

The Armenian SORT IT Course

Evaluation of IL-2, IL-10, IL-4 and γ -interferon levels in the oral fluids of patients with hepatitis C, B and HIV

Vahe Azatyan¹, Lazar Yessayan¹, Melanya Shmavonyan², Gayane Melik-Andreasyan³, Anush Perikhanyan⁴, Kristina Porksheyan⁵

¹ Department of Therapeutic Stomatology, Yerevan State Medical University (YSMU), Yerevan, Armenia

² Department of Infectious Diseases, Yerevan State Medical University (YSMU), Yerevan, Armenia

³ National Center of Disease Control and Prevention, The Ministry of Health of Republic of Armenia

⁴ Medical Affairs Department, FMD Contract Research Organization, Yerevan, Armenia

⁵ Nork Clinical Infectious Hospital CJSC, Yerevan, Armenia

Abstract

Introduction: Oral fluid cytokine levels can vary considerably during the onset of Inflammatory Periodontitis (IP) especially in people with hepatitis C virus (HCV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Aim of our study was to evaluate levels of oral cytokines during the onset of IP among HCV, HBV and HIV negative and positive individuals in order to evaluate local immunity state during these infections.

Methodology: This was a case control study with 3 groups of virally infected individuals and control group. All had IP including control group. Results: 45 patients (51.7%) had HCV, 18 (20.7%) HBV and 24 (27.6%) HIV. For IL-2 we received significant difference for all groups compared with control -2.83; HBV-31.1 ($p < 0.001$), HCV-25.99 ($p < 0.001$) and HIV-24.57 ($p < 0.001$). For IL-10 significant difference was observed between control -0.94 and HCV-3.63 ($p = 0.027$), HBV-8.38 (15.51) groups ($p < 0.001$). IL-4 was significantly higher in control group 14.29 compared to HCV 0.2 ($p < 0.001$) and HIV 0.21 ($p = 0.037$) group. The adjusted analysis where we consider age as possible confounder revealed that only IL-2 significantly differs for all groups compared with control group: control vs HCV ($p = 0.001$); control vs HBV ($p = 0.024$); control vs HIV ($p = 0.004$).

Conclusions: Evidence for significant differences when comparing oral fluid cytokines of individuals with HCV, HBV and HIV with non-viral individuals was more obvious for IL-2. IL-2 levels were significantly higher in all 3 groups vs non-viral group even when age is confounder.

Key words: Oral fluid; cytokines; HIV; HCV; HBV.

J Infect Dev Ctries 2019; 13(5S):069S-074S. doi:10.3855/jidc.10919

(Received 05 October 2018 – Accepted 13 February 2019)

Copyright © 2019 Azatyan *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

400 million people in the world live with hepatitis C and B infections and 1.4 million people die every year from complications of viral hepatitis in the world [1,2]. Number of people living with HIV as of 2017 was about 3400, incidence number was 0.06 (0.04-0.08) per 1000 uninfected population [3]. Armenia is a country with income below the average and has 3-5% prevalence of HCV among the general population. For this indicator Armenia is on the 3rd place among the post-soviet countries. The prevalence of HBV in Armenia is 2% [4]. Based on recent data it demonstrates an increasing trend [5]. Symptoms of infection can be recognized by dental health-care workers in oral cavities of infected individuals [6].

The introduction of viral antigens and antibodies through the oral fluid as an alternative to venipuncture has led to many researches. In comparative study by

