The Levels of Salivary IgA and Lactoferrin and Some Salivary Parameters in Waterpipe Smokers and Cigarette Smokers

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Abstract

Background: Waterpipe and cigarette are two types of tobacco consumption, their smoking is associated with many adverse effects it has a detrimental effect on saliva and causes a reduction in salivary pH and flow rate in addition to their controversial effect on salivary IgA and lactoferrin. **Materials and Methods**: unstimulated saliva was collected from 84 subjects in the early morning between 8-10 am. Subjects were divided equally into three group'swaterpipe smokers, cigarette smokers, and non-smokers all of the participants are adult male aged between 25-60 years, Sandwich ELISA technique was used for detection and estimation the level of salivary IgA and lactoferrin. **Result:** IgA and Lactoferrin results showed significantly($p \leq 0.01$), higher concentration in cigarette and waterpipe smokers group than non-smokers Results also showed that Salivary flow rate(SFR) and pH was higher in the non-smoker's group followed by waterpipe and cigarette smokers groups with a highly significant difference ($p \leq 0.01$), The correlation between Lactoferrin and salivary IgA also showed a significant ($p \leq 0.01$),moderate positive correlation in the cigarette group only. **Conclusions**:Smokingincrease Salivary IgA and lactoferrin concentrations while it reduces SFR and pH of saliva, a significant positive correlation was founded between lactoferrin and salivary IgA only in cigarette smokers

Keywords*: salivary IgA, lactoferrin, salivary parameters waterpipe smokers*

Introduction

Tobacco is a product obtained from the leaves of an annually-grown herbaceous plantknown as Nicotiana tabacum, there are twotypes of tobacco consuming either smoking like waterpipe, cigarette, and pipeand non-smoking chewing tobacco like kheni [1, 2].

Globally, there are about one billion men and 175 million women ages older than 15 are smoker currently, tobacco contains more than 7000 lethal chemicals compounds, including at least 70 known carcinogens compound that can damage nearly every organ in the human body [3],includecarbon monoxide, cadmium, cobalt, and polycyclicaromatic hydrocarbons [4]. A cigarette is the most favored product of tobacco used by about (82%) of tobacco consumers [5].

Another type of tobacco consumption is waterpipe which use has recently increased in popularity, and modern-day waterpipe smokers involve young teens, university students, and even high-school students [6]. Tobaccohas an adverse effect on saliva which consists of 99 %water while the remaining 1 % is composed of organic and inorganic molecules [7]. Saliva has important functions in the oral cavity which include maintaining a moist oral mucosa that is less susceptible to abrasion, neutralization of acids or bases, protecting against demineralization [8], it protects the oral mucosa from dryness and food debris, and it acts as alubricant during the process of mastication, it provides the fluid in which solid food

dissolves and distributesit to the locations of the taste buds (9).

Saliva also helps in the process of formation of plaque and protects the enamel of teeth at the same time [10, 11, 12]. Tobacco causes asignificant reduction in salivary pH and flows ratewith increased duration ofexposureand this increases the probability of more caries exposure [13, 14, 15]. As well as it hasmany adverse effects on both innate and adaptiveimmune responses (16).One of their implication was salivary IgA which is a dimeric (dIgA) composed of two IgA monomeric, secretory components (SC) and J (joining) chains [17]. sIgA neutralizing toxins and enzymes, preventing the binding of pathogens to mucosal surfaces and facilitating their removal in the mucosal layer [18], studies found that high-intensity caries is associated with increased levels of salivary sIgA which is combined with specific epitopes of cariogenic bacteria, resulting in a locally specific immune response [19].

Tobacco showed a controversial effect on salivary IgA levels, some revealed higher s-IgA in smokers in comparison to non-tobacco consumersmay be due to irritation of tobacco to mucosa so IgA defends these membranes from multitudes of soluble antigens [20, 21, 22]. While others studies showedthat the levels of IgA decrease in smokers when compared with non-tobacco users [23]. Another salivary parameter affected by tobacco is Lactoferrin (Lf) whichis an iron-binding protein.

