Examination of Salivary TNF-A and Antioxidant Capacity in Smokers Who Switch to Non-Combustion Product: A Randomized-Controlled Trial

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Abstract

Cigarette smoke contains a large number of oxidants and plays an important role in disturbing the oxidative balance due to the content of free radicals in it, which is responsible for many adverse effects in the oral cavity. Chronic inflammation accompanied by increased TNF-α can lead to increased production of free radicals in the body. A high total antioxidant capacity can help neutralize free radicals and reduce oxidative stress, which in reducing TNF-α production. This study aimed to evaluate the effect of switching from tobacco smoking to using non-combustion products on gingival health in terms of TNF-α levels in saliva, and total antioxidant capacity in saliva in smokers with gingivitis.

This study was a randomized controlled trial. A total of 34 subjects (17 smokers and 17 switching to non-combustion products) were involved in the study according to the inclusion criteria. Saliva samples were taken at month 0 and month 3 to examine TNF-α levels and total antioxidant capacity (TAOC).

TAOC in the switch group increased after 3 months (p=0.006) compared to the smoker group. TNF-α in the switch group decreased after 3 months compared to the smoker group, but there was no significant difference (p=0.473). Switching smoking behavior from conventional cigarettes to non-combustion products has the potential to increase TAOC levels and decrease TNF-α after 3 months.

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Introduction

It is still not easy to overcome the issue of nicotine dependence. The harmful effects of tobacco use are known to have detrimental effects on the health of individuals. To prevent people from smoking, various countries have enacted regulations and implemented smoke-free zones. Despite the various efforts being made by health agencies to prevent people from smoking cigarettes, they still have not been able to achieve satisfactory results.[1] A study conducted by West et al in four countries revealed that only 52% of the participants were able to stop smoking. One of the most effective ways to stop smoking is through a type of nicotine replacement therapy known as nicotine replacement therapy.[2] Electronic cigarettes are becoming a popular alternative to smoking. These devices use batteries to deliver the desired amount of nicotine in vapor. According to the WHO, this type of device is an *electronic nicotine delivery system* (ENDS).[3]

According to a study conducted by Bergstörm et al, the use of tobacco products can suppress the inflammatory responses in the tissues of the gingival. This condition triggered several studies comparing the effects of electronic cigarettes and other types of tobacco products on the oral cavity, including periodontal tissue.[4] Tatullo et al (2016) found that people who switched from regular cigarettes to electronic ones had a positive impact on the

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periodontal health status of smokers.[5] Another study was conducted by Wadia et al (2016). also revealed that those who used electronic cigarettes had an increase of gingival inflammation.[6] Another study revealed by Amaliya et al (2023) stated that the use of e-cigarettes with nicotine vapour did not mask the clinical features of inflammation.[7] The findings of the study suggest that the metabolic changes that occur in the tissue of the periodontal condition can be affected by the use of electronic cigarettes. This study supports the idea that these types of cigarettes can be used in various ways to help people stop smoking.[8]

One of the most important factors that can affect the development and maintenance of periodontal disease is the presence of a certain type of cytokine known as Tumor Necrosis Factor Alpha (TNF-α). This cytokine plays a role in the various inflammatory responses that can occur during the process. The presence of elevated levels of this cytokine can contribute to the development of periodontal inflammation. It can also cause bone resorption and tissue damage. This is why it is important that examinations of saliva with elevated levels of this cytokine are performed.[9]

One of the most important components of tobacco is its free radical content. This substance plays a significant role in disrupting the balance of free radicals in an organism, which is characterized by a fine equilibrium. The free radical destruction and formation in an organism occur in this equilibrium, and if the balance is not stable, it can lead to damaging effects.[10] One of the main factors that contribute to the development of oxidative stress is exposure to tobacco smoke. It is known that the presence of numerous oxidants in this substance can cause various negative effects.[10–12]

A study conducted by Karademirci and colleagues (2018) revealed that the total antioxidant value of smokers was lower than that of non-smokers. In addition, the index of oxidative stress and the total oxidant value were higher in smokers. It is believed that the lower antioxidant values of smokers are due to the high levels of free radicals that are present in cigarette smoke. This causes the body to exhaust its antioxidant reserves.[13] The lack of clarity regarding the effect of switching to vaping from tobacco cigarettes motivated the researchers to conduct this study. This study aimed to evaluate the effect of switching from tobacco smoking to vaping on gingival health in terms of salivary TNF-α levels and its total antioxidant capacity in smokers with gingivitis.

