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ORIGINAL ARTICLE

THE EFFECTS OF DUAL SMOKING ON PERIODONTAL HEALTH AND SALIVARY TUMOR NECROSIS FACTOR ALFA AMONG SUBJECTS FROM DUHOK CITY.

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ABSTRACT

Background: Smoking has been considered a risk factor for morbidity and mortality among both teenagers and adults. Waterpipe smoking has harmful effects on oral health, which might be similar to those of cigarette smoking. Dual smoking appears to be more deleterious on health than cigarette or Waterpipe smoking alone. The effects of dual smoking on clinical periodontal parameters and inflammatory biomarkers remain inadequately studied in our region.

Aim: The study aimed to evaluate the effects of dual smoking on periodontal health by the means of clinical periodontal parameters and a selected inflammatory biomarker.

Materials and methods: One-hundred and thirty six volunteered participants were involved in the study. A questionnaire was used to collect demographic data. Plaque index (PI), gingival index (GI), periodontal probing depth (PPD), and clinical attachment loss (CAL) were used to evaluate periodontal health. ELISA assay was used to measure salivary tumor necrosis factor (TNF-α). Statistical analysis was performed using the software IBM SPSS Statistics (Version 29.0.2).

Results: The current study discovered that the PI, PPD, and CAL indices were significantly higher among the dual smokers (DS), cigarette smokers (CS), and Waterpipe smoker (WPS) groups as compared to the non-smokers (NS) ($p< 0.05$). The level of salivary TNF-α of DS group was statistically higher than NS and WPS groups ($p< 0.05$), though salivary TNF-α level of DS group was higher than CS group; yet the difference was not statistically significant ($p>0.05$).

Conclusion: The current study concluded that dual smoking has more effects on clinical periodontal parameters than cigarette or waterpipe smoking alone. On the other hand, the level of salivary TNF-α among the dual smokers was statistically higher than the non-smokers which might be consider as a potential risk factor for periodontal disease.

Keywords: Dual smoking, periodontal health, salivary TNF-α, cigarette smoking, and waterpipe smoking.

INTRODUCTION

Consumption of tobacco represents a major threat to health, which exerts a huge burden on public health services [1]. Tobacco users have significantly higher rates of oral cancer, oral mucosal lesions, periodontal disease, dental caries, and implant failure than non-smokers [2].

Nowadays, waterpipe smoking (WPS) represents an emerging health issue worldwide, particularly in Eastern Mediterranean countries [3]. The spread and popularity of WPS among different communities might be attributed to several factors such as the misperception of its harmlessness or being less harmful than cigarette smoking, social acceptance, availability of various flavors, and the low cost of hookah consumables [4]. Psychological factors especially stress are among the other risk factors for the tendency

toward WPS [5]. In addition, evidences show that WPS brings happiness and euphoria for people; therefore, they use waterpipe (WP) to avoid sadness, stress, forget problems and feel happy [4]. WPS frequently practiced by men more than women; however, this habit is growing among females in some societies [3]. Clinical evidence has shown that oral inflammatory conditions such as periodontitis, peri-implantitis, and oral cancer [6,7]. Systemic diseases such as bronchitis, lung cancer, and cardiovascular diseases are more often manifested in waterpipe smokers than those who had never smoked [8].

Inflammatory biomarkers are measurable indicators in the body that can signal the presence and severity of inflammation [9]. Inflammatory biomarkers frequently used to detect or confirm presence of a disease or condition [10].

Concurrent use of waterpipes and cigarettes smoking appears to be more deleterious than cigarette or waterpipe smoking alone [11]. However, the effects of dual smoking on salivary inflammatory biomarkers and periodontal health indices remain understudied especially in Iraqi-Kurdistan region and whole Iraq.

The present study hypothesized that the different methods of smoking "cigarettes alone, waterpipe alone, and dual cigarette and waterpipe smoking" might directly or indirectly affect the inflammatory biomarkers secreted into saliva, thereby, affecting the oral mucosa and periodontal health status. The general research question guiding the present study is "Are there any differences in salivary TNF-α levels and periodontal health among cigarette smokers (CS), waterpipe smokers (WPS), dual smokers (DS), and non-smokers (NS)?"

