THE NEW ARMENIAN MEDICAL JOURNAL
Volume20 (2026), Issue 2 p.56-64

DOI: https://doi.org/10.56936/18290825-2026.20v.2-56

EVALUATING THE EFFECTS OF CIGARETTE SMOKING AND HEATED TOBACCO PRODUCTS ON THE ORAL MUCOSA AND PERIODONTIUM IN PATIENTS WITH HEPATIT C VIRUS IN ARMENIA: A PILOT STUDY

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Received 01.10.2025; Accepted for printing 14.05.2026

ABSTRACT

Since the last decades of the twentieth century, there has been an increase in the incidence of hepatit C virus. Currently, diseases of the oral mucosa and periodontium are an important problem in dentistry. Interest in the study of combined pathology in recent years is explained by the accumulation of new facts, such as cigarette smoking and harmful chemicals released during smoking, which have a negative impact on the health of the oral cavity. Tobacco consumption is a global public health problem. In addition, in recent years, new types of products such as heated tobacco products have emerged that are marketed as a means of harm reduction, it is important to note that in Heated Tobacco Products products, the tobacco is not directly burned. The aim of this study is to evaluate the impact of cigarette smoking and heated tobacco products on the oral mucosa and periodontium in patients with hepatit C virus. The study included 192 patients with hepatit C virus, who were divided into groups: Cigarete smoking with Hepatit C virus infection (main group, n= 96) and control group Hepatit C virus infection using heated tobacco products n=96. The average age of patients was 52.05 ± 12.25. A clinical examination of the oral cavity, including the status of the oral micosa and periodontium, was conducted. Hemorrhages on the buccal mucosa and hard palate were detected in 92.7% of the examined Cigarete smoking with Hepatit C virus infection patients (p<0.001). Hyperemia and edema of the gums were observed with a high degree of reliability (p<0.001) in the group of patients with Cigarete smoking with Hepatit C virus infection. Abundant growth of microorganisms was observed in almost all patients in the Smokers with Hepatitis C virus infection group P. gingivalis was cultured 89.1%, T. forsythia 93.5%; E. corrodens 87% and A. actinomycetemcomitans 95.7% cases which had a statistically significant difference (p<0.001) in data compared to the heated tobacco users with Hepatitis C virus infectiongroup. Thus, the oral mucosa and periodontium status of Hepatit C virus infection patients using the Heated Tobacco Products system were significantly better than that of the patients Hepatit C virus infection smoking cigarettes. In the Cigarete smoking with Hepatit C virus infection group, periodontopathogenic microorganisms were observed in 100% of pathological gingival pockets cases. Further studies with larger sample sizes are needed to better understand the oral health of hepatit C virus patients.

KEYWORDS: *Oral mucosa, periodontium, Hepatit C virus infection (HCVI), cigarette smoking, heated tobacco products(HTPs), heated tobacco users with Hepatit C virus infection (HHCV).*

CITE THIS ARTICLE AS:

AZATYAN V.YU., YESSAYAN L.K., SHMAVONYAN M.V., MURADYAN A.A. (2026). Evaluating the Effects of Cigarette Smoking and Heated Tobacco Products on the Oral Mucosa and Periodontium in Patients with Hepatit C virus in Armenia: A Pilot Study; 2026.20v.2-56

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Introduction

Since the last decades of the twentieth century, there has been an increase in the incidence of Hepatit C virus infection (HCVI) [*Azatyan VYu et al., 2022*]. According to World Health Organization (WHO), 71 million people are chronically infected with Hepatit C virus infection [*WHO, 2025*]. Currently, diseases of the oral mucosa and periodontium are an important problem in dentistry. This is due to the widespread prevalence of oral mucosa and periodontal lesions, the diversity of nosological forms and their relationship with systemic pathologies, difficulties in diagnosis and treatment, the success of which depends on the correctness of the diagnosis [*Iglesias-Sancho M et al., 2020; Luo Z et al., 2020*]. Interest in the study of combined pathology in recent years is explained by the accumulation of new facts, such as cigarette smoking and harmful chemicals released during smoking, which have a negative impact on the health of the oral cavity. Tobacco consumption is a global public health problem as more than a billion people from different ethnic groups and socioeconomic backgrounds smoke across the planet, with high prevalence of tobacco use observed in low- and middle-income countries [*Neuberger J, 2021; Le Foll B et al., 2022; Ankily MA et al., 2024*]. Tobacco consumption remains one of the most widespread health problems of humanity, causing millions of deaths every year, as its use is associated with a wide range of health complications such as lung cancer, cardiovascular diseases, respiratory diseases and oral health problems [*Varghese J, Gharde PM, 2023; Ankily MA et al., 2024*].

