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Original Article

Dentistry Section

Comparison of Taste Threshold in Smokers and Non-Smokers Using Electrogustometry and Fungiform Papillae Count: A Case Control Study

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ABSTRACT

Introduction: Smoking in long term is not only responsible for cancerous changes but is also one of the reasons of altered taste sensation in smokers. These taste changes are hypothesized to be due to reduction in density of fungiform papillae on the dorsum of the tongue.

Aim: The aim of this study was to assess the relationship between fungiform papillae count, blood Red Cell Distribution Width (RDW) and electrogustometric thresholds in smokers and non-smokers.

Materials and Methods: Fungiform papillae count was assessed using digital photography and imaging software while electrogustometric thresholds were assessed using modified Transcutaneous Electric Nerve Stimulation (TENS) machine in 30 smokers and 30 non-smokers. The subjects also underwent RDW evaluation. The data collected was analyzed using Pearson's correlation coefficient.

Results: Fungiform papillae counts in smokers were less than those of non-smokers and an inverse relationship was detected between smoking and fungiform papillae count. Electrogustometric thresholds were more in smokers than non-smokers and showed direct relationship with smoking. RDW was significantly more in smokers compared to non-smokers. An inverse relationship was observed between fungiform papillae count and RDW.

Conclusion: Our results suggest that smokers have a high taste threshold because of decrease in the number of fungiform papillae on the tongue and RDW values do show an inverse relationship with fungiform papillae density which depicts subclinical nutritional deficiency bringing atrophic changes in tongue.

Keywords: Fungiform papillae, Metallic taste, Red cell distribution width, Smoking, Transcutaneous electric nerve stimulation, Taste threshold

INTRODUCTION

Taste is a specific sensory function performed by the tongue along with its mechanical function which aids mastication [1]. The fungiform papillae are found only on the anterior two thirds of the tongue. Data based on counting all papillae on a series of cadaver tongues, suggest an average of approximately 200 fungiform papillae per tongue. They are more numerous on the tip (29/cm^2) and fewer in the middle region (7/cm^2 to 8/cm^2) [2,3].

Smoking is the chief and the most common cause of inducing changes in taste functionality. The simplest test to quantify this taste loss is electrogustometry. This method makes use of electrical impulses to detect taste sensitivity. Advantages of this method are its easiness, the short time required and its quantitative character [4]. Earlier studies were based on the use of chemical solutions to assess whole mouth taste threshold [4]. This generated a lot of controversy. A number of studies reported a greater taste threshold in smokers as compared to the non-smokers [4].

Taste variation is one of the oral features of anaemia and diagnosis of anaemia is made using Red Cell Distribution Width (RDW) [5]. Results from various reports suggest different levels of RDW where tongue depapillation occurs [6]. Only a few studies have quantified tongue papillae and this being the only study in which the relationship of RDW was assessed with fungiform papillae count [3,4].

AIM

Therefore this study was conducted to assess the possible effect of smoking on fungiform papillae count and electrogustometric thresholds of the tongue.

MATERIALS AND METHODS

Source of Data: The present case control study design was presented to the ethical committee and the approval for the same was obtained from the Institutional Review Board of Coorg Institute of Dental Sciences, Virajpet, Karnataka. Patients visiting Department of Oral Medicine, in the age range of 25 to 45 years and who provided a written consent were selected for the study. The rationale behind selecting the above age group was to include only those subjects in which the habit had induced sufficient changes. Also, the fungiform papillae are found to get degenerated beyond the age of 45 years [1]. The study was conducted over a period of six months during April to October 2013. The study design conformed to the Declaration of Helsinki for Medical Research involving human subjects. All the subjects in the study were males because there were not sufficient female smokers matching the criteria.

