ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 2, Issue – 5, September – October - 2019, Page No. : 191 - 197

Estimation and Correlation of salivary thiocyanate levels and gingivitis in healthy subjects non-smokers and smokers- A cross-sectional study.

Kirti Raina[1], Anuridhi Choudhary[2], Meenakshi Thakur[3]

MDS Public Health Dentistry, Jammu, India

Corresponding Author: Kirti Raina, MDS Public Health Dentistry, Jammu, India

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Context: Saliva is considered to be critical for the maintenance of healthy oral mucosa. It is the first biological fluid to encounter cigarette smoke which is a major risk factor for oral cancer. Thiocyanate (SCN) is a metabolic product of cyanide found in serum, saliva etc.

Aims: The aim of this study was to estimate and correlate salivary thiocyanate (SCN) levels and gingivitis in healthy subjects, non-smokers and smokers.

Methods and Material: A cross-sectional study comprised of 150 study subjects in the age group of 18–55 years that was further divided into three groups: Control, non-smokers, and smokers. Gingival status was assessed by using (GI) (Loe and Silness-1963), and estimation of SCN was performed by colorimeter at 447 nm wavelength.

Statistical analysis used: ANOVA test and Pearson's correlation test using SPSS version 21.1 software.

Results: Results showed statistically significant increase in SCN levels in smoker's i.e. 11.569±3.851 in smokers and very low in two other groups. Significantly gingivitis was seen in non-smokers but not in smokers. A negative correlation observed between the GI and thiocyanate levels.

Conclusions: The present study revealed a significant increase in SCN levels and less gingival bleeding in smokers as compared to other groups

Keywords: Salivary Thiocyanate, Smokers, Gingivitis, Saliva

Introduction

Oral health is multifaceted and includes the ability to speak, smile, smell, taste, touch, chew, swallow and convey a range of emotions through facial expressions with confidence and without pain, discomfort and disease of the craniofacial complex. It reflects the physiological, social, and psychological attributes that are essential to the quality of life.[1]

The oral cavity is kept moist by a film of fluid called saliva that coats the teeth and mucosa. Saliva is a complex fluid, produced by salivary glands, the most important function of which is to maintain the well-being of the mouth. It is a mirror of the body. It is considered to be critical for the maintenance of healthy oral mucosa and is the first biological fluid to encounter cigarette smoke.

Smoking is a major risk factor for oral cancer. Tobacco usage is a major preventable cause of death and disease worldwide, irrespective of whatever form it is being used. Tobacco consumption is a major risk factor for mortality [2]. After China, India is the second largest nation in the

world, with respect to tobacco production and also consumption.[3]

In India, beedi smoking is the most popular form of tobacco smoking, followed by cigarette smoking. Chemical measures such as Salivary thiocyanate how promising results in obtaining accurate, quantitative information on smoking habits.[4]

Thiocyanate (SCN) is a metabolic product of cyanide, found in organic and inorganic compounds and is a normal constituent of body fluids such as serum, saliva, tears and urine.[5]

Salivary thiocyanate has a property to induce cancerous changes in epithelium. Salivary thiocyanate is secreted in saliva and has a long half life of 10-14 days in normal adults and is in continuous contact with epithelium through blood and saliva. Hence, it is important to know about the SCN being liberated in different forms of tobacco consumed by humans.[6] So, the present study was conducted aiming to investigate the salivary thiocyanate levels and gingivitis in healthy subjects, non-smokers and smokers.

Methodology

A cross-sectional study was carried out on 150 study subjects. Ethical clearance was obtained from the Institutional Review Board (ITSCDSR/L/2017/079/12/9/17)before starting the study. Informed consent was taken from every patient and demographic details were recorded and later on clinical examination and sample collection was conducted at the same time**.** A pilot study was conducted on 20 patients to assess the sample size and feasibility of the study.

The study population was divided into three groups with 50 participants in each group.