Despite the fact that cigarette smoking (CS) is one of the most common habits that have a negative impact on health, the advertising strategies of tobacco companies have contributed to its widespread use [*Nazir MA et al., 2019; Owens VL et al., 2019*]. In addition, in recent years, new types of products such as heated tobacco products (HTPs) have emerged that are marketed as a means of harm reduction [*Saravia I, Pisinger C, 2020; Kim SC, Friedman TC, 2022*]. It is important to note that in HTPs products, the tobacco is not directly burned. The temperature applied to the tobacco varies from ambient temperature to 330 °C - 350 °C, depending on the device [*Willemsen MC et al., 2015; Vukas J et al., 2023*]. Heated tobacco products products produce less sidestream smoke and less toxic combustion products than heated cigarettes, resulting in a reduction in chronic diseases associated with traditional smoking such as lung cancer, coronary heart disease, and chronic obstructive pulmonary disease [*Znyk M et al., 2021*]. The long-term risks of these alternatives remain controversial as the main harmful compounds are present in tobacco smoke emissions [*Ratajczak A et al., 2020; Upadhyay S et al., 2023*]. New hazards continue to emerge as the consumption of these alternative smoking methods continues to spread among populations around the world [*Znyk M et al., 2021; Luca A-C et al., 2023*]. Their effectiveness in promoting smoking cessation remains largely uncertain, and their impact on smoking initiation among young people is a matter of debate [*Czoli CD et al., 2020; Camoni N et al., 2023*].

Both forms of tobacco are associated with oral mucosa and periodontal diseases, with cigarette smoking worsening gingival health, increasing total gram-negative anaerobes due to chronic tissue hypoxia induced by nicotine, decreasing tissue blood flow, reducing the immune response to infections, increasing the release of inflammatory mediators due to frequent exposure to harmful and potentially harmful products from smoking, and impairing gingival healing [*Komar K et al., 2018; Jiang X et al., 2019; Zhang Y et al., 2019; Pouly S et al., 2021; Silva H, 2021; Yoshioka T, Tabuchi T, 2021*]. Cigarette smoking has been associated with benign mucosal diseases including periodontal diseases such as gingivitis [*Johnson NW et al., 2000; Calsina G et al., 2002; Moimaz SAS et al., 2009*].

Since cigarette smoking enters the body through the oral cavity, proper functioning of the oral epithelium is important as a first defense and requires a balance between proliferation, differentiation and desquamation or death of its cells [*Zanetti F et al., 2016*].

Although cigarette smoking exposure did not affect the cell cycle in oral keratinocytes, it altered the numbers of cells with early and late apoptotic features, indicating effects on oral epithelial biology [*Sever E et al., 2023*]. Moreover, smoking leads to xerostomia, decreased saliva production, and decreased salivary self-cleansing and acid-buffering capacity [*Kakoei S et al., 2021*].

Saliva is a complex biological fluid consisting of various electrolytes, peptides, glycoproteins, lipids and water. Its functions include protection of the oral mucosa, digestion, taste sensations and maintaining pH balance. It is the first biological fluid to come into

contact with tobacco, which causes structural and functional changes in it, expressed in a decrease in its quantity. The main component of tobacco is nicotine, which can stimulate taste buds, some cholinergic receptors, and blood flow to the salivary glands, causing changes in salivary secretion. Mechanical, chemical, and thermal stimulation of the salivary glands by cigarettes during smoking can cause a short-term increase in saliva. Some studies have shown a short-term increase in salivation as a result of stimulation, but the long-term impact of tobacco use remains unclear [*Petrušić N et al., 2015; Saputri D et al., 2017*].