Ravi *et al* aimed to evaluate the presence of hepatitis B surface antigen in saliva and its sensitivity and specificity through Enzyme Linked Immunosorbent Assay (ELISA) methodology, the authors concluded that use of oral fluid samples makes it a satisfactory alternative to a number of very sensitive and specific serologic tests, since the possibility to detect immunity using body fluids can be easily done by self-collection, and will facilitate the investigation, the follow-up of outbreak and the surveys of immunity in representative samples of the general population. Several studies showed that saliva and oral fluid can be effectively used for large-scale HBV detection [7-9]. Another study results revealed that screening for active HCV infection, testing oral mucosa transudate (OMT) is a safer, noninvasive, easy to use alternative. In most of the cases, sensitivity and specificity of oral fluid testing do not considerably differ from paired serum testing

and oral fluid considered an important biological sample for HCV antibody testing [17–19]. Based on literature review we found that oral fluid represents a valuable source for identification of cytokines involved in IP and immune functioning [10,11,12-18]. Concurrently with the development of testing methods several studies explored sensitivity of proinflammatory salivary cytokines. The periodontal changes for HCV patients occur more frequently and more visible than for healthy control group. Several pathological changes boost proinflammatory cytokines production [19,20]. Periodontal disease occurs when the ecological balance of the oral cavity is disrupted and periodontal bacterial pathogens start triggering an inflammatory reaction. Subsequently the inflammation becomes chronic and causes the periodontal tissues' dissolution. Pathological mechanism of the disease can be influenced by impairment of the patient's immune system. This not only causes a shift in oral bacterial species but also creates an inadequate inflammatory reaction [12,21,22].

Periodontal diseases are prevalent both in developed and developing countries and affect about 20-50% of global population [23]. Periodontal disease is a component of the global burden of chronic disease, and chronic disease and periodontal disease have the same essential risk factors. In addition, severe periodontal disease is related to poor oral hygiene and to poor general health (e.g. the presence of diabetes mellitus, chronic infections and other systemic diseases) [24]. The spreading of various forms of parodontitis was revealed with almost 70% of the examined among Armenian population [25].

There are several publications highlighting associations between severity of PI and infections [22,26]. Awareness of the increased prevalence of periodontitis associated with HIV, HCV and HBV infection among infected patients and health-care professionals could significantly improve oral health and quality of life of infected patients.

The aim of our study was to evaluate the levels of oral cytokines, specifically IL-2, IL-4, IL-10 and γ -interferon during the onset of IP among negative and positive for HCV, HBV and HIV individuals in order to evaluate the local immunity state during these infections.

Methodology

Study Design

A case control study using oral fluids of virally positive and negative individuals with IP.

Study settings

This research study was conducted in Stomatology Policlinic #1 of Yerevan State Medical University after M. Heratsi.

Study Population and sampling

HCV, HBV and HIV patients were enrolled from Nork Clinical Infectious Hospital. Healthy volunteers were enrolled from Stomatology Policlinic's database. They were individuals with HCV, HBV, HIV admitted for IP treatment and individuals with negative for HCV, HBV and HIV again admitted for IP treatment. No special method was applied for the sample size determination. All consecutive patients admitted in 2017 and who gave their agreement were included in the study.

Data collection Instrument

For analysis we harvested non-stimulated mixed saliva (oral fluid) by means of sterile syringe. The samples were frozen at -20 °C degree. Before the analysis the samples were thawed back to the ambient temperature and centrifuged by the laboratory technician. The content of cytokines was determined by the method of solid-phase enzyme-linked immunosorbent assay (ELISA) "Vector-Best" (Vector-Best JSC, Novosibirsk, Russia) test systems and photometer registration StatFax 303 Plus (Awareness Technology, Inc. Palm City, FL, USA).