LF is present in several mucosal secretions such astearsand saliva [24] Lactoferrin is a multi-functional compound due to its high affinity to ferric iron, which deprives microbes of the free iron required for their growth and its tendency to bind with microbial and target host cell surfaces. [25, 26]. Tobacco showed a controversial effect on salivary lactoferrin,whether an increased or decreased level [27, 28].

Materials and Methods

The subjects who participated in this study were 84 divided equally into three groups two smokers groups (waterpipe smokers, cigarette smokers) and one non-smokers group), all subject were adult male between 25 and 60 years old with a duration of smoking more than 5 years. Subjects with systemic diseases, such as diabetes mellitus, chronic heart disease, and acquired immunodeficiency syndrome or those who took antibiotics, within the last 3 months; or reported having periodontal treatment during the last 6 months had been excluded.

Unstimulated saliva was collected from subjects in the early morning between 8-10 am. Before collecting the saliva; the subjects were asked to avoid eating or drinking for three hours,the subjects were asked to spit saliva into the sterilized cups that possess graduationsin order to determine SFR (29). The pH was measured directly using a pH Meter to prevent any degeneration of the sample.

Determination of salivary Lactoferrin and salivary IgA was conducted by using sandwich ELISA technology, the ethical approval had been obtained from the College of Dentistry- University of Baghdad,

Statistical Analysis

Data were analyzed using SPSS (statistical package of social science) software version 25. In this study the following statistics were used:

Descriptive statistics: including means, medians, standard deviations, standard errors, mean ranks, minimum and maximum values, and statistical tables and figures.

Inferential statistics: including:

a) Kruskal-Wallis H test: to compare the measured variables among the groups.

b) Mann-Whitney U test: to test any statistically significant difference between every two groups.

c) Spearman's rank correlation coefficient test (r): to test the relation between the measured variables in each group.

Results

Table 1: The differences between, waterpipe smokers, cigarette smokers, and non-smokers groups considering salivary IgA, lactoferrin, and other salivary parameters.

Variables	Groups	Descriptive statistics		Group difference					
		Median	Mean Rank	KWH test	p-value	Groups		Mann-Whitney U test	p-value
Salivary IgA (µg /ml)	Control	69.90	20.875	36.181	0.000	Control	Waterpipe	132.5	0.000
	Waterpipe	153.15	47.518				Cigarette	46	0.000
	Cigarette	173.45	59.107			Waterpipe	Cigarette	273	0.051
Salivary Lactoferrin (µg /ml)	Control	0.87	28.589	15.103	0.001	Control	Waterpipe	202	0.002
	Waterpipe	1.09	45.571				Cigarette	192.5	0.001
	Cigarette	1.25	53.339			Waterpipe	Cigarette	288	0.088
SFR	Control	0.90	61.250	31.641	0.000	Control	Waterpipe	185	0.001
	Waterpipe	0.70	41.089				Cigarette	74	0.000
	Cigarette	0.50	25.161			Waterpipe	Cigarette	224.5	0.005
pH	Control	7.00	60.571	47.553	0.000	Control	Waterpipe	255	0.022
	Waterpipe	6.90	49.357				Cigarette	23	0.000
	Cigarette	6.60	17.571			Waterpipe	Cigarette	63	0.000

SFR= salivary flow rate, P≤0.05 Significant, P>0.05 Non-significant

Table 1 showed that IgA results in (µg /ml) had thehigher median values in cigarette smokers group (173.45) and waterpipe smokers group (153.15) followed by non-smokers (69.9) with a highly significant difference (p≤0.01), Mann-Whitney test clarified that even the median value of cigarette group was higher than waterpipe group but statistically there was no significant differences (P>0.05) between them.

Also, lactoferrin results in (µg /ml) revealed that the higher median values were in the cigarette smokers group (1.25) and waterpipe smokers group (1.09) followed by non-smokers (0.87) with a highly significant difference (p≤0.01), the median value of

cigarette group was higher than waterpipe group but statistically, there were no significant differences (P>0.05) between them,as clarified by Mann-Whitney test.