2. MATERIAL AND METHODS

2.1 Ethical approval

The clinical aspect of the study protocol was approved by the Research Ethics Committee of Duhok university and Duhok Directorate general of Health (Reference number: 25012023-1-3) at the 25th of January 2023. The study was applied in accordance with the Declaration of Helsinki of 1975, as revised in 2013. Enrollment in the study was completely voluntary. Before acceptance and signing the consent form for participation, the study was explained in simple language to all candidates and all of them were welcomed to ask any question regarding the study. Upon acceptance and signing the consent form, all participants were informed that their data and identities shall be anonymized and that they can withdrew from the study at any time without consequences.

2.2 Inclusion and exclusion criteria

2.2.1 Exclusion criteria

Volunteered participants were excluded if they: Had less than 20 teeth, underwent periodontal therapy or surgery within the last 6 months, underwent major or minor oral surgery within the last 6 months, had hard or soft tissue oral swelling, undergoing orthodontic treatment, had any systemic disease or condition, took (or still taking) medications such as antibiotics, non-steroidal anti-inflammatories or steroids within the last 3 months, or Alcohol drinkers.

2.2.2 Inclusion criteria

Any volunteered participants, supposedly healthy, male participants, ranging from 18-45 years old. Started cigarette smoking, waterpipe smoking, or dual smoking habit before at least three years and continuing the habit until/while participating in the study. Cigarette smokers smoke 10 cigarettes or more per day within the last 3 years. Waterpipe smokers performed waterpipe smoking three times or more per week within the last three years. Dual smokers who smoked 10 cigarettes or more per day & performed waterpipe smoking three times or more per week within the last three years. Non-smoker subjects, any participants who had never smoked or used any tobacco products during their lives.

2.3. Study design

A cross-sectional study designed to analyze data obtained from clinical periodontal parameters and measurement of salivary TNF-α's levels collected from four study groups.

2.4 Recruitment of volunteered participant

Recruitment of participants commenced at February 2023, ended at January 2024. The first author, on behalf of the second, third, and fourth authors, carried out this procedure, after the team had set the inclusion/exclusion criteria and study protocol. Participants were selected among the out patients attending the clinics of the college of Dentistry/ Duhok University, as well as, customers of several cafes in Duhok city/ Iraqi-Kurdistan Region. Participants were selected according to the inclusion/exclusion criteria, dividing them into 4 groups based on their smoking status, including: cigarette smokers (CS), waterpipe smokers (WPS), dual smokers (DS) and non-smokers (NS). At the beginning, the first author met the candidates at the aforementioned sites, explained the study and its protocol to them verbally in a simple plain language, as well as, using brief participant information sheet if needed. Some of the candidates nominated friends or relatives who may fit the study criteria, and they gave the first author their numbers so he called them to briefly explain the study to them in a simple plain language, upon initial acceptance they were invited to the clinics of the Duhok College of Dentistry for further explanation of the study. Secondly, following initial acceptance by the selected participants, they were invited into an isolated dental clinic specified for the study at the Duhok College of Dentistry, then each participant filled and signed a consent form to participate voluntarily in the study, ensuring that each one of them may withdraw from the

study at any time without consequences.

On the same appointment for signing the consent form, the study protocol (procedure) was explained in details for each participant using a simple plain language, informing them about how to get ready for obtaining saliva samples, as well as, how to examine their mouths for the clinical periodontal parameters on a separate appointment for each participant. Additionally, each participant was informed about the harmful effects of smoking on oral and systemic health. The participants' identities and personal information were encoded and stored anonymously by the first author (figure 2.1).

2.5 The study groups

While carrying out the study, we have met hundreds of candidates, among which 136 subjects met the inclusive criteria, agreed to participate voluntarily and signed consent forms to join the study. Those 136 subjects were divided into four groups including: 34 participants as Cigarette smokers (CS) group, 34 as waterpipe smokers (WPS) group, 34 as dual smokers (DS) group (DS), and 34 as Non-smokers (NS) representing the negative control group.