There is evidence that long-term smoking is one of the external factors that reduces saliva production and causes hyposalivation. Hyposalivation can be caused by many factors, such as salivary gland diseases, various organic diseases, chemotherapy, radiation therapy, and can also be a side effect of various drugs [*Kauss AR et al., 2022; Kopa PN et al., 2020*]. Cigarette smokers often experience subjective feelings of xerostomia and halitosis. These oral health consequences highlight the importance of individual awareness of smoking-related complications and seeking support for smoking cessation to maintain oral health [*Myagmar-Ochir E et al., 2021*]. In addition, cigarette smoking significantly worsens liver damage and increases the risk of liver cancer in people with Hepatit C virus infection by promoting fibrosis and interacting with the effects of the virus. This combination creates a multiplicative risk of liver cancer, and smokers with Hepatit C virus infection often experience worse health outcomes. Therefore, concurrent tobacco cessation treatment and Hepatit C virus infection treatment are critical to maximise health benefits and prevent severe complications of chronic liver disease [*Cohen JF et al., 2024*].

Similarly, heated tobacco products, although perceived as a potentially less harmful alternative to traditional cigarettes, may also cause oral changes [*Haiduc A et al., 2020; Ye P et al., 2021*]. In addition, the heat generated by heating tobacco may damage oral organs by reducing saliva production and contributing to more intense lesions [*Patil PB et al., 2013*].

Consumption of heated tobacco products and cigarette smoking, although somewhat different in mechanisms and consequences, have a significant impact on oral health [*Wiesmann-Imilowski,N et al., 2024*]. Based on the above, we consider it appropriate to conduct this study.

The aim of this study is to evaluate the impact of cigarette smoking and heated tobacco products on the oral mucosa and periodontium in patients with viral hepatitis C.

Material and methods

The study included 192 patients with Hepatit C virus infection, who were divided into groups (Group I patients with main group (n= 96) cigarette smokers with HCV infection (SHCV)), Group II - patients with Hepatit C virus infection using heated tobacco products (HHCV) n=96 (control group)). The average age of patients was 52.05 ± 12.25 (mean ± SD), hospitalized in the period 2024-2025 in the infectious diseases clinic ‘’Mikaelyan Institute of Surgery’’ Yerevan State Medical University (Yerevan, Armenia), 129 (67.2%) men and 63 (32.8%) women. The final diagnosis of Hepatit C virus infection was established based on the detection of hepatitis C virus Ribonucleic acid (RNA) in the blood by polymerase chain reaction (PCR).

To assess the condition of the oral cavity and periodontium, a clinical examination was conducted, including an external examination of the corners of the mouth, an assessment of the condition of various areas of the OM and periodontium (color, relief, presence of pathological elements, consistency, bleeding, pathological gingival pockets, dental plaque), as well as the condition of the tongue (color, plaque, foci of epithelial desquamation).

Microbiological study: Microbiological studies were conducted from the pathological gingival pockets in 92 patients with Hepatit C virus infection. The material for the microbiological study was gingival fluid taken with sterile paper points from the pathological gingival pockets. The points were immersed in test tubes with 5 *ml* of nutrient broth and delivered to the laboratory for examination no later than 2 hours after taking the material. Microscopy was performed on the original material to assess the overall picture of the microflora. The diluted samples were seeded by the drop method on selective and differential diagnostic media: Endo agar, Chocolate agar, Sabouraud medium, 5% blood agar - (Casein Soybean Digest Agar with neutralizer and Tryptic Soy Agar with Lecithin and Tween. Lecithin, Tween®, UNSPSC Code: 41106231; Columbia agar with 5% Sheep Blood, Catalogue Numbers 63784, 12016409. Corporate

Headquarters: Bio-Rad Laboratories Inc. 1000 Alfred Nobel Drive Hercules, CA 94547 USA; Legal Entity / Contact Address: Bio-Rad Laboratories Pty Ltd u1A, 62 Ferndell Street, South Granville NSW2142 Australia). The incubation mode of microorganisms on dense selective and differential diagnostic media was adopted as follows: for Endo agar medium - 37 °C for 24 hours, Sabouraud - 37 °C - 48 - 72 hours, blood agar - 37 °C - 24 - 48 hours, respectively. For an approximate assessment of the quantitative growth of microorganisms in the association, it is advisable to use the following criteria: 104 -105 - moderate growth; 106 -108 - abundant growth (indicate a pathological process).