Study group: Total 30 subjects with the habit of smoking at least 10 cigarettes per day (as this study was conducted in India many cigarette manufacturers market the product in a pack of

10. Choosing subjects smoking minimum of 10 cigarettes would therefore ease in calculating pack per years as shown below) were randomly included in the study group. After recording demographic data they were subjected to assessment of habit, medical and dental history followed by clinical examination. All the patients were subjected to electrogustometry and tongue photography (for fungiform papillae assessment). Also, blood samples was collected under sterile aseptic conditions.

Control group: Age and gender matched 30 subjects without the habit of smoking and who were willing to give their consent for the study were included. The protocol above was also followed for the control group.

Inclusion criteria: Patients in the age range of 25 to 45 years with a habit of smoking minimum 10 cigarettes or beedis per day for minimum of six years.

Exclusion criteria: Patients suffering from otolaryngological diseases, chronic rhinitis and sinusitis were excluded from the study. Patients with history of cardiac surgery for pacemaker were also eliminated from the study. Patients with ankyloglossia, geographic tongue, median rhomboid glossitis or any other atrophy or malignancy of tongue were definitely excluded from the study sample for the obvious reason of difficulty in counting the fungiform papillae. Patients with history of metabolic disorders and anti amoebicides, antihelmintics, local anesthetics, clofibrate, chlorpheniramine maleate, diuretics, antidiabetic drugs like sulfonylureas and calcium channel blockers, drugs for treating Parkinson's disease were too eliminated from the study as the above drugs known to result in taste alterations [7].

Quantification of Smoking: A number of studies on correlating lung cancer with habit smoking have used pack-year as a unit to quantify smoking over a long period [8,9].

Calculation

Number of pack-years = (packs smoked per day) × (years as a smoker)

OR

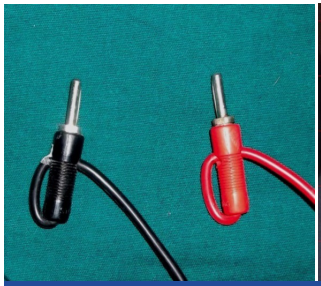
Number of pack-years = (number of cigarettes smoked per day × number of years smoked)/10

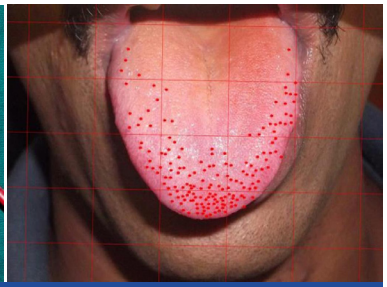
One pack has 10 cigarettes according to Indian manufacturing standards.

Procedures

Electrogustometry

- All the subjects were instructed not to drink, eat or smoke for an hour prior to the beginning of the test.
- An imaginary division of anterior 2/3rd of dorsum of tongue into four quadrants was done.
- In each quadrant electrode with a diameter of 4mm was placed and electrogustometric analysis was carried out on the subjects by using modified Transcutaneous Electrical Nerve Stimulation (TENS) machine.
- The TENS machine was modified by removing the rubber pads at the end of the electrodes and exposing the steel electrodes [Table/Fig-1].





[Table/Fig-1]: Modified electrodes of TENS machine.**[Table/Fig-2]:** Counting of fungiform papillae using BIOWIZARD® software.

- Circuit was completed by asking the patient to hold the positive electrode in one hand and the negative was placed on the tongue 1cm from the midline on four locations on the tongue dorsum on either side.
- A minimal current frequency of 10 Hz and a pulse width of 1 micro second were kept constant and the current was increased till the patient feels acidic taste.
- This current reading at which the patient feels metallic or acidic taste sensation was recorded and the average reading of the four quadrants was calculated.

Training the subject: Manual method of electrogustometry was done in which the subjects were verbally appraised about the procedure and then demonstrated by increasing the current gradually and asked to raise their hand to indicate if they felt sour / metallic taste with a 'yes' or 'no' response.