Materials and methods

This study is a randomized-controlled trial, and has been registered at UMIN with number UMIN000051684 and also has received ethical approval from the research ethics commission of Universitas Padjadjaran Bandung with number: 643/UN6.KEP/EC/2021, issued on August 13, 2021. Data were taken from the target population, namely smokers aged 18–65 years, who came to the Oral and Dental Hospital, Faculty of Dentistry, Universitas Padjadjaran, who suffered from gingivitis in the period July 2022–December 2022. The inclusion criteria were that they had gingivitis which is known by examining the pocket depth of not more than 3 mm, had been smokers for at least 1 year, smoked as much as 10–30 cigarettes a day, still had 24 natural teeth (excluding the third molar), and were willing to be randomized into the smoker group and the group switching to non-combustion product. While the exclusion criteria were having an acute illness that required treatment (subjects who had a viral infection) within 4 weeks before the enrolment visit, having oral soft tissue disease or any type of gingival overgrowth other than those caused by plaque, suffering from periodontitis, having a history of tooth loss due to periodontitis, being treated with anticoagulant therapy in the previous six months, using prostheses or orthodontic appliances, history of alcoholism or drug/chemical abuse, taking any drug or substance (other than tobacco) that interferes with the cyclooxygenase pathway (e.g. anti-inflammatory drugs including aspirin and ibuprofen) in the 14 days prior to the enrolment visit, taking antibiotics in the 14 days prior to the enrolment visit, breastfeeding or pregnant women.

Potential smoker subjects must meet the requirements through the examination of carbon monoxide levels from exhaled breath at ≥ 7 ppm through a CO meter. Potential subjects will receive smoking cessation counseling at the beginning of the study and during the study procedures. Participation in this counseling program is mandatory for each subject in order to always get information about the effects of

smoking on health and gradually reduce addiction to nicotine. Consort flow diagram is illustrated in Fig.1. It explains that three subjects Unwilling to switch, two people were on a pregnancy program and three smokers for less than 1 year, the rest survived until the end of the study.

Subject Withdrawal Criteria

Subjects may be withdrawn prematurely from the study for reasons such as experiencing intolerable side effects, suffering from illnesses for which the researcher discourages participation in the study, or wishing to withdraw from the research procedure.

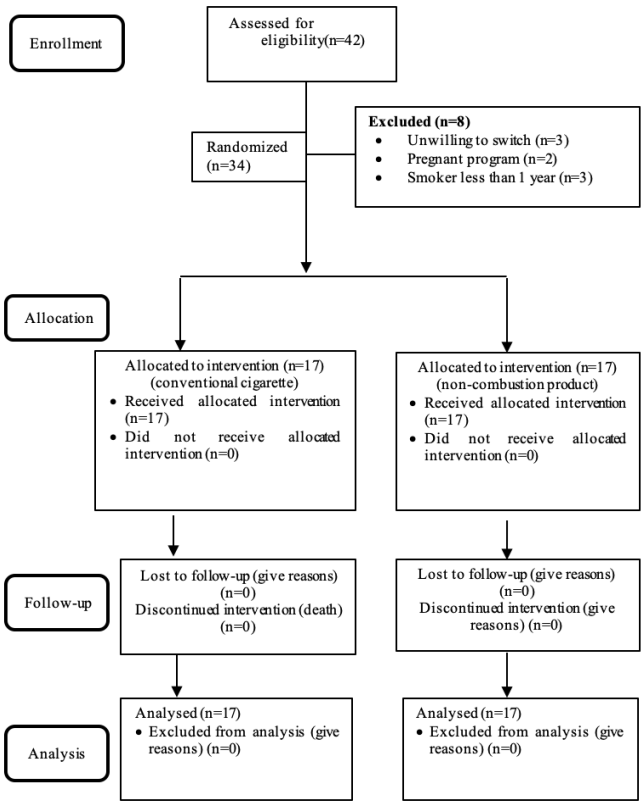

Figure 1. Consort Flow Diagram.