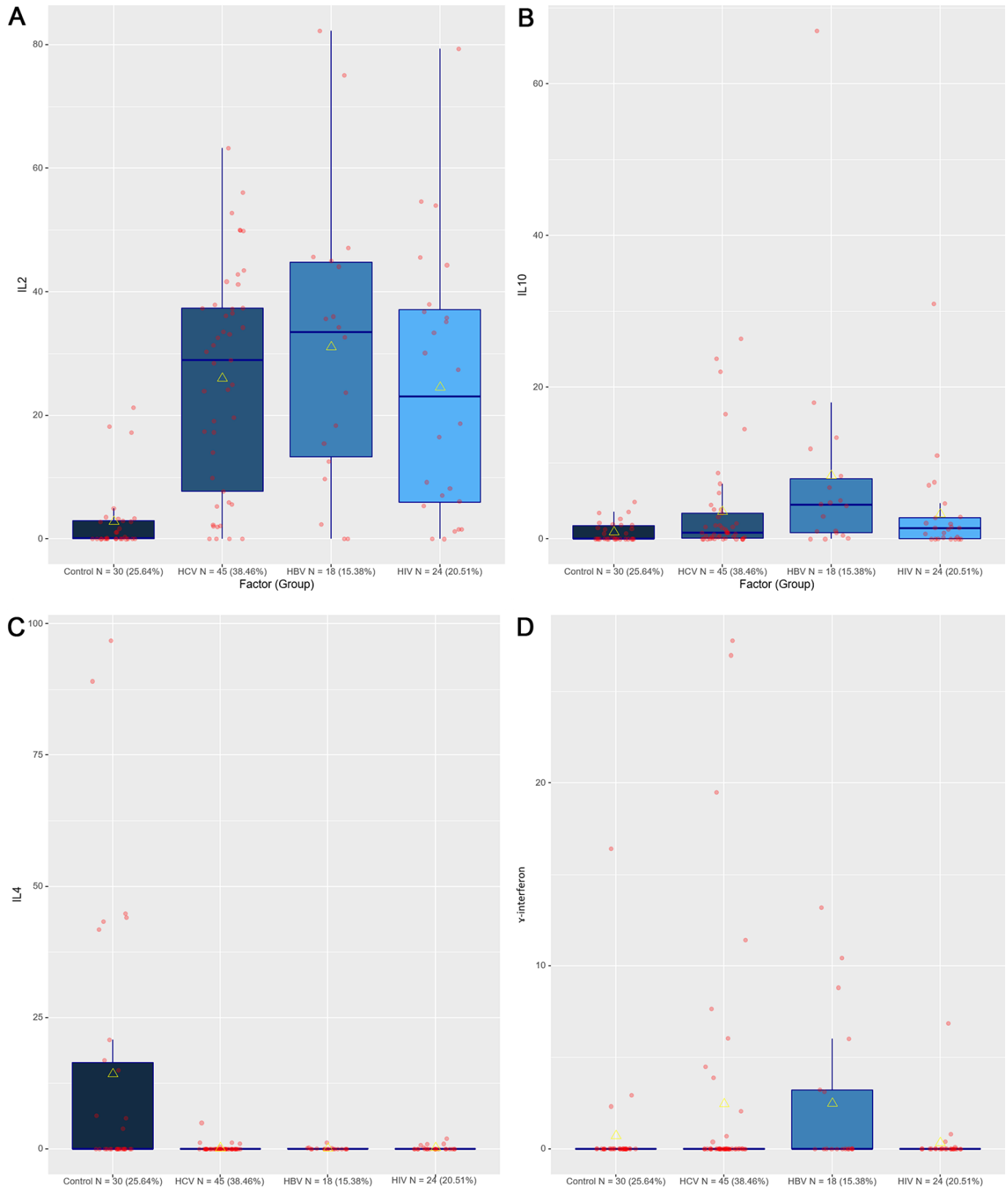
Statistical analysis

Descriptive analysis (Mean \pm SD for continuous and frequencies/proportion for categorical variables) were computed 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. Spearman correlation was performed for determination of relationships between continuous variables. Analyses were conducted using Excel 2013 and R software.

Ethics

This study was approved for conduct by the Institutional Review Board of Yerevan State Medical University after M. Heratsi. Informed consent was obtained from each participant.

Figure 1. Oral fluid cytokine levels (IL-2, IL-4, IL-10 and γ -interferon) of HCV, HBV, HIV and control groups.



X axis- groups: Control n = 30 (25.64%), HCV n = 45 (38.46%), HBV = 18 (15.38%), HIV = 24 (20.51%), Y axis -1) IL2,2) IL-10,3) IL-4,4) γ -interferon.