Tongue photography and fungiform papillae count assessment

- The patients tongue was cleaned with cotton gauze.
- The patient was asked to protrude the tongue maximally in a relaxed state.
- Photograph of the tongue along with a centimetre scale was taken using Fujifilm AV 150 digital camera with Fujinon 3X optical zoom lens (14 megapixels) in macro mode.
- The photograph was transferred to a computer in which the fungiform papillae were counted using software for image analysis called BIOWIZARD® (Dewinter Inc., U.S.A).
- Grid from the software was superimposed onto the image and stretched to coincide with the centimetre scale markings in the photograph [Table/Fig-2].
- This is to have a uniform magnification of the image.
- The numbers of fungiform papillae were counted in each grid box of the image using the mouse cursor and clicking onto each papilla which marked the papilla with a red arrow.
- The number of fungiform papillae were averaged to give mean number of fungiform papillae on the tongue per square centimetre of the tongue.

Parameter	FPAP	EGM	RDW
Age	r=-0.699	r=+0.473	--
	p=0.03	p=0.04	--
Pack-yr	r=-0.403	r=+0.420	r=+0.186
	p=0.027	p=0.021	p=0.325
FPAP	r=1	r=-0.612	r=-0.349
	p=0.00	p=0.001	p=0.059
RDW	r=-0.349	r=+0.143	r=1
	p=0.059	p=0.452	p=0.00

[Table/Fig-3]: Pearson's correlation and independent sample t-test values in smokers.
'r'= Pearson's correlation; 'p'= t-test significance; fpap= Fungiform papillae count; EGM= Electrogustometric threshold; RDW= Red cell distribution width.

Parameter	FPAP	EGM	RDW
Age	r=-0.290	r=+0.172	--
	p=0.04	p=0.05	--
FPAP	r=1	r=-0.590	r=+0.175
	p=0.00	p=0.001	p=0.356
RDW	r=+0.175	r=+0.002	r=1
	p=0.059	p=0.452	p=0.00

[Table/Fig-4]: Pearson's correlation and independent sample t-test values in non-smokers.
'r'= Pearson's correlation; 'p'= t-test significance; fpap= Fungiform papillae count; EGM= Electrogustometric threshold; RDW= Red cell distribution width

Blood sample collection: A 2ml to 3ml of blood sample was withdrawn into a vacuum tube coated with EDTA (Ethylene Diamine Tetra Acetic Acid) using venipuncture method and sent to the laboratory for red blood cell distribution width assessment by electrical impedance using Erma PCE-210® cell counter.

STATISTICAL ANALYSIS

Descriptive statistics, Crosstabs (Contingency table analysis), Independent samples't' test and Pearson's correlation coefficient were the statistical methods applied in the study. All the statistical calculations were done through SPSS for windows (v 16.0).

RESULTS

Study sample: A total of 60 study subjects were included in the study. Thirty subjects formed the experimental group (smokers) and 30 subjects in control group (non-smokers) and all the subjects were males in the age range of 25 to 45 years.

In the present study a moderate direct correlation (r=0.473) of age exists with the electrogustometric threshold and a strong negative correlation (r = -0.699) exists between age and fungiform papillae in smokers [Table/Fig-3]. But in non-smokers the correlation between age and electrogustometric threshold was weak (r=0.172) and the relationship between age and fungiform papillae was also weak (r=-0.290) [Table/Fig-4].

The smokers had a mean 15.88±13.14 pack-year with a majority of them having 10 pack-years.

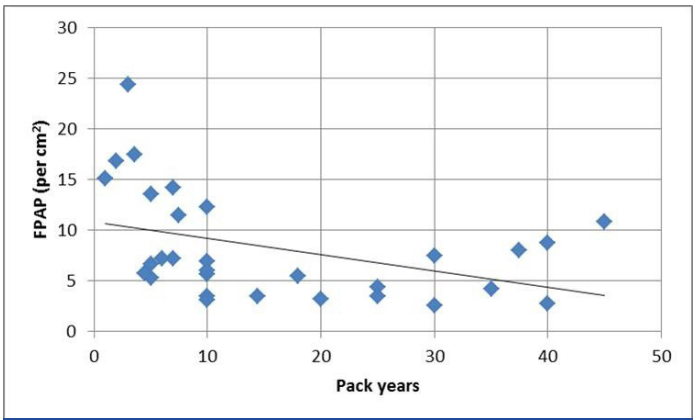
Relationship between smoking and fungiform papillae count: The mean fungiform papillae count in smokers was 8.22±5.30 papillae/cm^2 and in non smokers it was 12.42±4.18 papillae/cm^2. There existed a significant difference (p=0.001) [Table/Fig-5] between the two groups in relation to fungiform papillae count.