General aspects of the subject

All subjects must agree to: avoid scaling and polishing procedures during the study period (except for research protocol procedures); not change their own oral hygiene habits (e.g., mouthwash, dental floss) during the study period, refrain from oral hygiene procedures (brushing, mouthwash, dental floss) at least 8 hours before each visit; refrain from eating and drinking (except water) at least 2 hours before each visit; and not smoke for 2 hours before each visit.

Randomization

Potential subjects who met the criteria would be randomized into 2 groups, namely the conventional smoker group (control) and the switch to vaping group (test). Smokers randomized to the test group would be asked to try and familiarize themselves with vaping. They were trained and counseled on vaping. Subjects would be instructed to brush their teeth at least once a day to maintain optimal gingival health. Subjects are requested not to change toothpaste brands during the study period. However, if there should be a change in the brand of toothpaste used, the subject should inform the researcher of the change.

Product Usage

The conventional smoker group would continue to smoke their own brand of conventional cigarettes as usual. Subjects in the switch group would have the option to try and choose to use pods (RELX®) or tobacco heat sticks (IQOS®). Subjects who choose to use tobacco heat sticks would receive one kit and a refill supply of 200 tobacco sticks. When the refill is almost used up, the subject will immediately report to the researcher to get a refill of tobacco sticks. Subjects who choose to use pods would receive a kit of pods and five e-liquid cartridges. When the refill is almost used up, the subject immediately reports to the researcher to retrieve the e-liquid cartridge refill.

Research procedure

Prospective subjects who met the inclusion criteria were first scaled before examining the study variables. After completion of the scaling procedure, polishing was carried out on the teeth with the help of a brush that was given prophylactic paste and run with contra angle low speed, then irrigated with Chlorhexidin 0.12% mouthwash. One week after scaling, the subjects began enrollment, which is the collection of saliva. This enrollment procedure was also performed at the 3rd month examination.

Saliva was collected at the enrollment visit and after 3 months with no stimulation. Whole, unstimulated saliva was collected from each subject between 8 and 10 a.m. to avoid circadian variation. No oral stimulation was performed 90 minutes prior to saliva collection. Subjects were asked not to smoke two hours prior to saliva collection. In a sitting position, the subjects were asked to swallow saliva, then remain motionless and allow saliva to flow passively for 10 minutes into a sterile plastic vial. The collected saliva sample of approximately 5

ml was immediately sent to the Clinical Pathology Laboratory of Hasan Sadikin Hospital Bandung to be frozen at -80°C and stored for further analysis.14

The sample size was calculated according to Federer's formula:

(t-1)(n-1)>15, where t = number of treatment groups; and n = number of samples per group, because it is known that t = 2, so the following calculation is obtained:

(2-1)(n-1)>15 1 (n-1)>15

n>16 It is concluded that the number of samples per group must be >16. Therefore, the number of samples per group was set to 17.

Results

This study was conducted from July 2022 to December 2022 and obtained 17 subjects who continued to smoke (control) and 17 subjects who switched to vaping (test), with characteristics as shown in Table 1.

Characteristics	Smokers (control) n=17	Switch (test) n=17
Age (years)		
18-33	12	11
34-48	5	5
49-65	0	1
Gender		
Male	15	16
Female	2	1
Length of smoking (years)		
1-5	2	2
6-10	7	7
11-15	5	5
>15	3	3

Table 1. Subject Characteristics.

Total Antioxidant Capacity (TAOC) In Saliva

Comparing the total antioxidant capacity of the control group and the experimental group can be accomplished by analyzing the difference in total antioxidant capacity (TAOC) changes. Based on Table 2, the results of the Independent Sample T Test obtained p=0.006 (<α=0.05), so it was concluded that there was a significant difference in the total antioxidant capacity of the Test group compared to the control group at the 95% confidence level.

		levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	(2-tailed)	n Difference	td. Error Difference	Confidence Interval of the Difference Lower er
OC	al variances	69	684	944	2	.006	-.08000	.02717	.13535
	assumed al variances not assumed			944	673	.006	-.08000	.02717	.13537

Table 2. Comparison of total antioxidant capacity between control group and groups test after three months.