Study of cytokines in oral fluid (OF): Cytokines of the oral fluid were tested among 75 patients with HCVI. The test material was unstimulated mixed saliva – oral fluid, obtained without stimulation and collected with a sterile syringe into steril tubes. Samples were frozen and stored at –20 °C. The samples after were thawed at room temperature, centrifuged at 5000 rpm in the cold. Mucin was precipitated using 6 units of Lydase per 1.0 ml of oral fluid by patented method (Patent Republic of Armenia No. 3295 A dated at May 16, 2019) [Yessayan L, Azatyan V, 2019]. The concentration of proinflammatory cytokine IL-2 and antiinflammatory IL- 4 was determined by the method of solid-phase enzyme-linked immunosorbent assay (ELISA) using the Vector-Best test systems (Vector-Best JSC, Novosibirsk, Russia) and was registrated on a Statfax 303 Plus photometer (Awareness Technology, Inc. Palm City, FL 34990, USA).

Statistical analysis: Descriptive analysis (Mean ± SD for continuous and frequencies/proportion for categorical variables) were calculated for all variables of interest. Differences between two groups were evaluated using “chi-square” or “Fisher’s exact” tests for categorical variables and “Wilcoxon signed rank test” for continuous variables. Spearmen correlation was performed for determination of relationships between continuous variables. P-value was considered significant at <0.05 and <0.001 for highly significant results. Analyses were conducted using Excel 2013 and R software software and program Vassar Stats to calculate odds ratio (OR) and 95% confidence intervals (CI).

Results

The results of the clinical examination of the oral cavity, including the dental status of the oral mucosa and periodontium, are presented in Tables 1 and 2.

The frequency of detection of cracks in the corners of the mouth in the SHCV group was 53.1%, which was significantly and reliably higher than in the HHCV group (p<0.001). Disorders in the mucous membrane relief were observed in 91.7% of the SHCV group, which significantly exceeded the incidence of this indicator in HHCV patients (p<0.001). Hemorrhages on the buccal mucosa and hard palate were detected in 92.7% of the examined SHCV patients, which statistically differed significantly from the HHCV group (p<0.001). Examination of the tongue also revealed symptoms that were absent in the control group. The presence of coated on the surface of the tongue was found in 83.3% of the examined patients with SHCV, and foci of epithelial desquamation on the surface of the tongue were found in 19.8% (p<0.001).

Hyperemia and edema of the gums were observed with a high degree of reliability (p<0.001)

Table 1.

Clinical examination data on the oral mucosa in patients with Heated tobacco products and Cigarete smoking with Hepatit C virus infection groups

Sign	HHCV n=96		SHCV n=96		p-value*
	n	%	n	%	
Cracks in the corners of the mouth					
Absent	95	99	45	46.9	<0.001
Present	1	1	51	53.1	
Disorders in the mucous membrane relief					
Absent	94	79.9	8	8.3	<0.001
Present	2	2.1	88	91.7	
Hemorrhages on the buccal mucosa and the hard palate					
Absent	96	100	7	7.3	<0.001
Present	0	0	89	92.7	
Coated tongue					
Absent	96	100	16	16.7	<0.001
Present	0	0	80	83.3	
Foci of epithelial desquamation on the surface of the tongue					
Absent	96	100	77	80.2	<0.001
Present	0	0	19	19.8	

Notes: * *p-value test result from the comparison between HHCV and SHCV groups, SHCV – Cigarete smoking patients with Hepatit C virus infection, HHCV- Heated tobacco users with Hepatit C virus infection.*

Table 2.

