongue, a few days after primary signs and symptoms.⁴ Reactivation of the virus can cause shedding into saliva and oral secretions which is an important risk factor in transmission of the disease. Fever, UV, stress and menstruation are some common stimulants of virus

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reactivation.⁷ Another common stimulant is trauma that may occur during dental procedures, specially, in gingival manipulations.³ The reactivation of HSV leads to shedding into saliva, lesion formation or virus transmission, furthermore, gingival or periodontal pockets act as a reservoir of virus during recurrent herpetic infection periods.⁸ Sunde et al. claimed that anti-viral treatment in patients with recurrent periodontitis who had a high level of herpes virus in sub gingival area, has successfully decreased the number of viruses and improved periodontal condition.⁹ Miller et al. mentioned prophylactic valacyclovir is a way of reducing the number of EBV in patients' saliva after dental procedures.¹⁰

Since no similar studies have so far evaluated the level of HSV-1 in saliva after dental procedures, we aimed to evaluate the level of HSV-1 in the saliva of patients who took under crown lengthening surgery, before and after the procedure and it's relation with age and sex.

2. Materials and Methods

52 HSV seropositive patients who agreed to cooperate and needed crown lengthening surgery in one session, were included in this study. 30 patients agreed to have their second specimen taken, eventually. Patients with a history of systemic disease (diabetes, immunosuppressive disease, leukemia, AIDS, infectious and febrile diseases, etc.), taking medicine during past month (anti-viral, immunosuppressive or hormones, antibacterial mouthwash before or after surgery), during menstrual cycle and patients who received dental therapies during past two weeks were excluded from the study.

The whole procedure was explained to the participants and an informed consent was taken from all of them. Patients were prohibited from drinking water 1 h before sampling and they were waiting 30 min on dental unit. Patients spit in a sterile dish and 1.5 ml of non-stimulated saliva was taken from each patient after filling their documents (age, sex, etc.). Surgical procedure was then performed by a periodontologist. Second sample of the saliva was taken 24 h after surgery under the same conditions. Samples were collected in 1.5 ml microtubes and sent to virology laboratory of microbiology research center. The specimens were stored in -70 °C until use.

Laboratory Procedures.

The specimens were suspended in 600 ml of buffer (10 mM Trihydrochloride, 1 mM EDTA with pH = 8) and homogenized by vigorous mixing on a vortex. The viral DNA extracted and precipitated using InviTrap® Spin Blood RNA mini kit (Premix EX taq probe qpcr-TAKARA COMPANY-China) as per the manufacturer's instructions; the viral DNA was eluted in 100 µL of elution buffer. The extracted samples quantified using commercially available viral kits (Genome Diagnostics Pvt. Ltd., Hague, Netherland). For each virus the final volume setup of 25 µL Taq Man PCR (including 5 µL of specimen, 12.5 µL Mater Universal mixtures, 5 pmol primer, 4 pmol Taq Man probe) was adjusted. Limited PCR quantification was conducted using plasmid dilution (one-copy per mL). The evaluation process was as follows: 95 °C for 10 min, followed by 50 cycles of 94 °C for 10 s, 60 °C for 32 s, and 72 °C for 25 s. The reactions were performed in a 7500 RT-PCR system tool (Applied Biosystems, USA).

The data were processed by SPSS software (version 22), Wilcoxon, Mann-Whitney test and spearman's correlation were used to analyze the data and the level of significant differences was considered 0.05.

3. Results

Patients including 18 women and 12 men with an average age of 39 ± 6.36 (age range: 28–49) participated in the study (Table 1). HSV has been found in 27 saliva samples out of 30.

Table 1
Demographic details.

sex	Female	male
quantity	18	12
age	average age:39 ± 6.36 (age range: 28–49)	

The level of virus in saliva did not have any significant differences before and after surgery (p = 0.18).

Salivary level of HSV before surgery between male and female had not significant differences (p = 0.22), however the viral level before and after surgery was different between these two groups (p = 0.003). In fact, the level of virus before and after surgery had a relation with sex (Fig. 1).

The level of virus in women was significantly different before and after surgery (p = 0.01) and viral load was significantly increased after surgery but no important statistical difference was observed in men (p = 0.2) (Table 2).