Group I – Healthy subjects

Group II- Non-smokers

Group III – Smokers

Sample Size Determination- Sample size for three groups at 5% level of significance and 80% power

$26\ s^2/d^2 + 1$**.**

Where s=standard deviation

D=effect size (Expected difference between the groups)

Taking s=1.5 and d=0.5 the sample size comes out to be 46 for each group

The patient were selected by convenience sampling method who met the following criteria:

Inclusion criteria

-Subjects with > 20 natural teeth.

-Subjects who did not have any systemic disease such as Diabetes Mellitus, Atherosclerosis etc.

Exclusion criteria

-Subjects with <20 natural teeth.

-Subjects suffering from any systemic disease such as Diabetes Mellitus, Artheriosclerosis etc.

-Subjects on any antimicrobial or anti-inflammatory medication within the previous 3 months for any reason.

- History of regular use of mouth washes.

A prevalidated, pretested, structured and self-administered questionnaire in English was used. This questionnaire was used for an initial pilot study to check if they were suitable for the Indian population. Suitable changes were made and a final questionnaire was administered to the study subjects. Face validity was done and Cronbach's alpha was 0.89 indicating good reliability.

The study Performa consisted of 3 parts-

First part consisted of questionnaire for collecting demographic details. The second part consisted of tobacco usage status, type of Tobacco used, frequency in current users, amount of consumption, duration. The third part consisted of clinical examination in which gingival index was used to access gingival condition.

Clinical examination: The patients were seated comfortably in a chair with the examiner standing in front of the chair and clinical examination were carried out under good illumination.

The Gingival status was assess by using gingival index (Loe H and Sillness 1963). The data was collected from the month of **October 2017 to April 2018**. Each day about 10 subjects were examined and sample was collected.

Collection of Saliva Samples: Each subject was asked to accumulate saliva in the mouth for about 2minutes, after which he was asked to spit the accumulated saliva in a sterile plastic container. The unstimulated saliva thus collected was refrigerated at-20°c, and processed within 24 hours.

Protocol

Principle

Tobacco smoke contains HCN gas. Once this gas is inhaled, it is dissociated into its components according to the following equilibrium equation: HCN (aqueous) + H^+ (aqueous) + CN^- (aqueous). The CN^- ion is then converted into the SCN ion when it reaches the liver. Ferric ions (Fe3+) present in ferric nitrate (Fe(NO3)3 react with thiocyanate in the saliva to form a deep-red colored complex thiocyanate iron (III) ion (FeSCN2+) which was measured calorimetrically at 447 nm wavelength The intensity of the color is directly proportional to the concentration of cyanide and also proportional to the level of thiocyanate exposure[24].

$Fe^{3+} + SCN = FeSCN^{2+}$

Reagent used

1) SCN standard

a) Stock solution- To prepare the stock solution we weighed 50 mg of KSCN and dissolved it in 50ml of distilled water. Then the stock solution of 50 mg/50ml were obtained.

b**) Working solution:** From the above solution we took 10ml and added to 40ml of distilled water which gave us 10mg/100ml working KSCN (Potassium Thiocyanate) solution. This was used to put the standard.

2) Ferric nitrate solution: To make ferric nitrate solution 50gm of crystalline ferric nitrate was dissolved in 500ml of distilled water, and 25ml of concentrated HNO_3 was added to make 1litre of solution with distilled water.

3) Blank solution: The blank solution was 25ml of concentrated HNO_3 diluted to 1 litre of solution with distilled water.

Calibration: We took 5 tubes:-Tube 1 contains 9ml distilled water without KSCN solution and 1ml of ferric nitrate was dissolved.

Tube 2 contains 8.5ml distilled water with 0.5ml KSCN and 1ml ferric nitrate was dissolved.

Tube 3 contains 8ml distilled water with 1ml KSCN and 1ml ferric nitrate was dissolved.

Tube 4 contains 7.5ml distilled water with 1.5 ml KSCN and 1ml ferric nitrate solution was dissolved.