On Pearson's correlation analysis, a significant moderate negative correlation (r=-0.403) (p= 0.027) exists between the pack-year and fungiform papillae count [Table/Fig-3]. There exists an inverse relationship between pack-year and fungiform papillae count. This means that as the quantity of smoking is increased the fungiform papillae count is decreased. This shows the negative effect of smoking on the quantity of fungiform papillae [Table/Fig-6].

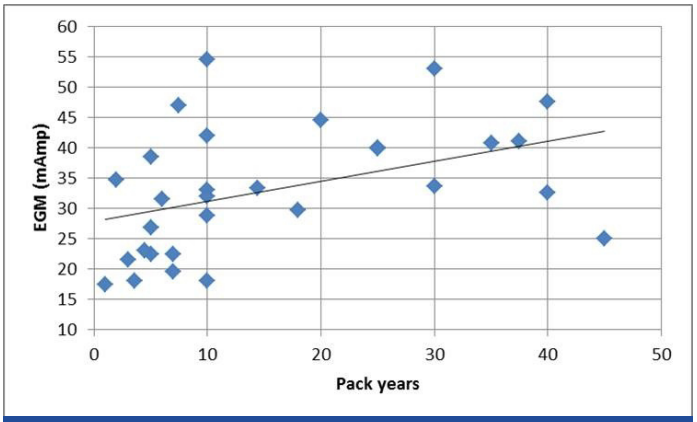
Relationship between smoking and electrogustometry (EGM): In contrast, the mean EGM for smokers was 33.06±10.49 mAmp and for non-smokers 24.85±8.99 mAmp. The two groups showed a significant difference (p = 0.002) with respect to EGM [Table/Fig-5]. The EGM showed a significant direct relationship (p=0.021) and a positive moderate correlation (r=+0.420) with the pack-year [Table/Fig-3,7]. This indicates that as the quantity of

Factor	Group	N	Mean	Std. Deviation	Std. Error Mean	Significance 'p' value
FPAP (per cm^2)	Smokers	30	8.22	5.30	0.967	0.001
	Non smokers	30	12.42	4.18	0.764	
EGM (mAmp)	Smokers	30	33.06	10.49	1.916	0.002
	Non smokers	30	24.85	8.99	1.642	
RDW (%)	Smokers	30	15.42	1.22	0.223	0.039
	Non smokers	30	14.85	0.82	0.150	

[Table/Fig-5]: Independent sample t-test of various parameters.
FPAP =fungiform papillae count; EGM= electrogustometric threshold; mAmp= milli amperes.



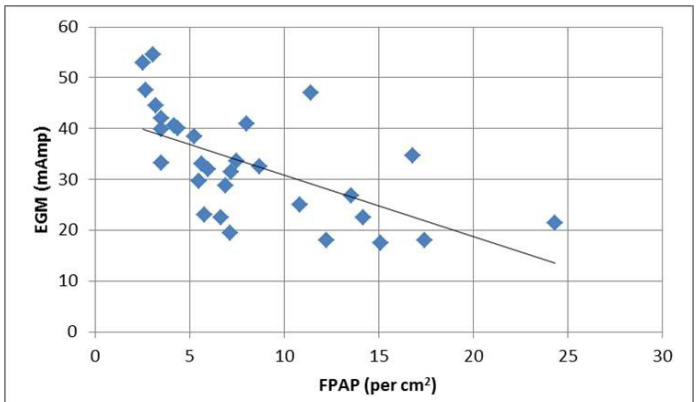
[Table/Fig-6]: Relationship between pack-year and fungiform papillae count (fpap) in smokers.