Results

Descriptive Data

This study included 87 virally positive patients with IP and 30 patients with IP (control group), but virally negative. From 87 virally positive patients 45 patients (51.7%) had HCV, 18 (20.7%) HBV and 24 (27.6%) HIV. Considering cytokines reactivity, they can vary considerably and show nonparametric distribution: IL-2- 20.55 (\pm 20.40); IL-10-3.60 (\pm 8.12); IL-4-3.80 (\pm 14.46) and γ -interferon- 1.59 (\pm 4.73).

Comparative Analysis

The mean age was significantly different between the groups: control -26.93 (\pm 6.81); HCV-50.33 (\pm 14.17); HBV-43.17 (\pm 13.04); HIV-45.58 (\pm 10.38) ($p < 0.001$). However, correlation analysis between age and cytokines did not reveal any significant relationship between both ($p > 0.05$ for all cytokines analyzed in each group separately). In terms of gender distribution, all 4 groups were homogenous: Males in control-23 (77%); in HCV-30 (67%); in HBV-14 (78%); in HIV-20 (83%) ($p > 0.05$).

The mean level of IL-2 was significantly higher in all 3 groups compared to the control group -2.83 (median = 0.09); HBV-31.1 (median = 33.47) ($p < 0.001$), HCV-25.99 (median = 28.95) ($p < 0.001$), HIV-24.57 (median = 23.03) ($p < 0.001$). In case of IL-10 significant difference was observed between control -0.94 (median = 0) and HCV-3.63 (median = 0.8) ($p = 0.027$) and HBV-8.38 (median = 4.5) ($p < 0.001$) groups. For control and HIV group 3.29 (median = 1.4) ($p = 0.063$) the difference was not significant. IL-4 was significantly higher in control group 14.29 (median = 0) compared to HCV 0.2 (median = 0) ($p < 0.001$) and HIV 0.21 (median = 0) ($p = 0.037$) groups. In HBV it was also decreased 0.11 (median = 0), but not significantly when compared to the control group ($p = 0.19$). In general, all significant differences in regards to cytokines were observed between control and HCV, HBV, HIV groups. Between HCV, HBV and HIV groups the difference was not highly significant, except for the marginal difference for IL-10 between HCV and HBV groups ($p = 0.049$). Figure 1 depicts the box plots

with means and medians of all cytokines in 4 groups. Statistical comparison with Means and odds ratio (OR)/p-values of cytokines are provided in the Table 1. All significant differences in regards to cytokine levels were observed between control and HCV, HBV, HIV groups.

Additionally, we have performed adjusted analysis to find the influence of age on cytokine levels between control and each viral group. The results showed that only IL-2 significantly differs when we consider age as confounder: control versus HCV ($p = 0.001$); control versus HBV ($p = 0.024$); control versus HIV ($p = 0.004$).

Discussions

Inflammatory periodontitis can cause significant functional disorders of maxillofacial area due to tooth loss. According to World Health Organization (WHO) reports the IP causes significant disorders 5 times more often than complicated forms of caries [23,27]. In the pathogenesis of IP both local and common causes are important, including those caused by lesions of internal organs and systems, pathology of the liver and biliary system is not an exception [27,28].

There are many research studies related to investigation of pro-inflammatory and anti-inflammatory cytokine levels in the oral fluid during different pathological disorders. Of note, these studies provide with disputable results. The data acquired from control groups is not an exception as it is difficult to establish standards for levels of cytokines in the oral fluid of healthy individuals. In general, there is a scarcity of research studies concerning the state of cytokines in oral fluids/saliva among patients with HCV, HBV and HIV. Therefore, for the first time in Armenia we aimed to investigate the oral state of cytokines particularly in patients with HCV, HBV and HIV.

