

Original Research Article

Evaluation of Peri-Implant Immunological Parameters amongst Subjects Vaping E-Cigarettes and Non-Smokers

Dr. Kumar Ravishankar¹, Dr. Afifa Rasheed Shervani², Dr. Meenakshi M. Singh³, Dr. Mamta Singh (MDS)^{4*}

¹Senior lecturer (MDS), Department of Periodontology, Aditya Dental College, Beed- 431122

²Post Graduate Trainee Second year Department of Prosthodontics and Crown and Bridge Subharti Dental College and Hospital Uttar Pradesh India

³Post graduate Trainee Third Year, Department of Pedodontics And Preventive Dentistry, Kothiwal Dental College and Research Centre, Mora Mustaqueem, Kanth Road, Moradabad, 244001, Uttar Pradesh, India

⁴Senior Lecturer Department of Periodontics, Kothiwal Dental College and Research Centre, Mora Mustaqueem, Kanth Road, Moradabad, 244001, India

***Corresponding Author**

Dr. Mamta Singh (MDS)

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Abstract: *Aim:* The present study aimed to evaluate peri-implant immunological parameters along with clinical and radiographic parameters amongst subject's vaping e-cigarettes and non-smokers (NS). Two immunological parameters that were included were measurement of tumor necrosis factor alpha (TNF- α) and interleukin (IL)-1 β . **Materials and Methods:** A statistically significant no. of subjects were included in the study and further divided into two groups: Group-1: Vaping e-cigs (n=47) Group-2: Non-smokers (n=45). A structured questionnaire was used at baseline for data collection related to demographic and peri-implant data of each individual. Baseline parameters included Plaque Index, Bleeding on probing, probing pocket depth and peri-implant bone loss (PI, BOP, PD and PIBL respectively) for those vaping e-cigs and non-smokers with the aid of standardized digital radiographs. ELISA kit was used to assess immunologic parameters using sulcular fluid collected from peri-implant region (PISF). **Results:** Bleeding on probing (BOP) was found significantly high in non-smokers compared to the vaping group. But probing depth greater than 4mm and PIBL scores were significantly higher in vaping group than non-smokers. Mean concentrations of immunologic parameters were significantly higher in vaping group than in non-smokers. TNF- α levels and IL-1 β levels were found to have a positive correlation with bone loss around implants (PIBL). Besides TNF- α also had a positive correlation with bleeding on probing in vaping group compared to non-smokers. **Conclusions:** Owing to the effect of nicotine on the peri-implant tissue, levels of inflammatory mediators as detected by ELISA test were found to be higher showing a greater amount of localized inflammatory tissue destruction and a compromised peri-implant area in vaping subjects. PISF concentrations were also found relatively higher than non-smokers.

Keywords: TNF- α , IL-1 β levels, Electronic cigarettes, interleukin, peri-implant bone loss.

INTRODUCTION

Electronic cigarette (e-cig) is a latest fed amongst nicotine users. It is a device which is battery operated, and releases nicotine in vaporized form simulating the effect of an actual cigarette. It has gained quite popularity among youth of today ranging from age group of 18 to 25 years. Nevertheless, it poses deleterious effect on systemic health of vaping individuals [1].

E-cigs are now available in various forms ranging from a simple pen-like device to a USB like portable device that operates on a battery, has a metallic element that heats up covered by stainless steel casing from outside. There is a cartridge inside, an atomizer that is directly connected to the battery. The metal element on heating changes the solution filled in the cartridge that contains nicotine and flavoring agents mixed in a chemical solution. Other additives are

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propylene glycol, glycerines etc [2]. Many people consider e-cigs as more sophisticated version of smoking tobacco with less deleterious effects [3]. However, this is contrary to the findings put forward by several researchers. They have drawn our attention to the harmful aerosols liberated during vaping these electronic cigarettes that increase oxidative stress, enhance release of inflammatory mediators, changes in cellular function of liver and cause damage in DNA constitution [4, 5].

From the perspective of oral health, vaping nicotine in any form has shown the precipitating factor for serious oral conditions like oral submucous fibrosis with marked reduction in mouth opening and damage to the integrity of periodontal tissues thus resulting in periodontal tissue- breakdown and in turn periodontal diseases [6, 7].

Sundar *et al.* [8] in their laboratory study drew the conclusion that oral epithelial cells and gingival fibroblasts have been negatively affected by aerosols of e-cig via damage to DNA and enhanced release of inflammatory mediators like cyclooxygenase as well as prostaglandins (COX-2 & PGE2). The present study aimed to evaluate immunological parameters (TNF- α) and (IL)-1 β between vaping individuals and non-smokers and draw a comparison between clinical and radiographic parameters in the peri-implant structure.

MATERIALS AND METHODS

Ethical clearance

The present study was done in accordance with 2013 revised Helsinki Declaration protocol and was approved by institutional ethical review board. Participants were explained the study protocol thoroughly and a written informed consent was taken from each subject taking the study. Individuals had the discretion of leaving the study as deemed fit by them at any phase of the study.