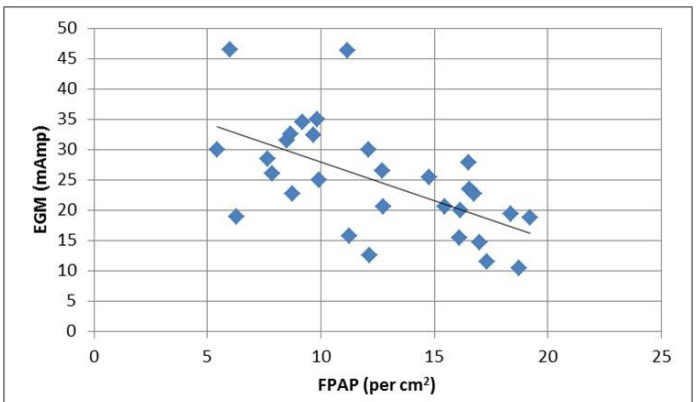
[Table/Fig-7]: Relationship between pack-year and electrogustometric thresholds (EGM) in smokers.

Parameter	FPAP	EGM	RDW
FPAP	r=1	r=-0.667	r=-0.256
	p=0.00	p=0.001	p=0.048
RDW	r=-0.256	r=+0.186	r=1
	p=0.048	p=0.156	p=0.00

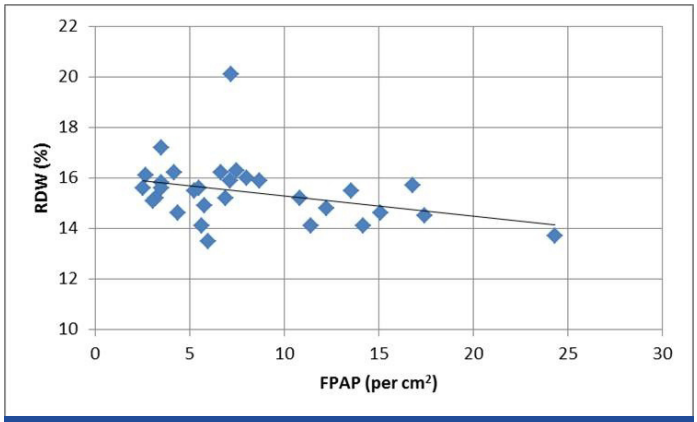
[Table/Fig-8]: Pearson's correlation and independent sample t-test values in smokers & non-smokers combined.
'r'= Pearson's correlation; 'p'= t-test significance; FPAP = Fungiform papillae count; EGM= Electrogustometric threshold; RDW= Red cell distribution width.



[Table/Fig-9]: Relationship between electrogustometric thresholds (EGM) and fungiform papillae count (FPAP) in smokers.



[Table/Fig-10]: Relationship between electrogustometric thresholds (EGM) and fungiform papillae count (FPAP) in non-smokers.



[Table/Fig-11]: Relationship between RDW and fungiform papillae count (FPAP) in smokers & non-smokers.

smoking is increased the taste thresholds of the subjects will also amplify.

Relationship between EGM and fungiform papillae count: An overall (inclusive of smokers and non-smokers) Pearson's analysis showed a highly significant (p=0.001) strong negative correlation (r=-0.667) between the EGM and fungiform papillae count [Table/Fig-8]. Subjects with more fungiform papillae count showed a lower taste threshold than those with fewer papillae. Subjects with fewer fungiform papillae showed an increased taste threshold. A similar trend was noted among smokers [Table/Fig-3] (p=0.001; r=-0.612) [Table/Fig-9] and non-smokers [Table/Fig-4] (p=0.001; r=-0.590) [Table/Fig-10].