Mean age of our participants in groups with HCV, HBV and HIV did not differ considerably as compared to the control group. This can be explained by the fact that it was almost impossible to select a control group within the age range of 40-50 years old without

Table 1. Oral fluid cytokine levels (IL-2, IL-4, IL-10 and γ -interferon) in HCV, HBV, HIV and control groups.

| Cytokine | Group Statistics | | | | | | |
|----------------------|--|--|-----------------|--|------------------|--|------------------|
| | Control N = 30 Mean/median (\pm SD) | HBV N = 18 Mean/median (\pm SD) | OR/ p value | HCV N = 45 Mean/median (\pm SD) | OR/ p value | HIV N = 24 Mean/median (\pm SD) | OR/ p value |
| IL-2 | 2.83/0.09 (5.67) | 31.1/ 33.47 (23.59) | -28.28(< 0.001) | 25.99/ 28.95 (17.86) | -23.17 (< 0.001) | 24.57/23.03 (21.58) | -21.75 (< 0.001) |
| IL-10 | 0.94/0 (1.33) | 8.38/4.5 (15.51) | -7.44 (< 0.001) | 3.63/0.8 (6.58) | -2.69 (0.027) | 3.29/1.4 (6.55) | -2.35 (0.063) |
| IL-4 | 14.29/0 (26.11) | 0.11/0 (0.3) | 14.18 (0.12) | 0.2/0 (0.79) | 14.09 (0.001) | 0.21/0(0.48) | 14.08 (0.037) |
| γ -interferon | 0.72/0 (3.04) | 2.49/0 (4.24) | -1.77 (0.04) | 2.46/0 (6.52) | -1.74 (0.11) | 0.34/0 (1.4) | 0.38 (0.561) |

discernible changes in the oral cavity. Infected and control groups were not comparable by age, however, in our opinion, this was reasonable, as based on the literature the composition of saliva usually changes with aging [29]. Considering the age influence we additionally performed adjusted analysis by categorizing individuals to 3 age groups taking into account that elderly are the individuals between 60 and 75 years old and young people are individuals below 45 age. We received that age appeared to be significant confounder for all cytokines, but not for IL-2. Therefore, we can assume that IL-2 can be used for has a distinctive informative value for determination of the state of the local immunity at individuals with HCV, HBV and HIV. However, more research is pending. In the reviewed literature regarding oral cavity cytokines we either didn't find any attestation of IL-2 significance or IL-2 was not the part of their objective as a cytokine of interest. However, those studies were focused on periodontitis rather than periodontitis in individuals with HCV, HBV and HIV diseases and similar study articles were not available for judgment of our study validity [16-18,30,31].

Research participants with HCV, HBV and HIV were included from Nork Clinical Infectious Hospital registry and with consideration of their agreement to participate, we encountered with limitation for larger sample selection.

Conclusion

Evidence for significant differences when comparing oral fluid cytokines of individuals with HCV, HBV and HIV with non-viral individuals was observed for IL-2. Anti-inflammatory cytokines IL-10 and IL-4 showed less predictable behavior: IL-10 significantly increases in viral hepatitis C and B, IL-4 decreases during HCV and HIV. Pro-inflammatory cytokine α -interferon increases during HBV only. Between to hepatitis groups the only significant difference was seen for IL-10. During HBV levels of IL-10 increased more than in HCV group. Levels of IL-2 were significantly higher in all 3 groups versus non-viral group even when age was included in adjusted analysis considering its confounding effect on oral fluid composition. Results will be important for the choice of tactics for complex treatment of IP at patients with HCV, HBV and HIV.

In respect of future steps it is recommended to take biopsy samples from patients 'oral mucosa and alveolar gum (after extraction of the tooth with medical prescriptions) involved in this research for immunohistochemical studies (definition of the

markers CD 20, CD 3), which will allow to have more detailed study of the local immunity state of oral cavity at patients with HCV, HBV and HIV.

Acknowledgements

The online lectures and materials of the Structured Operational Research and Training Initiative (SORT IT), a global partnership led by the Special Programme for Research and Training in Tropical Diseases at the World Health Organization (WHO/TDR), provided guidance for the authors to conduct this research and develop the manuscript(<https://www.theunion.org/what-we-do/courses/online-and-multimedia-training/sort-it%20>). The online materials were jointly developed by: WHO-TDR; the Operational Research Unit (LuxOR), Médecins Sans Frontières, Brussels Operational Center, Luxembourg; the Centre for Operational Research, International Union Against Tuberculosis and Lung Disease, France.