2.6 Questionnaire

A study-specific and standardized questionnaire was developed and applied to gather information and data from each participant, giving each participant a personal code number for anonymous purpose. The study questionnaire included the following sections:

1. Demographic data (such as participant name, age, gender, occupation, phone number, and marital status).

2. Details about smoking and smoking methods (CS, WPS, DS, and NS), the smoking habit initiation age, duration, and number of times performing the habit per day or per week.

3. Medical history (such as general health status, history of medications, previous surgeries or admissions to hospital).

4. Past dental history regarding previous dental, periodontal and oral surgical treatments.

5. Oral hygiene status regarding details about self-care procedures such as age of brushing initiation, number of brushing per day, duration of brushing model. The study investigated 72h responses at concentrations at different fixed ratios *i.e.*, 1:1, 1:2, 2:1, 3:1, 1:3, 1:4, 4:1 respectively. The Bliss Independence model allowed quantitative classification of interactions as synergistic, additive, or antagonistic.

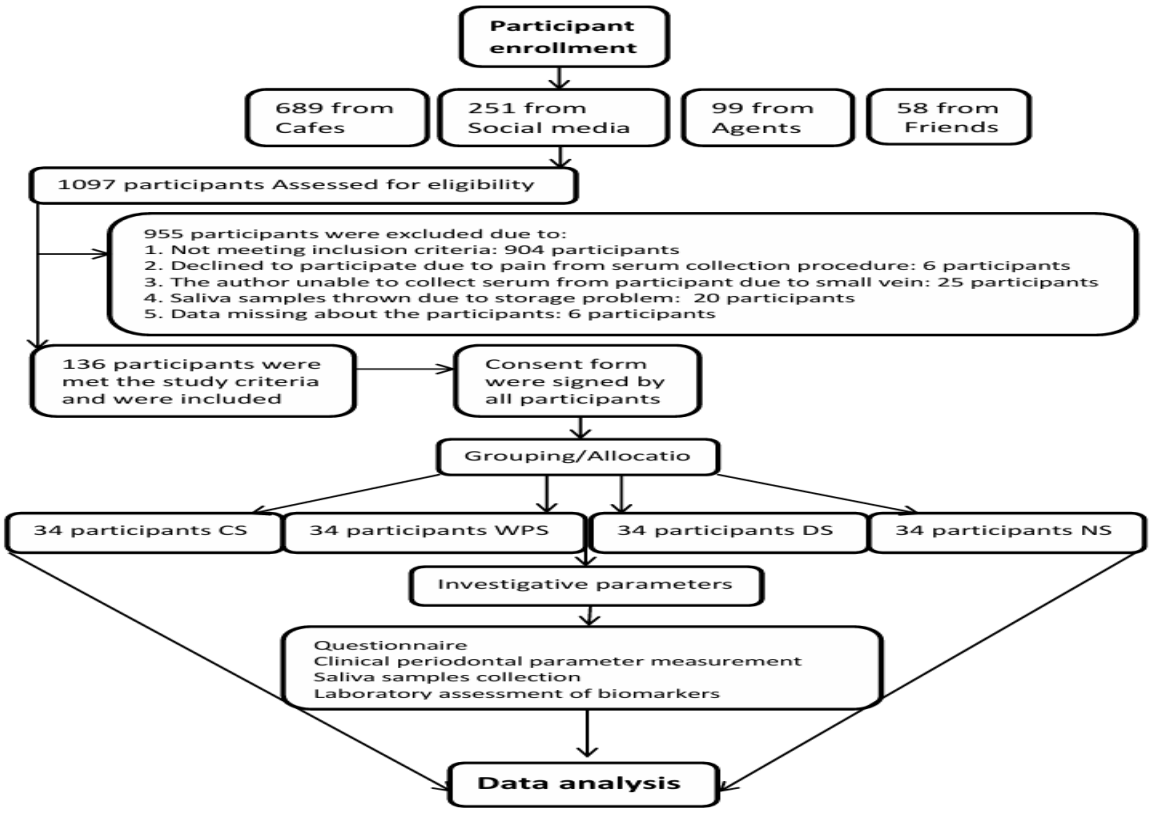

Figure 2. Participant enrollment, derivation of study groups and the study map.

