Saliva of Tobacco Smokers a Profile of C3, IgA, Amylase and Total protein

Zahraa Hussein M. Kadri[1], Hazima Mossa Alabassi[2], Ahlam Jassim Taher[2]

[1]Ass. Lecturer, [2]Ass. Prof., Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Iraq

Abstract

Objective: The present study aimed to shed light on the role of narghileh and cigarette smoking on immunity status of oral cavity by assess (C3 complement component, Immunoglobulin A, Total protein, α-Amylase and EBV IgG antibody).

Method: Saliva levels in two smokers groups the first include 28 narghileh smokers and the second include 32 narghileh and cigarette smokers as well as 30 non-smokers consider as control.

Results: As compared control, the levels of C3, IgA and total protein were significantly decreased, and the highest decreased was observed in saliva of narghileh and cigarette smokers, the result was (C3= 0.400 ± 0.194µg vs. 9.728 ± 3.561µg; IgA= 2.460 ± 0.492mg/dl vs. 5.048 ± 0.937mg/dl; Total protein= 170.20 ± 45.93mg% vs. 452.20 ± 136.57mg%, respectively) while the level of α-amylase was slightly dropped but with a non-significant, the result was (246.37 ± 122.47,243.56 ± 178.69 vs,213.51 ± 101.88) respectively.

Conclusion: The narghileh and cigarette can alter the microenvironment of oral cavity and influence the immunity of mucosa tissue, then increase the risky for many diseases such as blood hypertension, heart diseases, and lung diseases and may contribute to a variety of cancer.

Keywords: *Smoking, complement component, total protein, amylase, EBV.*

Introduction

Narghileh, argileh, hookah, shisha, hubble-bubble, water-pipe and goza all of these nomenclatures refer to the same way of smoking. Narghileh is an old habit that has been use for 400 years, created in Asia and India. Narghileh smokers are exposed to many carcinogenic compounds toxic materials like nicotine, carbon monoxide, heavy metals and many toxic materials depend on puffing time, number of puffs and smoke inhalation[(1)].

Smoke inhalation has also been associated with elevation in the total white blood cells count, platelet activation and increased expression of proinflammatory cytokines (IL-6 and TNF-alpha) in the bronchoalveolar lavage fluid. A study performed by researchers Al-sawalha and his coworkers in 2017 on a mouse model showed an association between narghile and airway inflammation and it raises blood pressure and heart rate so this may increase the risk of heart attack and stroke and may contribute to a variety of cancer, heart disease and lung diseases[(2)].

Saliva as obtained from the oral cavity is a mixture containing the contribution of the various salivary glands, the oral tissues microorganism, and ingested substance, optimum PH is 8.57. It has many important roles; the most important one is the protection of oral environment from the numerous microbes that constantly exposed to through our mouth. Plasma B cells reside in the salivary gland and produce IgA antibody which is the common saliva Immunoglobulin, proline-rich protein, cystatins lysozyme, salivary amylase, peroxidase, and cationic peptides (i.e defensine, lactoferrin, and cathelcidine). IgA is the major class of salivary antibodies which constitute about (80-90%) of the salivary antibodies[(3)].

Salivary lysozyme can influence human granulocyte and lymphocyte function it seem to be active against bacteria, fungi and exerts antiviral properties as well as may induce lysis of tumor cells[(4)], α-amylase is a

highly abundant protein in saliva that perform a direct inhibitory effect on the growth of certain bacteria [5], and may also exert virus inhibitory properties. α-amylase initiate the digestion process in oral cavity and modulate the bacterial growth and adhesion on intraoral surfaces and it is consider as a physiological marker [6]. As well as complement function can be enhanced when meet saliva at the gingival margin, the potential of the complement system by saliva may play a role in neutralizing certain viral infections on mucosal surface. In addition increase or decrease saliva biomarkers contribute directly to describing the oral health also for diagnosis and monitoring oral diseases or prognosis for many systemic diseases in the future [7].

EBV is an orally transmitted human herpesvirus that infect epithelial cells and establishes latency in memory B lymphocyte .EBV exhibit a biphasic life cycle it's dual tropism for B lymphocyte and epithelial cells, which allow the virus to be transmitted within oral lymphoid tissues. The ability of EBV to immortalize B cells and its prevalence in a subset of cancer has implicated EBV as a carcinogenic cofactor in cellular context where the viral life cycle is altered .Conferring malignant phenotype observed in EBV –positive cancer. Given that oral cavity serve as the main site of EBV residence and transmission [8].

The topic of this study is to evaluate the important saliva component that may contribute to maintain oral immunity in narghile smokers.