Authors' Contribution

Study concept and design: Vahe Azatyan, acquisition of data: Vahe Azatyan, Lazar Yessayan, Melanya Shmavonyan; analysis and interpretation of data: Vahe Azatyan, Gayane Melik-Andreasyan, Anush Perikhsyan, Kristina Porksheyanyan

References

1. World Hepatitis Alliance, World Health Organization (WHO), The Scottish Government (2015) Paving the way to eliminate viral hepatitis. Infographic. World Hepatitis Summit. Available: <https://www.who.int/hepatitis/publications/world-hepatitis-summit-infographic.pdf?ua=1>. Accessed: 4 September 2015.
2. World Hepatitis Alliance, World Health Organization (WHO) (2015) Glasgow declaration on viral hepatitis. Available: <http://www.who.int/hepatitis/publications/world-hepatitis-summit-infographic/en/>. Accessed: 4 September 2015.
3. Global Health Workforce Statistics, World Health Organization (2018) Global health observatory data repository. Available: <http://apps.who.int/gho/data/node.main.HIVINCIDENCE?lang=en>. Accessed: 6 June 2018.
4. Ghazinyan H, Melik-Andreasyan G, Asoyan A, Mkhitarian A (2014) Markers of hepatitis B and C viruses among population in Armenia. *Hepatol Int* 8 Suppl 1: 157.
5. Movsesyan T (2016) Establishing hepatitis C patients' surveillance system in the Republic of Armenia. Master of Public Health Integrating Experience Project. American University of Armenia, Yerevan. 73p.
6. Garbin CA, de Souza NP, de Vasconcelos RR, Garbin AJ, Villar LM (2014) Hepatitis C virus and dental health workers: an update. *Oral Health Prev Dent* 12: 313–321.
7. Ravi BS, Gurram Shankar Vidya (2014) Saliva as a diagnostic tool for hepatitis B infection-A comparative ELISA study. *IJSRP* 4: 1–3.
8. Hutse V, Verhaegen E, De Cock L, Quoilin S, Vandenberghe H, Horsmans Y, Michielsens P, Damme PV, Vlierberghe HV, Claeys F, Vranckx R, Oyen HV (2005) Oral fluid as a medium