Red cell distribution width (RDW): The mean RDW values of smokers (15.42±1.22 %) was significantly higher (p=0.039) than RDW values of non-smokers (14.85±0.82 %) [Table/Fig-5]. All the values were in normal range (11.6 – 14.6 %).

Relationship between RDW and fungiform papillae count: On Pearson's correlation analysis of all subjects together, a significantly weak negative correlation (p=0.048; r = -0.256) was seen between the FPAP and RDW [Table/Fig-8]. This indicated that RDW was inversely related to and fungiform papillae count [Table/Fig-11]. However, a similar relationship in individual groups of the smokers (p=0.059) [Table/Fig-3] and non-smokers (p=0.356) [Table/Fig-4] could not be established. There was no significant (p=0.325) correlation (r=+0.186) between RDW and smoking.

Relationship between RDW and electrogustometry (EGM): No significant (p=0.156) correlation (r=+0.186) was found between RDWand EGM in combined groups [Table/Fig-8]. Similar trend persisted on analyzing RDW and EGM in individual groups (Smokers p=0.452; r=+0.143) [Table/Fig-3] (nonsmokers p=0.99; r= +0.002) [Table/Fig-4].

DISCUSSION

The simplest test for evaluation of taste is EGM. EGM was introduced in the clinical assessment of taste sensitivity during the 1950s. It has good test retest reliability [4]. Compared to tests based on chemical solutions, EGM is an efficient clinical tool, used in the evaluation of taste disorders. Increased use of this technique is due to its easiness, the short session required and its measureable character. However, very few experimental studies provide data about the effects of smoke on the number of fungiform papillae. Therefore, the effect of smoking on fungiform papillae density and electrogustometric thresholds was evaluated.

Age: In the present study a moderate direct correlation of age exists with the electrogustometric threshold and a strong negative correlation exists between age and fungiform papillae in smokers. But in non-smokers the correlation between age and electrogustometric threshold was weak and the relationship between age and fungiform papillae was also weak.

The mean age of smokers was 38.13±8.16 years and that of non-smokers was 32.7±8.35 years. The subjects in the study were carefully selected to be in the age range of 25 to 45 years because a reduction in density of fungiform papillae (number of papillae per unit area of tongue) was reported on the human tongue from 40-55 years of age [10].

Effect of smoking on fungiform papillae count: As the quantity of smoking is increased the fungiform papillae count is decreased. There is also a significant decrease in the fungiform papilla density in smokers as compared to non-smokers. A study on burning mouth syndrome patients obtained a count of fungiform papillae as 27.55±2.12 fungiform papillae/cm^2 for burning mouth syndrome patients and 31.57±3.11 fungiform papillae/cm^2 for controls [11].

In a similar study on smokers and non-smokers, the papillae count in non-smokers was reported as 27±7.6; 26±6 fungiform papillae/cm^2 and in smokers as 25.7±4.2; 24.9±5.9 fungiform papillae/cm^2 [4]. This shows the negative effect of smoking on the quantity of fungiform papillae.

The relationship between the number of fungiform papillae and taste function is never ideal. Fungiform papillae are relatively stable anatomical structures whose pattern of distribution and number depend on genetic factors [12]. However, taste sensations depend not only on the number of fungiform papillae but also on the integrity of taste buds within the papillae as well as the nerve carrying information from papillae to brain. Saito et al., reported that there was no difference in the number of fungiform papillae between the two sides of the tongue patients in whom the chorda tympani nerve has been severed unilaterally. The loss of fungiform papillae seen in human subjects who suffer damage to both the chorda tympani and trigeminal nerves is due to damage to trigeminal nerve [13].

Effect of smoking on electrogustometric thresholds: As the quantity and duration of smoking increased, a directly proportional increase in electrogustometric taste thresholds was seen. The reason behind above finding is the reduction in the count of fungiform papillae on the dorsum of tongue in smokers. Contact endoscopic findings of the smokers' tongues suggest alteration in the morphology of fungiform papillae and alterations in their vascular supply which cause higher electrogustometric thresholds [4].