Materials and Method

Subjects and saliva samples: A total of 90 Iraqi (healthy males) aged (18-25) years. They were distributed in to two groups the first include 28 Narghileh smokers, the second include 32 Narghileh and cigarette smokers as well as 30 non-smokers (control). Individuals in the study were fasting and asked to drink water. After ten minutes saliva samples were collected by spitting into a sterile universal tube. The samples centrifuged for 10 minutes at 4000 RPM and collected saliva were frozen at -80°C until assessment[9].

C3, IgA and total protein were determined by means of Automated Biosystem A15 while α-amylase were determined in saliva using (Human α-amylase; K LAB) kit. EBV IgG antibody was determined by means of ELISA using commercially available kits (Human EBV VCA IgG; IMMUNOLAB GmbH).

Data Analysis: The statistical analysis was performed using SPSS version 13. Their data were given as mean ± standard deviation (SD.), and differences between means were assessed by ANOVA, and then followed by LSD or Duncan test A p-value of ≤0.05 was considered a significant.

Results and Discussion

Numerous studies related to oral health among narghile smokers have been done to evaluate the effect of cigarette and narghile smoking on oral health among the practice people particularly youth, our study is one of these studies which aimed to the same target.

In this study different salivary constituent were assessed in saliva of narghile and cigarette smokers these factor included (C3, IgA Ab, total protein, α-amylase and specific IgG EBV Ab). As shown in table 1, which reveal the saliva level of C3 complement component in the three understudying groups (Non-smokers; NS, Narghile smokers; NAS and Cigarette smokers; CS) respectively the result of mean ± SD. was (9.728 ± 3.561, 3.60 ± 3.082 and 0.400 ± 0.194μ/ml) respectively.

Table 1: C3 level in saliva of smokers and non-smoker

Groups	C3 (micrograms/ml)		
	Mean±SD	Minimum	Miximum
Non-smokers (No.= 30)	9.728 ± 3.561^{C}	4.80	15.00
Narghileh smokers (No.= 28)	3.608 ± 3.082^{B}	0.80	9.00
Narghileh and Cigarette smokers (Mo.=32)	0.400 ± 0.194^{A}	0.12	0.64

*Different letters represent significant difference between means in columns ($P \leq 0.05$).

The result revealed a highly significant decreasing among the three groups particularly between Non-smokers vs. (Narghileh smokers & Cigarette smokers) $p<0.05$. our finding is disagree with study performed by Frial G. at 2008 which recorded an increasing level for both (C3 & C4) complement component in smoker groups compared with control [10].

A study carried out by Kew (Kew et al. 1985) [11] which also recorded an increasing level for C3 complement component, this finding explained by Kew who said that the increasing of complement serum level may related to the incorporation between smoke and C3 so the thioester bond may be intact, then he suggested that aqueous whole cigarette smoke solution can modify C3 and activate the alternative pathway of complement system. Furthermore a study which performed by (Alabassi and Al Nadawi 2020) [12], revealed an elevation in complement component C9 serum level in asthmatic patients exposed to smoking the result was (118.08 ± 5.45 pg/ml) as compared to control (94.47 ± 6.29pg/ml) while patient not exposed to smoking recorded a significant ($p<0.05$) complement C9 serum level (104.61 ± 2.64pg/ml) as compared to smoke exposed control which recorded (80.07 ± 2.25pg/ml).

The conflict between the finding of the present study with previous studies may related to type of specimen used in our study, Frial [10], Kew [11] (carried out their study on serum sample while the present study was carried out by using saliva samples. And the explanation of decreasing C3 saliva level may contribute to the depletion of C3 in plasma and tissue as a consequence of the activation of alternative complement pathway of the narghile smokers. In regard to IgA saliva level, the present study recording a significant decreasing of IgA saliva level among the three understudying groups and the highest lowering in the third group (narghile and cigarette smokers). Table 2 -demonstrate the IgA saliva level in three groups (NS, NAS and CS). The mean ± SD. was (5.048 ± 0.937, 3.560 ± 0.038 & 2.460 ± 0.492 mg/dl) respectively. There was a significant decreasing among the three understudying groups $P\leq0.05$. This finding is agree with previous studies (Sahib and Radhi 2018) [13] which observed a decreasing IgA saliva level, this decreasing may related to the effect of tobacco that may lead to impairment of body immune function. In addition to the impairment of Ag mediating signaling of T cell leading to immunosuppression.