- for the detection of hepatitis B surface antigen. *J Med Virol* 77: 53–56.
9. Arora G, Sheikh S, Pallagatti S, Singh B, Singh VA, Singh R (2012) Saliva as a tool in the detection of hepatitis B surface antigen in patients. *Compend Contin Educ Dent* 33: 174–176, 178.
 10. Laheij A, Kistler JO, Belibasakis GN, Välimaa H, de Soet JJ, European Oral Microbiology W (2012) Healthcare-associated viral and bacterial infections in dentistry. *J Oral Microbiol* 4: 10.
 11. Pallares C, Carvalho-Gomes A, Hontangas V, Conde I, Di Maira T, Aguilera V, Benloch S, Berenguer M, Lopez-Labrador FX (2018) Performance of the OraQuick Hepatitis C virus antibody test in oral fluid and finger stick blood before and after treatment-induced viral clearance. *J Clin Virol* 102: 77–83.
 12. Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnich J, Vernal R, Hernandez M, Gamonal J (2015) Host response mechanisms in periodontal diseases. *J Appl Oral Sci* 23: 329–355.
 13. Zein Elabdeen HR, Mustafa M, Ali R, Bolstad AI (2017) Cytokine profile in gingival crevicular fluid and plasma of patients with aggressive periodontitis. *Acta Odontol Scand* 75: 616–622.
 14. Zhang J, Zhang AM, Zhang ZM, Jia JL, Sui XX, Yu LR, Liu HT (2017) Efficacy of combined orthodontic-periodontic treatment for patients with periodontitis and its effect on inflammatory cytokines: A comparative study. *Am J Orthod Dentofacial Orthop* 152: 494–500.
 15. Javed F, Ahmed HB, Mikami T, Almas K, Romanos GE, Al-Hezaimi K (2013) Cytokine profile in the gingival crevicular fluid of rheumatoid arthritis patients with chronic periodontitis. *J Investig Clin Dent* 5: 1–8.
 16. Bostanci V, Toker H, Senel S, Poyraz O, Akpınar A, Gorgun EP, Bakar O (2017) Evaluation of IL-1 β , IL-1 α , and IL-10 levels and outcome of periodontal therapy in chronic periodontitis with familial Mediterranean fever. *Clin Oral Investig* 21: 469–475.
 17. Stadler AF, Angst PD, Arce RM (2016) Gingival crevicular fluid levels of cytokines/chemokines in chronic periodontitis: a meta-analysis. *J Clin Periodontol* 43: 727–745.
 18. de Lima CL, Acevedo AC, Grisi DC, Taba M (2016) Host-derived salivary biomarkers in diagnosing periodontal disease: systematic review and meta-analysis. *J Clin periodontol* 43: 492–502.
 19. Rhodus NL, Cheng B, Bowles W, Myers S, Miller L, Ondrey F (2006) Proinflammatory cytokine levels in saliva before and after treatment of (erosive) oral lichen planus with dexamethasone. *Oral Dis* 12: 112–116.
 20. Gheorghe DN, Foia L, Toma V, Surdu A, Herascu E, Popescu DM, Surlin P, Vere CC, Rogoveanu I (2018) Hepatitis C infection and periodontal disease: Is there a common immunological link? *J Immunol Res* 2018: 8720101.
 21. Meurman JH, Pyrhönen S, Teerenhovi L, Lindqvist C (1997) Oral sources of septicaemia in patients with malignancies. *Oral Oncol* 33: 389–397.
 22. Carrozzo M, Scally K (2014) Oral manifestations of hepatitis C virus infection. *World J Gastroenterol* 20: 7534–7543.
 23. Nazir MA (2017) Prevalence of periodontal disease, its association with systemic diseases and prevention. *Int Health Sci* 11: 72–80.
 24. Petersen PE, Ogawa H (2000 2012) The global burden of periodontal disease: towards integration with chronic disease prevention and control. *Periodontol* 60: 15-39.
 25. Kankanian AP (1996) The role of risk factors in the development of a hemoendothelial imbalance in periodontal diseases. *Stomatologia* 75: 21-25.
 26. Groenewegen H., Bierman WFW., Delli K., Dijkstra PU., Nesse W., Vissink A., Spijkervet FKL (2019) Severe periodontitis is more common in HIV-infected patients. *J Infect* 78: 171-177.
 27. Grudyanova AI (2009) Periodontal Disease. Medical Information Agency. Moscow. 339 p [Book in Russian].
 28. Dmitrieva LA (2007) Periodontology. National Guide. Moscow, 324 p [Book in Russian].
 29. Taba M, Jin Q, Sugai JV, Giannobile WV (2005) Current concepts in periodontal bioengineering. *Orthod Craniofac Res* 8: 292–302.
 30. Toker H, Gorgun EP, Korkmaz EM, Yuce HB, Poyraz O (2018) The effects of IL-10 gene polymorphism on serum, and gingival crevicular fluid levels of IL-6 and IL-10 in chronic periodontitis. *J Appl Oral Sci* 26: 1-7.
 31. PirimGorgun E, Toker H, Korkmaz EM, Poyraz O (2017) IL-6 and IL-10 gene polymorphisms in patients with aggressive periodontitis: effects on GCF, serum and clinic parameters. *Braz Oral Res* 31: e12.

Corresponding author

Anush Perikhanyan Pharm D, MPH, Project Manager
 FMD Contract Research Organization, Medical Affairs
 Department
 Komitas 1, 0033 Yerevan, Armenia
 Tel: +374 94811523
 Email: anush.peri@gmail.com

Conflict of interests: No conflict of interests is declared.