2.7 The study samples

2.7.1 Collection of unstimulated whole saliva samples

Whole unstimulated saliva samples were collected from each volunteered participant. Before saliva sample collection, each participant was instructed not to eat and abstain from any oral hygiene for at least 2 hours prior to sample collection (12). All participants were instructed to rinse their mouths with water for 30 seconds at least 10 min prior to saliva collection to get rid of any remained food particles from last meal (13). With their head leaned forward, all participants sat motionless on a regular chair and in a quiet room (26). Each participant was left quietly to accumulate saliva passively in his mouth, and then the passively collected saliva was drooled passively or spitted into an ice-cooled graduated tube until 5 mL of saliva were collected (12). Saliva supernatants were obtained by centrifugation at 4,000 ×g for 15 minutes at 4°C using a centrifuge with thermostat (12). Then, 0.5 ml aliquots of the saliva supernatants were stored separately in Eppendorf tubes at − 80 °C until analysis (13).

2.7.2 Measurement of clinical periodontal parameters

The clinical periodontal indices (PI, GI, PPD, and CAL) were measured for all participants in the present study as followed by this study (14). CAL was measured on four surfaces (buccal, mesial, distal, lingual or palatal) for all teeth except the third molar (15, 16, 17), while; the PI, GI, PPD indices were measured in selected teeth of selected sites depending on Ramfjord teeth (18). Ramfjord (18) recommended the assessment of six 'index teeth' which later on known as the 'Ramfjord teeth'. These teeth are Maxillary right first molar, maxillary left central incisor, Maxillary left first bicuspid, Mandibular left first molar, Mandibular right central incisor and Mandibular right first bicuspid (18). Four areas of each tooth were selected (mesial, distal, buccal, and lingual or palatal) which make up the total circumference of the marginal gingiva of each tooth (15, 17). It could be felt that a thorough examination of the periodontal status using index teeth will make a valid basis for an evaluation of the periodontal condition of participant subject (18). The clinical periodontal parameters assessment was carried out for all participants, and the procedure was performed on dental chair under the standard conditions of light, using a disposable mouth mirror and dental probe, as well as, the periodontal probe CP15 University of North Carolina (19, 20). The thickness of plaque was recorded according to the PI (21) and given a score from 0–3. The gingival inflammation extent and severity was measured according to the GI (15), by thorough inspection with naked eye and gentle probing on gingival surfaces of the examined tooth and given a score from 0–3 (20). PPD was measured by gentle insertion of the periodontal probe from the gingival margin to the base of the sulcus (22, 6). CAL was recorded by measuring the distance from the cementoenamel junction to the base of the pocket using periodontal probe (6). The probing measurements were made by using a light force approximately 30 gram (23).

2.7.3 Assessment of salivary TNF-α

Tumor necrosis factor-α levels were measured in saliva samples according to manufacturer's instructions using the quantitative human TNF-α ELISA kit provided by Solarbio company/China (catalog number SEKH-0047).

2.8 Statistical analysis

Statistical analysis was performed using SPSS Statistics software version 29.0.2.0 (From IBM, USA). According to the normality test, the study data were not normally distributed ($p<0.05$, Shapiro-Wilk test), therefore, median and quartile ranges (Quartile1, Quartile3) were used to present the data, and non-parametric tests for comparisons. The Kruskal-Wallis test was used for comparisons among the groups with Bonferroni post-hoc correction and Mann-Whitney U test was used for comparisons between the groups. Level of significance was set at $p\leq 0.05$.