Table 2: IgA level in saliva of smokers and non-smoker

Groups	IgA (mg/dl)		
	Mean±SD.	Minimum	Miximum
Non-smokers (No.=30)	5.048 ± 0.937^{C}	4.00	6.11
Narghileh smokers (No.=28)	3.560 ± 0.0381^{B}	2.90	4.00
Narghileh and Cigarette smokers (Mo.=32)	2.460 ± 0.492^{A}	1.80	3.00

*Different letters represent significant difference between means in columns ($P \leq 0.05$).

Table 3: Total protein level in saliva of smokers and non-smoker

Groups	Total protein (mg%)		
	Mean±SD	Minimum	Miximum
Non-smokers (No.=30)	452.20 ± 136.57^{C}	212.00	600.00
Narghileh smokers (No.=28)	306.20 ± 70.872^{B}	194.00	411.00
Narghileh and Cigarette smokers (Mo.=32)	170.20 ± 45.93^{A}	117.00	221.00

*Different letters represent significant difference between means in columns ($P \leq 0.05$).

In respect to the total protein saliva level, and as shown in table 3, this research finding record a significant decreasing of saliva total protein among the three understudying groups the result was (306.20 ± 70.872 and 170.20 ± 45.93 mg%) respectively as compare to non-smokers (452.20 ± 136.57mg%). This finding was disagree with study carried out by (Zainulabdeen and Alak 2014) [14], which recorded a high elevating total

serum protein in smokers vs. non-smokers. The conflict between our results may relate to the type of specimen. Also the decreasing of saliva total protein is parallel to the decreasing of saliva constituent which recorded decreasing in this investigation.

As shown in Table 4 the saliva level of α-amylase enzyme recorded a non-significantly increasing among the three groups P≤0.05 and result was (213.51 ± 101.88, 246.73 ± 122.47 and 243.56 ± 178.69 U/L) respectively. This finding is agree with the previous research in which the reduction of amylase activity of serum narghileh smokers is most probably due to the interaction between smoke aldehyde and SH-groups of the amylase molecule (Singh et al. 2018) [15]. Another study recorded a non-statistically significant difference in salivary α-amylase level between smokers and non-smokers this study is agree with the present study. While Greabu and colleagues (Greabu et al. 2007) [16] indicated that exposure to cigarette smoke decreased salivary amylase activities.

Table 4: Alpha-amylase level in saliva of smokers and non-smoker

Groups	α-amylase (U/L)		
	Mean±SD	Minimum	Miximum
Non-smokers (No.=30)	213.51 ± 101.88^{A}	67.22	343.99
Narghileh smokers (No.=28)	246.73 ± 122.47^{A}	130.48	470.52
Narghileh and Cigarette smokers (Mo.=32)	243.56 ± 178.69^{A}	126.52	581.23

* Similar letters represent no significant difference between these means (P > 0.05).

The last finding of our investigation is the level of saliva IgG EBV antibody in narghile smokers. Table-5-revealed the+ve and –ve IgG EBV antibody in just two groups which were (NS & NAS) the result was obtained by using ELISA technique which revealed a slight distribution percent among the NAS. The result was (3+ve out of 27 –ve with distribution percent 11.11% for NS and 5+ve out of 23 –ve with distribution percent 21.73% for NS. A study performed by Chen and his coworkers (Chen et al. 2018) [17] who investigate the association between cigarette smokers infect with EBV and has nasopharyngeal carcinoma such pointed that cigarette smoking appear to be as risk factor of nasopharyngeal carcinoma (NPC) in people with+ve EBV IgA antibody but the association remain unclear. Furthermore Hodstrom and his coworkers (Hodstrom et al. 2020)[18] reported that smoking increases EBNA-1 antibody levels and acts synergistically with EBV infection to increase Multiple sclerosis risk.

Table 5: Positive and negative for EBV IgG in narghile smokers and non-smokers

Groups	**EBV IgG**	
	Saliva	**Distribution of EBV(%)**
Non-smokers (No.=30)	(3)+ve, (27)-ve	11.11
Narghileh smokers (No.=28)	(5)+ve, (23)-ve	21.73

Conclusions

- Based on our finding the percentage of EBV IgG Ab (21.73%) and despite the slight elevation compared to the non-smokers but still consider a risk factor of initiation the nasopharyngeal carcinoma.
- The lowering of immune constituent of saliva in oral cavity may enhance the reactivation of EBV infection that may induce the abnormal growth in nasopharyngeal tissue.
- Narghile smoking affect oral health, narghile and cigarette smoking can changing the microenvironment of oral cavity which may influence the mucosa tissue of oral cavity also may become a risk factor for many type of diseases

Acknowledgment: The author would like to introduce his thanks and gratefulness to College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Iraq

Conflicts of Interest: The author declares that there are no conflicts of interest.