The level of virus before and after surgery was not significantly related to the age of patients (p = 0.9).

4. Discussion

HSV in one of the most prevalent infections of oral cavity, and since it appears as vesicles and ulcer, it makes patient uncomfortable.¹¹ The recurrent type of this disease might appear only as shedding of virus into saliva (without any signs or symptoms) which it can act as the way of infection transmission during dental procedures.¹² There are many factors that stimulate HSV, one of them is the trauma from dental procedures and intra oral surgeries might change the level of virus and lead to the appearance of herpetic lesions.¹³

In this study, the level of virus before and after crown lengthening surgery as one of the stimulating factors that can traumatize the oral cavity was evaluated. No significant difference was observed in the level of virus before and after surgery eventually. Therefore, it seems that crown lengthening surgery does not have a significant influence in increasing the level of virus in saliva. Since this procedure is counted as a minor surgery, the low level of stress and trauma from this type of surgery may have not affected the level of virus after the procedure. Yet this result may have obtained because of insufficient samples and abnormal distribution of data.

The results of this study showed a significant relation between sex and enhancement in level of virus after surgery (p = 0.003). A significant increase was observed in the level of virus after surgery in women (p = 0.1). Considering that hormonal changes and stress are two factors that stimulate virus proliferation and both are more common in women than men, we can justify changes in the level of virus in women.¹⁴ According to this finding, the need for prevention of saliva infection or incidence of lesions in women after surgery is evident.

Since to the best of our knowledge, no studies have been done based on the relation of salivary level of herpes virus type 1 and dental procedures so far, we cannot compare our results with other studies.

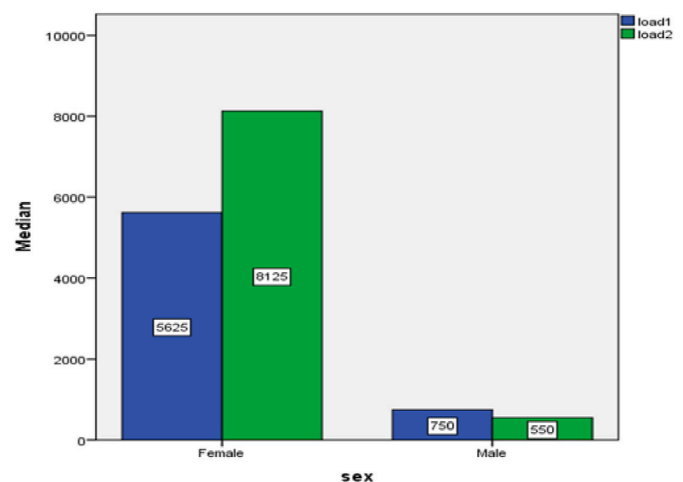


Fig. 1. Level of virus before and after surgery
Load1: virus level before surgery
Load 2: virus level after surgery.

Table 2
Level of virus before and after.

Viral level (median) sex	before surgery	Difference of viral level before and after surgery	P-VALUE
women	56/25	500	0/01 mention p value in decimal
men	750	-750	0/2

Nevertheless, we have compared the results of some researches with similar methods, here. Craig S. et al. (2004) evaluated the prophylactic effect of valacyclovir on presence of herpes virus DNA in human saliva after dental procedures, using PCR real time. They found that HHVs normally are present in the saliva of healthy people that they can facilitate transmission, and valacyclovir therapy could reduce the prevalence of EBV in saliva but it has little effect on HHV-6 and HHV-7.¹⁰ Craig et al. also had evaluated the level of virus in saliva after dental procedures but their result cannot be compared to ours since they had considered anti-viral therapies and different types of viruses have been evaluated.