3. RESULTS

3.1 Demographic data of the study population

Statistical analysis of the study data revealed that the median age of all participants was 27.5 (23, 35) years, additionally the median age for each group was 32 for CS group, 28.2 for WPS group, 25.18 for DS group, and 34 for NS group, with no significant differences among and between the groups (Kruskal-Wallis and Mann-Whitney U tests, $p>0.05$) (Table 3.1). The smoking habit median duration for each group was 18 (8, 22.5) years for CS group, 9.5 (6, 13) years for WPS group, and 6 (4, 9) years for waterpipe smoking, 9 (3.7, 12) years for cigarette smoking in the DS group respectively (Table.1). The medians of habit frequency were 20 (20, 28) CS/day for CS group, 7(7, 10) WPS/week for WPS group, and 5.5 (3, 7) WPS/w for waterpipe smoking, 20 (20, 26)/d for cigarette smoking in the DS group respectively (Table 3.1). The one session median for each group was 2.5 (1.6, 3) minutes for CS group, 1.5 (1, 2.6) hours for WPS group, and 1.5 (1, 2) hours for WPS, 2.5 (1.9, 3.2) m for cigarette smoking in the DS group respectively (Table 3.1).

Table 3.1 Demographic data of the study population.

Study parameters	Study groups			
	CS	WPS	DS	NS
Participant numbers	34	34	34	34
Age of participants/ years	32	28.2	25.18	34
Habit duration/ years	18 (8, 22.5)	9.5 (6, 13)	WPS 6 (4, 9) CS9 (3.7, 12)	NA
Habit frequency	20 (20, 28) CS/d	7 (7, 10) WPS/w	WPS 5.5 (3, 7)/w CS 20 (20, 26)/d	NA
One session of CS or WPS	2.5 (1.6, 3) m	1.5 (1, 2.6) h	WPS 1.5 (1,2) h CS 2.5 (1.9, 3.2) m	NA

CS: cigarette smoking, WPS: water-pipe smoking, DS: dual smoking, NS: non-smokers, NA: Not applicable, w: week, d: day, h: hours and m: minutes.

3.2 Oral hygiene status and clinical periodontal parameters among study groups

Statistical analysis of the data obtained from the clinical periodontal parameters used in the present study (PI, GI, PPD, and CAL) revealed that the median PI for the groups were 1.5 (1, 2), 1.85 (1.2, 2), 1.5 (1.15, 2), and 1 (0.5, 1.5) for CS, WPS, DS & NS groups respectively, and that the PI for CS, WPS, and DS groups were statistically higher than that for the NS group (Mann-Whitney U test, $p<0.05$ respectively), however, there was no statistically significant difference of PI among and between CS, WPS, and DS groups (Kruskal-Wallis and Mann-Whitney tests, $p>0.05$) (Table 3.2).

The median GI for the groups were 1.5 (1, 2), 2 (1.37, 2.5), 1.75 (1, 2), and 1 (0.35, 1.5) for CS, WPS, DS and NS groups respectively, and that the GI of both WPS and DS groups were statistically higher than those of CS and NS groups (Mann-Whitney U test, $p<0.05$ respectively), yet there was no statistically significant difference of GI between CS and NS groups, as well as, between WPS and DS groups (Mann-Whitney U test, $p>0.05$ respectively) (Table 3.2).

The median PPD for each of CS, WPS, DS and NS groups were 2.5 (2, 3) mm, 2.5 (2, 3) mm, 2.5 (2.15, 3) mm, and 2.1 (1.5, 2.5) mm respectively. The PPD of both DS and WPS groups were statistically higher than that of the NS group (Mann-Whitney U test, $p<0.05$ respectively). Though, the median PPD of CS group was higher than that of the NS group yet the difference was not statistically significant (Mann-Whitney U test, $p>0.05$). There were no statistically significant differences in the PPD among and between CS, WPS and DS groups (Kruskal-Wallis and Mann-Whitney tests, $p>0.05$) (Table 3.2).

The median CAL for each of CS, WPS, DS, and NS groups were 0.7 (0.07, 1.15) mm, 1 (0.6, 1.2) mm, 1 (0.7, 1.2) mm, and 0.25 (0.0, 0.9) mm respectively. The CAL of CS, WPS, and DS groups were statistically higher than that of the NS group (Mann-Whitney U test, $p<0.05$ respectively), however, there was no statistically significant differences of CAL among and between CS, WPS, and DS groups (Kruskal-Wallis and Mann-Whitney tests, $p>0.05$) (Table 3.2).