Funding: There is no source of any funding

Ethic Statement: The researchers already have ethical clearance from all required institution and laboratories.

References

1. Qasim H, Alarabi AB, Alzoubi KH, Karim ZA, Alshbool FZ, Khasawneh FT. The effects of hookah/waterpipe smoking on general health and the cardiovascular system. Environmental Health and Preventive Medicine. 2019; 58: 1-17.
2. Al-Sawalha NA, Migdadi AM, Alzoubi KH, Khabour OF, Nidal AQ. Effect of waterpipe tobacco smoking on airway inflammation in murine model of asthma. Inhalation Toxicology. 2017; 29: 46-52.
3. Fabian TK, Hermann P, Beck A, Fejerdy P, Fabian G. Salivary Defense Proteins: Their Network and Role in Innate and Acquired Oral Immunity. International Journal of Molecular Sciences. 2012; 13: 4295-4320.
4. Ibrahim HR, Thomas U, Pellegrini A. A helix-loop-helix peptide at the upper lip of the active site cleft of lysozyme confers potent antimicrobial activity with membrane permabilization action. Journal of Biological Chemistry. 2001; 276: 43767-43774.
5. Shugars DC, Wahl SM. The role of the oral environment in HIV-1 transmission. Journal of American Dental Association. 1998; 129: 851-858.
6. Rohleder N, Nater UM. Determinants of salivary alpha-amylase in humans and methodological considerations. Psychoneuroendocrinology, 2009; 34: 469-485.
7. Ridha HSh, Kadri ZHM. Assessment of some immunological biomarkers in saliva and serum of Iraqi patients with chronic periodontitis disease. Journal of Pharmaceutical Sciences and Research, 2018; 10(11): 2998-3000.
8. Jiang R, Scott RS, Hutt-Fletcher LM. Epstein-Barr Virus Shed in Saliva Is High in B-Cell-Tropic Glycoprotein gp42. Journal of Virology. 2006; 80:7281-7283
9. Almerich-Silla JM, Pastor S, Serrano F, Puig-Silla M, Dasí, F., (2015). Oxidative stress parameters in saliva and its association with periodontal disease and types of bacteria. Disease markers, 2015: 653537. doi: 10.1155/2015/653537
10. Frial G. Studing of immunoglobulins, C3, C4 and IL-8 concentrations in serum smokers and non-smokers. Journal of Kerbala University. 2008; 6: 16-19.
11. Kew RR, Ghebrehiwet B, Janoff A Cigarette smoke can activate the alternative pathway of complement in vitro by modifying the third component of complement. Journal of Clinical Investigation, 1985; 75(3): 1000-1007. doi: 10.1172/JCI111760
12. Alabassi HM, Al Nadawi W. (2020). The complement component C9 and nitric oxide serum level in Iraqi patients with asthma. Annals of Tropical Medicine and Public Health, 23(10). DOI: 10.36295/ASRO.2020.231023
13. Sahib AM, Radhi NJMH. Salivary constituent in relation to dental caries among coffee-shop workers in Najaf city/Iraq. International Journal of Medical Research & Health Sciences. 2018; 7: 86-92.
14. Zainulabdeen JA, Alak SA. Effects of Smoking on Proteins Level and Alpha Amylase Activity in Sera of Iraqi Narghile Smokers. Al-mustansiriyah Journal of Science. 2014; 25: 33-40.
15. Singh S, Sharma M, Rohilla N, Salgotra V, Kumar V,Sharma RK. Assessment of Salivary Catalase, a-Amylase, and Cotinine Levels in Chronic Smokers: A Comparative Study. Journal of Contemporary Dental Practice. 2018; 19: 253-256.
16. Greabu M, Battino M, Totan A, Mohora M, Mitrea N, Totan C, Spinu T, Didilescu A. Effect of gas phase and particulate phase of cigarette smoke on salivary antioxidants.What can be the role of vitamin C and pyridoxine? Pharmacology Reports. 2007; 59: 613-318.
17. Chen Y, Xu Y, Zhao W, Xiao X, Zhou X, Lin L, et al. Lack of association between cigarette smoking and Epstein Barr virus reactivation in the nasopharynx in people with elevated EBV IgA antibody titres. BMC Cancer. 2018; 18(1): 190. doi: 10.1186/s12885-018-4110-6.
18. Hodstrom AK, Huang J, Brenner N, Butt J, Hillert J, et al. Smoking and Epstein–Barr virus infection in multiple sclerosis development. Science Reports. 2020; 10: 10960. doi: 10.1038/s41598-020-67883-w.