Comparison of TAOC of Month 0 and Month 3 between Control Group and Test Group

The impact of the treatment on the control group's total antioxidant capacity after a three-month period was assessed using a paired t-test. The resulting p-value was found to be 0.450, which is greater than the predetermined significance level of α=0.05. Based on this analysis, it can be concluded that there is no statistically significant difference in the total antioxidant capacity of the control group after three months, with a confidence level of 95% (α=0.5). The impact of the treatment over a duration of three months can be observed through the application of a paired t-test on the experimental groups, employing a confidence level of 95% (α=0.05). The resulting data yielded a p-value of 0.000, which is less than the predetermined significance level of α=0.05. Consequently, it can be deduced that a substantial disparity exists in the overall antioxidant capacity of the Test group following the three-month period, with a confidence level of 95% (α=0.05).

TNF-α levels in saliva

The comparison between the control group and the test group (switched) can be seen by taking into account the difference in changes that occur in the TNF-α variable.

Comparison of TNF-α levels of Month 0 and Month 3 between Control Group and Test Group

The impact of a three-month treatment on TNF-α levels can be assessed by conducting a paired t test on the control group. Based on the Table 3, the resulting p-value of 0.473 (>α=0.05) indicates that there is no statistically significant difference in TNF-α levels within the control group after three months, at a 95% confidence level with α=0.05. In addition, based on the Table

4, the impact of the treatment on TNF-α levels in the test group was assessed during an interval of three months. The resulting p-value was calculated to be 0.186, which is greater than the predetermined significance threshold of α=0.05. Therefore, based on a 95% confidence level, it can be inferred that there is no statistically significant differences in TNF-α levels within the test group after the three-month period.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	TNFa_enrollment - TNFa_M3	-.23824	1.33627	.32409	-.92528	.44881			

Table 3. Comparison of TNF-α of Control Group Between Month 0 and Month 3.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Deviation	Std. Error Mean	% Confidence Interval of the Difference Lower	Upper			
Pair 1	TNFa_enrolment - TNFa_M3	1.96235	5.85577	1.42023	-1.04840	4.97311	1.382	16	.186

Table 4. Comparison of TNF-α of Test Group between Month 0 and Month 3.

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Fa_enrolment & TNFa_M3	17	.641	.006

Table 5. Correlation of TNF-A Levels in the Test Group between Month 0 and Month 3.

Based on the Table 5, the correlation observed in the Test group yielded a p-value of 0.006, which is less than the predetermined significance threshold of α = 0.05. Therefore, it can be inferred that there exists a statistically significant correlation between TNF-α levels in the Test group after a duration of three months, with a confidence level of 95%.

Discussion

The present study was conducted to evaluate the effect of switching from tobacco smoking to vaping on gingival health in terms of salivary TNF-α levels and its total antioxidant capacity in smokers with gingivitis. Potential subjects who met the criteria would be randomized into 2 groups, namely the conventional smoker group (control) and the switch to vaping group (test). Subjects in the switch group would have the option to try and choose to use pods (RELX®) or heat-not-burn (IQOS®). The pods (closed system) used are the 4th generation of e-cigarettes. The 4th generation is more efficient, because it can provide a greater amount of nicotine even though the liquid is less, thereby reducing aerosol exposure. Heat-not-burn was also used in this study because not all subjects who switched could adapt well to pods, besides that the shape was still similar to conventional cigarettes and they still used tobacco even though they were only heated and not burned, so using heat-not-burn was still can taste nicotine like using conventional cigarettes.

Smoking can affect an individual's antioxidant capacity, because cigarette smoke can cause free radicals and this can vary depending on various factors, such as smoking frequency and duration, general health, diet, and genetics. In order to prevent damage caused by free radicals, the body must have antioxidants. These substances help neutralize these harmful substances by complementing the lack of electrons. They can also prevent the formation of free radicals by blocking their chain reaction. An imbalance between the production and lack of antioxidants can lead to oxidative stress. Oxidative stress has been implicated in the pathogenesis of periodontal disease, and salivary total antioxidant capacity (TAOC) has been investigated as a potential biomarker of oxidative stress and antioxidant status in periodontal disease.[15]

In the examination of salivary total antioxidant capacity, it was found that the test group experienced a significant increase in TAOC for three months compared to the control group. These results are in line with research conducted by Kostelli et al (2020). which states that there is substantial evidence that the aerosol component of e-cigarettes can increase the formation of reactive oxygen species (ROS), which causes increased oxidative stress but is lower than that caused by conventional tobacco cigarettes.[16] Nevertheless, in Poulianiti's study (2016), tobacco and e-cigarette exposure did not acutely alter the antioxidant system response in either active or passive smoking conditions. Overall, there was no difference in total antioxidant levels between the effects of active and passive smoking of tobacco and e-cigarettes. This condition is clearly different

from the results of the present study because the Poulianiti study was only conducted for 7 days, while this study examined the change after three months of switching.[17]