The medians of starting age (initiation age) of tooth brushing for CS, WPS, DS, and NS groups were 13.5 (10.2, 18), 12.5, (10, 17.2), 12 (10, 16), and 13 (10.2, 20) years respectively. There was no statistically significant differences among and between the groups for the tooth brushing starting age (Kruskal-Wallis and Mann-Whitney tests, $p>0.05$) (Table 3.2).

Table 3.2 Oral hygiene status and clinical periodontal parameters among study groups:

Parameters	Smoking status			
	CS	WPS	DS	NS
Number of participants in each group	34	34	34	34
PI (0-3)	1.5 (1, 2)	1.85 (1.2, 2)	1.5 (1.15, 2)	1 (0.5, 1.5)
GI (0-3)	1.5 (1, 2)	2 (1.37, 2.5)	1.75 (1, 2)	1 (0.35, 1.5)
PPD in mm.	2.5 (2, 3)	2.5 (2, 3)	2.5 (2.15, 3)	2.1 (1.5, 2.5)
CAL in mm.	0.7 (0.07, 1.15)	1 (0.6, 1.2)	1 (0.7, 1.2)	0.25 (0.0, 0.9)
Starting age of tooth brushing	13.5 (10.2, 18)	12.5 (10, 17.2)	12 (10, 16)	13 (10.2, 20)
Frequency of tooth brushing/w	7 (3, 7.5)	7 (1, 7)	7 (1.7, 7)	7 (7, 12)
1 session of tooth brushing in m	2 (1, 3)	2 (1.3, 3)	1.5 (1, 2.5)	2 (1.5, 3)

CS: cigarette smoking, WPS: waterpipe smoking, DS: dual smoking, NS: non-smokers, PI: plaque index, GI: gingival index, PPD: periodontal probing depth, CAL: clinical attachment loss, w: week, & m: minutes.

The frequency of tooth brushing for each of CS, WPS, DS, and NS groups was 7 (3, 7.5), 7 (1, 7), 7 (1.7, 7), and 7 (7, 12) per week respectively. Again, there was no statistically significant differences among and between the groups for the tooth brushing frequency (Kruskal-Wallis and Mann-Whitney tests, $p>0.05$) (Table 3.2). Duration of one session of tooth brushing for CS, WPS, SHS, and NS groups was 2 (1, 3), 2 (1.3, 3), 1.5 (1, 2.5), and 2 (1.5, 3) minutes respectively. Once again, there was no statistically significant differences among and between the groups for the one session tooth brushing duration (Kruskal-Wallis and Mann-Whitney tests, $p>0.05$) (Table 3.2).

3.3 The salivary inflammatory biomarker (TNF-a) levels among smoking groups

The median levels of salivary TNF-α for CS, WPS, DS, and NS groups were 11.02 (9.8, 12.8) pg/ml, 10.61 (9.4, 11.7) pg/ml, 11.9 (10, 18.3) pg/ml, and 10.9 (10.5, 12.5) pg/ml respectively. The level of salivary TNF-α in the DS group was statistically higher than in the NS and WPS groups (Mann-Whitney test, $p<0.05$ respectively). Although the DS group TNF-α level was higher than that of the CS group, yet the difference was not statistically significant (Mann-Whitney tests, $p>0.05$) (Table 3.3).

Table 3.3 Salivary TNF-α level among the study groups:

Parameters	CS 34 participants	WPS 34 participants	DS 34 participants	NS 38 participants
Saliva TNF-α **in pg/ml**	11.02 (9.8, 12.8)	10.61 (9.4, 11.7)	11.9 (10, 18.3)	10.9 10.5, 12.5)

CS: cigarette smoking, WPS: waterpipe smoking, DS: dual smoking, NS: non-smokers, pg/ml: picogram per milliliter.

DISCUSSION

4. Discussion

Tobacco smoking is a preventable cause of morbidity and mortality worldwide. In recent years, hookah use increased mainly as an alternate tobacco smoking method, under the assumption of it being less harmful [24]. WPS is gaining popularity around the world, especially among young people and college students, which could have more serious adverse health impacts [25].