Clinical examination data on the oral mucosa in patients with Heated tobacco products and Cigarete smoking with Hepatit C virus infection groups

Sign	HHCV n=96		SHCV n=96		p-value*
	n	%	n	%	
Hyperemia					
Absent	95	99	28	29.2	<0.001
Present	1	1	68	70.8	
Cyanosis					
Absent	71	74	63	65.6	>0.308
Present	25	26	33	34.4	
Edema					
Absent	82	85.4	27	28.1	<0.001
Present	14	14.6	69	71.9	
Looseness of gingival papillae					
Absent	95	99	79	82.3	<0.005
Present	1	1	17	17.7	
Bleeding					
Absent	86	89.6	23	24	<0.001
Present	10	10.4	73	73	
Subgingival dental plaque					
Absent	59	61.5	28	29.5	**<0.001**
Present	37	38.5	67	70.5	
Pathological gingival pockets more than 3.5 mm					
Absent	84	87.5	24	25	**<0.001**
Present	12	12.5	72	75	
Purulent discharge from pathological gingival pockets					
Absent	91	94.8	47	49	**<0.001**
Present	5	9	49	51	

Notes: *(*) p-value test result from the comparison between HHCV and SHCV groups, SHCV – Cigarete smoking patients with Hepatit C virus infection, HHCV- Heated tobacco userrs with Hepatit C virus infection*

in the group of patients with SHCV. The clinical picture of cyanosis was almost the same in both groups of observations and no statistical difference was observed (p>0.308). Looseness of gingival papillae in the group of patients with Cigarete smoking with Hepatit C virus infection was observed in 17.7% of cases (p<0.005). Bleeding gums were observed in the main observation group in 73% of cases (p<0.001). Subgingival dental plaque was detected in 70.5% of patients in the main group (p<0.001). pathological gingival pockets greater than 3.5 *mm* and purulent discharge from pathological gingival pockets in the Cigarete smoking with Hepatit C virus infection group were detected in 75% vs 51% with a high degree of statistical significance (p<0.001).

The results of the microbiological study of the material obtained from the pathological gingival pockets showed that in the group of patients with HHCV, abundant growth of microorganisms was not observed in cultures of all the listed microorganisms, with the exception of P. gingivalis in 1 (2.2%) case (OR=0.01), which had a statistically significant difference (p<0.001) compared to the group of patients with SHCV. Abundant growth of microorganisms was observed in almost all patients in the SHCV group, namely: P. gingivalis was cultured in 41 (89.1%) cases (OR=0.01); T. forsythia – in 43 (93.5%) cases (OR=0.01); E. corrodens – in 40 (87%) cases (OR=0.01) and A. actinomycetemcomitans in 44 (95.7%) cases (OR=0.01), which had a statistically significant difference (p<0.001) in data compared to the HHCV group (Table 3).

An immunological study of the proinflammatory cytokine IL-2 and antiinflammatory cytokine IL-4 levels in oral fluid is presented in Table 4.

A comparative analysis of oral fluid cytokine levels in the HHCV and SHCV groups revealed that the level of the proinflammatory cytokine IL-2 in the SHCV group significantly increased by 9.04 times (p<0.001). The level of antiinflammatory IL-4 decreased sharply in the SHCV group by 71.5 times (p<0.001).

Discussion

This small pilot study examined the effects of heated tobacco products and cigarette smoking on the oral mucosa and periodontium in patients with Hepatit C virus infection. According to the literature, HSV, relative to heated tobacco products, has a lower

Table 3.

Microbial association of pathological gingival pockets in patients with Heated tobacco products and Cigarete smoking with Hepatit C virus infection groups

Microogranisms	Levels	HHCV n=46	SHCV n=46	OR	p-value*
		n(%)	n(%)		
P. gingivalis	10^4 -10^5	45(100%)	5(10.9%)	1.21	<0.001
	10^6 -10^8	1(2.2%)	41(89.1%)	0.01	
T. forsythia	10^4 -10^5	46(100%)	3(6.5%)	1.79	<0.001
	10^6 -10^8	-	43(93.5%)	0.01	
E. corrodens	10^4 -10^5	46(100%)	6(13%)	2.01	<0.001
	10^6 -10^8	-	40(87%)	0.01	
A. actinomycetemcomitans	10^4 -10^5	46(100%)	2(4.3%)	1.64	<0.001
	10^6 -10^8	-	44(95.7%)	0.01	

Notes: *(*) p-value test result from the comparison between HHCV and SHCV groups, OR- odds ratio,SHCV – Cigarete smoking patients with Hepatit C virus infection, HHCV- Heated users with Hepatit C virus infection.*