Whereas Salivary FR results presented that median values of the non-smoker's group (0.9)have a higher value followed by the waterpipe smokers group (0.7) and cigarette smokers group (0.5) with a statistically highly significant difference(p≤0.01)

finally, Salivary pH results showed that median values of the non-smoker's group (7.0)have a higher value followed by the waterpipe smokers group (6.9) and cigarette smokers group (6.6)with a statistically highly significant difference(p≤0.01)

Table 2: The correlation of salivary lactoferrin with salivary IgA

Variables		Groups		
		Control	Waterpipe	Cigarette
s-IgA	r	0.218	0.192	0.580
	P	0.266	0.327	0.001

P≤0.05 Significant, P>0.05 Non significant

Table:2 showed that the correlation of Lactoferrin with IgA was weakly positive in the non-smokers and waterpipe groups with non-significant statistical differences (P>0.05), while there was a moderate positive correlation in the cigarette smokers group with a highly significant statistical difference (p≤0.01).

Discussion

The results of the present study revealed that tobacco smoking in general increase salivary IgA as the median values of smokers groups was higher than non-smokers with a highly significant difference, as smokers have an IgA level that is about two-fold of that of non-smokers, this may be due to the stimulation effect of smoking on the mucosal immune system that drives to increase production of the s-IgA which protects these membranes against soluble antigens by preventing their adhesion to the surface of mucosal cells [21]. These results agree with previous research on IgA which stated that salivary IgA concentrations were significantly higher in tobacco smokers [20, 30] and disagree with the previous study which showed lower levels of IgA in smokers in comparison with the non-tobacco consumer [31], and other study said that there is no significant difference in the concentration of IgA between waterpipe smokers and non-smokers [32].

Many previous studies showed that waterpipe smoke and cigarette smoke contains many similar toxicants such as carbon monoxide, tar, and nicotine [33, 34], and this may explain why their IgA and lactoferrin levels were so close in this study. Lactoferrin results also showed that the smoker's group had highly significant median values in comparison with non-smokers groups this agree with other studies which found that both active and passive smoking has been associated with an increase in lactoferrin concentrations in human secretions [35, 36].

The results also showed that the median of SFR of non-smokers group had a higher level followed by the waterpipe smokers group and then cigarette smokers group as smoking diminished SFR, and this agrees with the results of previous researches which demonstrated that long-termconsumption of any type of tobacco is one of the risk factors for reducing SFR

[37, 38, 39]. Another study proved that smoking for a long duration causes a reduction in the secretion of saliva and changes their quality from serous to thick [40]. While it disagrees with a study that said that SFR does not affect bythe consumption of tobacco [41].

The present study also revealed that pH median was higher in control groups followed by waterpipe smokers group and then cigarette smokers group and this in consent with former studies [42, 43, 44] as the reduction of salivary pH of smokers may be due to the impairment of the salivary mechanism defense bytobacco usage which may eventually result in multiple mucosaland dental diseases [41] and also decreasing of bicarbonate secretion with decreasing in SFR may lead to alter salivary pH and turn it acidic that resulted in improvement in the growth of aciduric bacteria and creating uninhabitable conditions for the protective oral bacteria. This leads to a shift in the oral environmental balance to favor the growth of cariogenic bacteria which produce acid from sugar and further reduces the salivary pH [45, 13].

On the other hand, The correlation between salivary lactoferrin and IgA revealed a significant positive correlation in the cigarette smokers group, this could be due to the potential association between them against cariogenic bacteria, as in one previous study which showed a potential association between LF and s-IgA in a patient infected with human immunodeficiency virus [46], or could be due to their response to irritation caused by cigarette smoking, while there was a non-significant positive correlation between them in waterpipe smokers and non-smokers groups, this positive correlation agrees with the previous study done by Ide et al in 2016 demonstrated that even with a non-significant correlation between them, increase or decrease in value of one was associated with a slight tendency for the other to also be increase or decrease, respectively [47].

Conclusions

Waterpipe smoking and cigarette smoking diminished SFR and pH of saliva significantly,while salivary IgA and Lactoferrin concentrations were increased in the tobacco smokers groups, and a significant positive association was found between salivary IgA and lactoferrin levels on cigarette smokers only

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