In some other studies, researchers evaluated the level of different viruses in the saliva or tissues of patients with periodontitis.¹⁵ Some of them have mentioned a decrease in level of virus after periodontal treatments¹⁶ and others have mentioned a decrease in level of virus in saliva and periodontal indices after anti-viral therapies.^{17,18} The significant differences that have been mentioned in those studies cannot be compared to ours; Due to differences in methods, patients and treatment

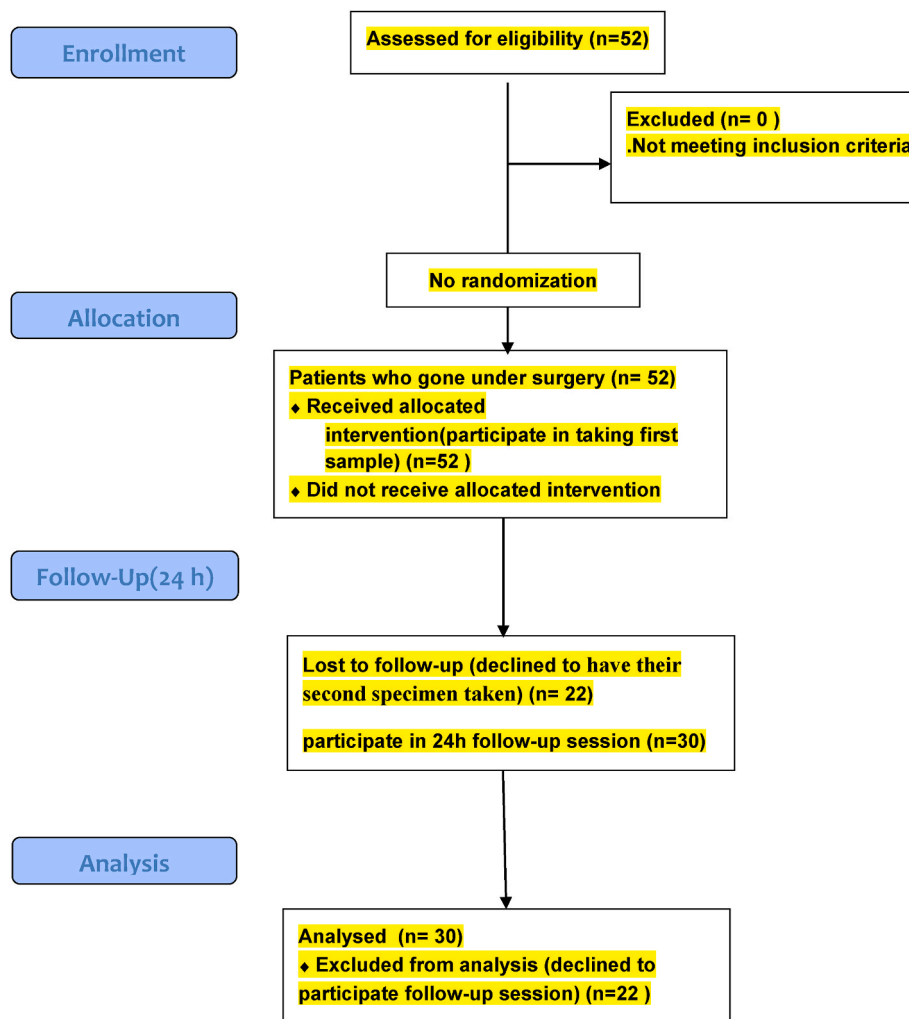
interventions.

This study was the first to evaluate one of the intra oral traumatic factors (crown lengthening), that have been known to stimulate the level of virus in the mouth precisely and quantitatively by using PCR real-time method.

Considering the limitations of this study like small sample size due to lack of patient cooperation (which led to loss of data), research's expensive costs and impossibility of sampling in longer periods (48 and 72 h after surgery), we suggest that future studies use newer and more precise kits and work on bigger sample sizes to appraise the virus. Also, evaluation of the virus in different time intervals after surgery in order to observe the changes in number of the virus will be helpful to determine the role of trauma from surgery in virus reproduction. age does not play an important role in changes of level of virus before and after the surgery.

5. Conclusion

Generally, the results of this study showed that crown lengthening procedure does not significantly change the level of virus in saliva; however, it was increased significantly in women after surgery. Therefore, the level of virus in the saliva is related to sex, but age does not play an important role in changes of level of virus before and after the surgery. Clinically, the result of this study suggests that women suffering from oral HSV lesions, could take anti-viral prophylactic treatment before crown lengthening surgery.



Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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