Study groups

Study subjects were selected from March 2018 to March 2019. 100 subjects were initially enrolled out of which 8 refused to participate so remaining 92 subjects were finalized for taking up the study. The following inclusion and exclusion criteria were devised for selection and further divided into two groups:

Group-1: Vaping e-cigs (n=47) Group-2: Non-smokers (n=45)

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria

- Group-1: Vaping e-cigs for a minimum of last year
- Group-2: Non-smokers those who never consumed any form of tobacco ever in their lives.

A minimum of one dental implant (functional for 3 years) in both of the groups

Exclusion criteria

Current smokers/ pipe smokers/ smokeless tobacco chewers

- Systemic disorders like diabetes, AIDS, heart and kidney dysfunction
- Subjects having edentulous ridges.
- Antibiotics/ NSAIDs for past 6 months
- Periodontal therapy for past 6 months

Study questionnaire

A structured questionnaire was used at baseline for data collection related to demographic and peri-implant data of each individual. They included

- Total no. of subjects per group (n)
- Sex (allmales) Mean age of each individual in years along with standard deviation
- Total number of implants placed in both arches
- Position of implant (maxilla/mandible)
- Duration of implants in months (mean \pm SD)
- Mean duration of habit (in years)
- Vaping frequency per day
- Duration of session (mean in minutes)
- Frequency of brushing either once or twice a day (%)

Clinical assessment

Baseline clinical parameters included plaque index, bleeding on probing, pocked depth (PI, BOP, PD) and were recorded by single examiner to avoid inter examiner bias. The kappa statistics for intraexaminer variation was 0.91 which was a very good score. 6 conventional periodontal sites were used for charting and probing pocket depth were calibrated to the nearest mm using UNC-15 periodontal probe.

Radiographic evaluation (PIBL)

A single masked evaluator recorded all radiographic parameters. The kappa statistics for intraexaminer variation was 0.86 and was considered as a decent agreement. Digital periapical radiographs were shot and assessed using an image analyzer on a standardized desktop screen. These RVGs were further scanned at 800 dots per inch using a cam-scanner. PIBL was assessed by measuring distance from crest of bone to the implant platform [9].

Collection of PISF

Supragingival oral prophylaxis was done using ultrasonic scalers from the crown surface judiciously and the peri-implant sites were isolated using dry and sterile cotton roles. Using GCF collecting periopaper/ paper strips PISF was collected in two separate vials. The paper was held still into the sulcus for 30seconds reaching 2mm into the sulcus/pocket. Any contamination with blood or saliva was checked by discarding PISF after every 15 minutes from particular site. Peritron was used for measuring PISF volume.

Measurement of TNF- α and IL-1 β in PISF

ELISA kit was used to analyze and quantify the inflammatory chemical mediators TNF- α and IL-1 β . PISF was centrifuged for 15 minutes at 5kg at 48 degree Celsius [10].

STATISTICAL ANALYSES

Statistical software version SPSS 23 was used for carrying out statistical analysis. Intergroup comparison was carried out using Kruskal–Wallis test. Mann-Whitney U test was used for comparison of inflammatory mediators. Multiple comparisons were done using Post hoc Bonferroni Test. A p-value of <0.05 was regarded as statistically significant.

Demographic and implant-related characteristics of the study groups

Table-1: Demographic and implant related characteristics of the study groups

Demographic Parameters	Vapers (group 1)	Non-smokers (group 2)
Total participants (n)	47	45
Sex (males)	47	45
Mean age \pm SD (in years)	34.6 \pm 6.1	44.8 \pm 2.5
Total number of implants	66	55
Implant position	45/21	35/20
(maxilla/mandible) Duration of implants in months (mean \pm SD)	53.2 \pm 13.7	51.2 \pm 19.5
Mean duration of habit (in years)	4.6 \pm 1.8	–
Daily frequency of habit	6.8 \pm 0.9	–
Mean duration of session (in minutes)	37.7 \pm 11.3	–
Brushing frequency (%)		
Once	54	58
Twice	46	42

Clinical and radiographic peri-implant parameters

Table-2: Mean clinical and radiographic peri-implant parameters among vaping individuals and NS

Peri-implant parameters	Vapers (group 1) n = 47	Non-smokers (group 2) n = 45
Plaque index (%)	54.6 ± 10.9	48.46 ± 9.76
Bleeding on probing (%)	23.7 ± 5.3 ^a	37.82 ± 17.81
Probing depth ≥ 4 mm (%)	5.87 ± 1.42 ^b	4.65 ± 0.71
Peri-implant bone loss (mm)		
□ Mesial	1.56 ± 0.7 ^b	0.98 ± 0.2
□ Distal	2.09 ± 1.0 ^b	1.12 ± 0.5
□ Total	1.78 ± 0.9 ^b	0.93 ± 0.3

a Compared with group 2 (P < .01). b Compared with group 2 (P < .05).