In line with this study, in 2015, Bakhtiari conducted a study which stated that smoking caused a decrease in salivary TAOC.[18] It is in agreement with results from the research of Mojtaba, et al (2014). which states that smoking is associated with a decrease in antioxidant capacity and oxidative stress.[19] Agreed with this study, Carnevale, et al (2016).stated that tobacco smokers and non-combustion product users have an unfavourable effect on oxidative stress markers although non-combustion product seems to have a smaller impact.[20]

Some reasons why antioxidant capacity in smokers tends to be lower include: 1) Smoking may reduce the intake of exogenous antioxidants, such as vitamin C, vitamin E, and polyphenolic compounds from food, as some of these compounds can be damaged by the heat and toxic substances in cigarette smoke..[14] 2) Tobacco consumption increases the production of free radicals and oxidative compounds in the body, necessitating the production of more endogenous antioxidants. However, although the production of endogenous antioxidants may increase, it may not be sufficient to neutralize all the free radicals generated by smoking..[21] 3) The activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase, and catalase, can be impaired by smoking tobacco. This can decrease the body's resistance to oxidative stress. [21]

Potential reasons for the increased antioxidant capacity of non-combustion product users is one of the primary differences between smoking tobacco and vaping is that smoking tobacco involves burning tobacco fuel while vaping entails heating the vape liquid. Tobacco combustion generates a large number of toxic compounds and free radicals, which can cause oxidative stress and diminish the body's antioxidant capacity. Nonetheless, vaping has the potential to reduce exposure to these noxious by products of smouldering tobacco.[22]
TNF-α release is a typical mechanism that results from metalloprotease activity that encourages cigarette smoke-induced inflammation. Immune system cells, particularly macrophages and T lymphocytes, produce the protein TNF-α. TNF-α participates in a number of inflammatory processes and the control of the immune system.TNF-α level analysis revealed in this study that although the test group (switched) looked to have lower levels after three months, this was not deemed statistically significant. Al-Tameemi et al.'s study found that smokers' TNF-α levels were significantly higher than non-smokers' when compared to smokers. Immune system cells release the cytokine TNF-α in response to inflammation. Chronic inflammation brought on by increased TNF-α production can be a factor in a number of illnesses.[23]

In this study, it can be seen that in the switch group, there was an increase in TAOC after three months, but TNF-α levels in the switch group also decreased, even though it was considered not statistically significant after three months. This is in line with research conducted by Sundawa et al (2022), which states that increased antioxidant administration has an inhibitory effect on TNF-α production.[24] Chronic inflammation accompanied by increased TNF-α can lead to increased production of free radicals in the body. High total antioxidant capacity can help neutralise free radicals and reduce oxidative stress, which in turn can reduce TNF-α production. In the test group (switched), there was a decrease in TNF-α levels after three months, although not significant, but judging from the TAOC levels, there was a significant increase after three months. This study is different from that conducted by Bunjaku, et al. (2021). Bunjaku reported that salivary TNF-α levels decreased significantly after 3 months of periodontal treatment. In the study conducted by Bunjaku, it did not involve smoking subjects while in this study the subjects were smokers who switched to non-combustion product.[25]

The finding of this result was related to the lack of discipline of the test group subjects because sometimes they still took the time to consume tobacco cigarettes in addition to vaping during the three-month study period. The reasons is that there was still some discomfort experienced when not smoking tobacco cigarettes after eating. Another reason is thought to be the short duration of this study, which was 3 months, so that the decrease in TNF-α levels was not significant. Cigarette smoke is just one of several variables

that can have an impact on the link between total antioxidant capacity and TNF-α. TNF-α production may rise due to increased oxidative stress brought on by smoking, and oxidative damage and inflammation may worsen due to poor total antioxidant capacity.[24]

Conclusions

Within the limitation of the present study, we concluded that after three months, the total antioxidant capacity of the saliva of smokers with gingivitis who switched to non-combustion product increased. On the other hand, TNF-α levels of saliva smokers with gingivitis who switched to non-combustion product decreased but not significantly after three months.

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Declaration of Interest

The authors report no conflict of interest.

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