Grant et al., in 1987 compared the evoked taste threshold in smokers and nonsmokers using electrogustometry and reported a significant elevation in taste threshold with age in both smokers and non-smokers. This was attributed to the decrease in number of papillae and number of taste buds per papilla with ageing [14].

Relationship between fungiform papillae and electrogustometric thresholds: In the present research a similar significant (p=0.001) inverse relationship is seen between the fungiform papillae count and electrogustometric thresholds both in smokers and non-smokers. The mean papillae density in smokers is significantly less than those of non-smokers. Alterations in morphology and vascularity of papillae due to smoking and subsequent atrophy can be the cause of such a finding [4]. Also the amount of keratinization of masticatory mucosa is increased in smoking and aging. In a study conducted on patients with burning mouth syndrome, the electrogustometric values were reported higher in subjects with burning mouth syndrome. This has been attributed to the decreased fungiform papillae count in burning mouth syndrome patients as compared to controls [11].

Relationship between RDW and fungiform papillae: RDW can give the idea of early changes in RBC, which is accompanied in iron deficiency anemia. In prelatent and latent iron deficiency, Hb% and MCV are normal. In latent iron deficiency RDW would be expected to increase because of microcytic population of cells [15].

In our study, an attempt was made to correlate the fungiform papillae count with the RDW values to assess if any subclinical changes possibly due to nutritional deficiencies could result in altered numbers of fungiform papillae of study subjects. There is an inverse relationship between RDW and the density of fungiform papillae.

Lu and Wu (2004) in their retrospective study investigated RDW and mean corpuscular volume (MCV) in patients with wide range sore mouth [5]. They reported atrophic changes in the tongue papillae of individual subjects with iron deficiency at RDW of 24.3%, 19.5% and 18.9%. Atrophic changes in the tongue papillae of individual subjects with vitamin B12 deficiency occurred at 15.1% and 15%. In subjects with combined deficiency of iron and vitamin B12, an RDW of 13.4% was reported [5]. Therefore, even for small changes in tongue papillae to be clinically appreciated, a greater variation from the normal range of RDW (11.6 – 14.6 %) is required [6]. In the present research there was not much of a variation in the RDW values in smokers group and non-smokers group and that most of the values were in normal range with only few exceptions. This could explain the weak association between RDW and fungiform papillae count.

Effect of smoking on red cell distribution width: RDW is a measurement of the variability in size of circulating erythrocytes. Although no statistically significant correlation is seen between smoking and RDW, a significant increase in RDW of smokers (15.42±1.22 %) is seen as compared to non-smokers (14.85±0.82 %). A similar result has been put forth by Kurtoğlu et al., (2013) [16].

Thus, elevated RDW may also be a surrogate measure of the chronic inflammatory process in smokers, which results in ineffective erythropoiesis causing immature RBCs to enter the circulation and in turn results in heterogeneity in the size of RBCs causing anisocytosis. Exposure to greater oxidative stress may be yet another potential contributing pathophysiologic mechanism linking higher RDW with smoking. A relationship between smoking and higher oxidative stress has been established [17]. It has been shown that oxidized RBCs lose their flexibility owing to a loss of lipid asymmetry and cytoskeleton rearrangement, causing them to be more rigid and thus develop anisocytosis [18].

LIMITATION

This study was unique because a modified TENS machine was used to measure the deleterious effect smoking on taste perception. However, the only drawback being was modified TENS machine could not be compared with an actual electrogustometer to calibrate the taste threshold readings. Therefore, further studies are required in this area to standardize this new methodology.

CONCLUSION

The results suggest that smoking has a negative effect on taste sensation and this is because of decreased number of fungiform papillae in smokers as compared to non-smokers.

RDW values show an inverse relationship with the number of fungiform papillae but do not correlate with the taste sensation.

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