Cytokine levels in PISF among study groups

Table-3: Concentrations of proinflammatory cytokines (TNF-α and IL-1b) in PISF among vaping individuals and NS

Immunological Parameters	Vaping individuals (group 1) n = 47	Never smokers (group 2) n = 45
PISF volume collected (in IL)	3.17 ± 0.6 ^a	1.5 ± 0.5
TNF- α (in pg/mL)	24.3 ± 32.4 ^b	6.7 ± 8.1
IL1b (in pg/mL)	205.2 ± 230.7 ^a	19.7 ± 22.3

a Compared with group 2 (P < .01). b Compared with group 2 (P < .001).

Pearson’s correlation analysis among proinflammatory cytokines and peri-implant parameters

Table-4: Pearson’s correlation analysis among proinflammatory cytokines and peri-implant parameters

Parameters	Vaping individuals (group 1)	Never smokers (group 2)
TNF- α		
PI		
Correlation coefficient	0.9111	-0.5268
P value	0.924	0.3871
BOP		
Correlation coefficient	0.1441	-0.1245
P value	0.0214	0.7847
PD		
Correlation coefficient	0.6127	-0.9572
P value	0.1728	0.4213
PIBL		
Correlation coefficient	0.3200	-0.4455
P value	0.0167	0.8914
IL-1b		
PI		
Correlation coefficient	0.5067	-0.4276
P value	0.7436	0.1983
BOP		
Correlation coefficient	0.8116	-0.3145
P value	0.2867	0.4810
PD		
Correlation coefficient	0.3345	-0.7669
P value	0.9723	0.0912
PIBL		
Correlation coefficient	0.4201	-0.3772
P value	0.0181	0.8168

Significant at P < .05

Bleeding on probing (BOP) was found significantly high in non-smokers compared to the vaping group. But probing depth greater than 4mm and PIBL scores were significantly higher in vaping group than non-smokers. Mean concentrations of immunologic parameters were significantly higher in vaping group than in non-smokers. TNF- α levels and IL-1 β levels were found to have a positive correlation with bone loss around implants (PIBL). Besides TNF- α also had a positive correlation with bleeding on probing in vaping group compared to non-smokers.

DISCUSSION

Cellular healing relatively is affected by smoking tobacco which is rich in nicotine content. Smokers show less bleeding tendency and was seen in present study too that bleeding on probing was seen more in non-smokers than in vapers. The potential threat thus posed by smoking tobacco remains clinically less evident as basic indicator of periodontal tissue breakdown i.e. Bleeding is not significantly appreciable. The mechanism behind reduced bleeding is the vasoconstriction caused by nicotine on the supra-periosteal blood vessels. Nonetheless, the higher concentration of sulcular fluid and increased amount of cytokines are indicative of rapid tissue destruction caused by nicotine. Not just smokers but vapers too remain unaware of potential threat e-cigs pose to the health of oral epithelium and gingival fibroblasts. It remains a common misconception that e-cigs are less harmful as strongly opposed by the findings of present study which showed a significant hike in the chemical mediators at the periimplant sites compared to those of non-smokers.

The findings of our study are clearly indicative that severity of periodontal tissue destruction is higher in individual's vaping electronic cigarettes. Both probing pocket depth and peri-implant bone loss was significantly higher in group 1 than in group 2. An increased amount of interleukins in the peri-implant area is well established indicator of advanced bone loss or osteoclastic activity and findings showed that IL-1 β was significantly higher in PISF sample of vapers than smokers. Pro-inflammatory cytokines TNF- α plays a pivotal and central role in destruction of peri-implant tissue. It is suggestive of gram negative microbes which liberate lipopolysaccharides and further advance destruction. Vaping e-cigs result in increase in advanced glycated end products AGEs in periodontal tissues, the AGE-RAGE interaction i.e. augmented interface of AGEs with their receptors liberates reactive oxygen species (ROS) creating an oxidative stress, inducing metabolic changes, altered chemotaxis, neutrophilic dysfunction, reduction in antibodies production, increased bacterial cell to cell adhesion and thus enhancing production of localized and systemic inflammatory mediators thus their expression is higher in sulcular fluid.

Further, such investigations should be carried out as to understand the mechanism behind increased peri-implant tissue destruction in vaping individuals. It is the responsibility of the dentists to properly understand the mechanism themselves and guide their patients about the hazards posed by vaping e-cigs. They should motivate their patients to quit their habit of vaping tobacco in any form and counsel their family members too. Success of implants largely depends on maintenance as well as quitting deleterious habits that cause destruction of peri-implant tissue. Besides, anti-tobacco campaigns should be conducted for explaining the hazards of tobacco smoking and educating the masses. Community health awareness camps can also be found beneficial for overall oral as well as general health.

CONCLUSIONS

Owing to the effect of nicotine on the peri-implant tissue, levels of inflammatory mediators as detected by ELISA test were found to be higher showing a greater amount of localized inflammatory tissue destruction and a compromised peri-implant area in vaping subjects. PISF concentrations were also found relatively higher than non-smokers.

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