Table 4.
Oral fluid cytokine levels in patients with Heated tobacco products and Cigarete smoking with Hepatit C virus infection groups (mean ± SD)

Cytokines	HHCV (n=30)	SHCV (n=45)	OR/MD	95% CI	p value*
IL2	2.75 ±4.98	24.87 ± 18.01	-22.12	[-28.32;-17.85]	<0.001
IL4	13.96 ±25.84	0.2±0.65	13.76	[4.43; 23.93]	<0.001

Notes: **(*)** *p-value test result from the comparison between HHCV and SHCV groups MD- mean difference, OR- odds ratio, HHCV-heated tobacco users with HCV infection*

content of potentially harmful and hazardous components and, therefore, a lower risk of developing smoking-related diseases compared to conventional cigarettes. To date, there are only a limited number of studies examining the effects of this alternative HSV on oral health, and further independent in vivo studies are needed [*Kaur G et al., 2018; Sever E et al., 2023*]. Currently, the global prevalence of conventional cigarette and HTP product use is higher among men. Awareness and use of Heated tobacco products products are steadily increasing. A large cross-sectional study of 7,714 people in Japan found that 5% of men and 2.2% of women used Heated tobacco products products. The authors concluded that young adults aged 20 to 30 years are more likely to smoke Heated tobacco products products than women and older adults. Our results partially support this conclusion, although in our study, 32.8% of women and 67.2% of men also use heated tobacco products products, the average age of which was 52.05 ± 12.25 years [*Myagmar-Ochir E et al., 2021*].

Cigarette smoking in patients with Hepatit C virus infection has a negative impact on oral health, mucosal integrity, and periodontal disease. According to the literature, inhaled components cause inflammatory and structural changes in the oral epithelium, as well as histopathological changes [*Zanetti F et al., 2017*]. Our studies are partially consistent with the literature data, namely, in patients with Hepatit C virus infection, cigarette smokers were found to have 92.7% hemorrhages on the buccal mucosa and the hard palate, disorders in the mucous membrane relief in the 91.7% cases. Among patients with Hepatit C virus infection who smoked cigarettes, hyperemia was detected in 70.8% and bleeding gums in 73% of cases.

According to Sever E. et al. (2023) cigarette smoke can affect the quantity and quality of saliva, and data on the effect of long-term smoking on the rate of saliva secretion are contradictory [Sever E et al., 2023]. Our study did not conduct research on the above-mentioned issue and does not have data for comparison.

Although several studies reported that cigarette smoking was associated with a higher incidence of Candida species and oral candidiasis, other studies, including the present study, failed to find an association between oral Candida load, traditional cigarette smoking, and tobacco heating system [*Vila et al., 2020*]. Our results revealed with statistical significance from pathological gingival pockets in cigarette-smoking patients with HCV, abundant growth of *P. gingivalis* in 89.1%, T. forsythia in 93.5%, *E. corrodens* in 87%, and A. actinomycetemcomitans in 95.7% of cases.

Regarding the immunological study of oral fluid that we conducted in patients with Hepatit C virus infection, there was no data in the literature for comparison with our study results.

One of the limitations of the study was that despite the fact that the Hepatit C virus infection group had 192 participants only 75 of them from the Hepatit C virus infection groups (cigarette smoking and heated tobacco products) agreed to pass the test of cytokines of the oral fluid, and microbiological study from the pathological gingival pockets 92 participants.

Another limitation was that the initial raw data from which the Excel database was created was a paper-based registry; and as the data were not double entered in the process of the data transmission errors could have happened.

Conclusion

The oral mucosa and periodontium status of Hepatit C virus infection patients using the heated tobacco products system were significantly better than that of the patients Hepatit C virus infection smoking cigarettes. In the Cigarete smoking patients with Hepatit C virus infection group, periodontopathogenic microorganisms were observed in 100% of pathological gingival pockets cases. Further studies with larger sample sizes are needed to better understand the oral health of Hepatit C virus infection patients.

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THE NEW ARMENIAN MEDICAL JOURNAL
Volume20 (2026). Issue 2

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THE NEW ARMENIAN MEDICAL JOURNAL

Volume 20 (2026) Issue 2

The Journal is founded by
Yerevan State Medical
University after M. Heratsi.

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