This cross-sectional study was the first in Iraqi Kurdistan region to evaluate the effects of dual smoking on periodontal health and the salivary inflammatory biomarker (TNF-α) in among dual smokers as compared to cigarette smokers, waterpipe smokers and non-smokers. In the present study, 136 volunteered participants met the study criteria and were

allocated in a four groups with 34 participants for each group. The sample size of the present study was decided in comparison to other relatively similar previous studies [26, 27, 28]. Due to challenges such as cultural norms and rarity of female smokers in the region society, the study participants were only male smokers and male non-smokers.

The present study hypothesized that the different methods of smoking "cigarettes, waterpipe, and dual smoking "cigarette and waterpipe smoking" might directly or indirectly affect the salivary inflammatory biomarkers thereby, affecting the oral mucosa and periodontal health status.

The PI of each of CS, WPS, and DS groups was statistically higher than NS group ($p<0.05$); however, there was no statistically significant difference of PI among CS, WPS, and DS groups ($p>0.05$). The high PI scores of CS, WPS, and DS in the present study indicated that the different smoking methods affect the PI and that the PI scores were concomitant with those of previous studies [6, 16, 26, 27] who also found high plaque accumulation among smokers. The PPD and CAL scores of CS, WPS, and DS groups of the present study were statistically higher than those of the NS group ($p<0.05$), a finding that is concomitant with that of previous studies (16, 26) who also reported high PPD and CAL among smokers as compared to non-smokers. Obviously most of the periodontal parameters were worse in the CS, WPS, and DS groups as compared to the NS group, although the differences in starting age of tooth brushing and the frequency of tooth brushing of CS, WPS, DS, and NS groups were statistically not significant ($p<0.05$), in other words, the age of starting brushing and brushing frequency were apparently similar in all study groups, a finding suggests that smoking methods worsen periodontal health status as compared to non-smokers.

The level of salivary TNF-α of DS group was statistically higher than both NS and WPS group ($p<0.05$ respectively); on the other hand, though the salivary level of TNF-α of DS group was higher than CS group, yet the difference was not statistically significant ($p>0.05$). Though Khan et al., (11) analyzed plasma and urine TNF-α; their study founded high levels of TNF-α in plasma and urine of waterpipe and dual smokers, a finding which might support the present study as regard to the higher levels of TNF-α in saliva of dual smokers, as well as, other smokers as compared to non-smokers. Hence, the salivary TNF-α results support the study hypothesis that different smoking methods including dual smoking has an impact on salivary levels of TNF-α, thereby affecting oral and periodontal health status negatively.

The most important strengths of the present study were; it was the first study in Iraqi Kurdistan Region with such a set of inclusion/exclusion criteria, well-designed study protocol and procedures to determine the effects of dual smoking as compared to other smoking methods and non-smoking on periodontal health and salivary levels of TNF-α. Among the limitations of the current study is that it was carried out on male participants only, therefore, it might be scientifically better to carry out such a kind of clinical studies on both male and female participants. Other potential weakness point of the present study was the participants' age range which was relatively young. Accordingly, future studies should include a more diverse and a wider age range study samples over extended periods.

5. CONCLUSIONS

The current study concluded that dual smoking has more adverse effects on periodontal health than cigarette or waterpipe smoking alone. On the other hand, the level of salivary TNF-α among dual smokers was statistically higher than the other smokers, as well as, the non-smokers, which might be considered as a risk factor for oral and periodontal diseases. Hence, dual smoking might have the double adverse effects of cigarette or waterpipe smoking alone.

DECLARATIONS

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Declaration of Interests

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 article.

Ethical Approval

According to the Research Ethics Committee of Duhok university and Duhok Directorate general of Health (Reference number: 25012023-1-3) at the 25th of January 2023, the author can say the following: It is not possible to share or display the study data due to legal, ethical, as well as, confidentiality considerations.

Consent for publication

No funding was received from any financially supporting body.

Competing interests

The authors declare no competing interests.

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