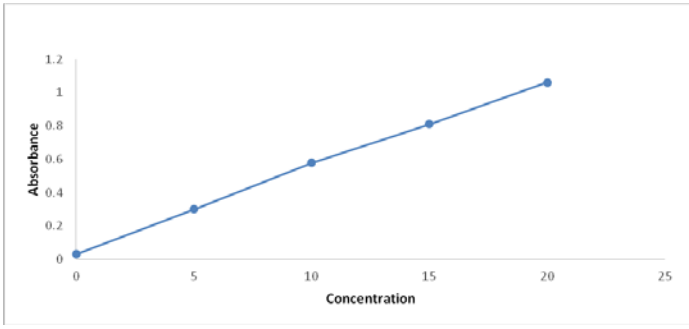
Tube 5 contains 7ml distilled water with 2ml KSCN and 1ml ferric nitrate was dissolved.

Then the absorbance readings was taken after 5minutes in colorimeter at 447nm wavelength. An average of the 5 readings was recorded as the standard reading.

Protocol for standard curve

Tube no	1	2	3	4	5
Working KSCN ion	0	0.5	1	1.5	2
Distilled Water	9	8.5	8	7.5	7
Ferric nitrate Solution					
Readings	0mg%	5mg%	10mg%	15mg%	20 mg%
	0.03	0.30	0.58	0.83	1.02

Standard curve with varying concentration of thiocyanate standard



The calibration on colorimeter revealed that the absorbance readings were directly proportional to the SCN in varying strengths of standard. The calibration curve was straight line. Therefore the use of a standard reading "S" obtained from readings solution containing 10mg of SCN ions per 100 ml is valid within the range.

Procedure

1) All the tubes used in the procedure was thoroughly washed by distilled water before use.
2) Saliva sample was centrifuged at 4000 rpm for 10 min and clear saliva was separated from the impurities.
3) The unknown solution (the saliva sample whose SCN concentration has yet to be determined) was made by placing one half of the clear saliva sample i.e. 0.5ml of clear saliva was taken into separate test tube. 4ml of distilled water was added to it and mixed thoroughly. Then 0.5ml of ferric nitrate reagent was added to it slowly with shaking.
4) The other half of clear saliva sample- i.e. 0.5ml of clear saliva was placed into a separate test tube. 4ml of distilled water was added to it and mixed thoroughly. Then 0.5 ml of blank solution (nitric acid with distilled water) was added to it slowly with shaking (blank solution).
5) After 5 minutes the reading at 447 nm was taken on colorimeter.

Calculation: The reading of blank solution was subtracted from the reading of unknown to obtain the true reading of unknown i.e. final optical density and since 10mg per 100 ml solution was used, the reading were calculated as:

Reading of unknown (U)

---------- X 1 0 mg of SCN^- per 100ml of saliva

Reading of standard (S)

Where reading of standard= 0.58

U/Sx10=mg of SCN^- per100 ml of saliva i.e. U/0.58x10=mg of SCN^- per 100 ml of saliva

Results

It represents the mean distribution of gingival index scores among study groups in which it was noticed that Group I has mean score of 0.00 ± 0.000, Group II has mean score of 2.62 ± 0.490, Group III has 1.30 ± 0.767 which means less gingival bleeding was seen in smokers.(Table 1).

It depicts the inter-group comparison of gingival index score between different groups in which we found out that there was a statistically significant difference among all the groups.(Table 2)

It shows the mean distribution of salivary thiocyanate concentration among study subjects in which Group I has mean score of 0.358±0.299, Group II has mean score of 1.793±0.908, Group III has 11.569±3.851 which means smokers has high salivary thiocyanate levels among all.(Table 3).

It shows the intergroup comparison of salivary thiocyanate concentration among study subjects in which we found out that there was highly statistically significant difference among all the groups. (Table 4)

It depicts the correlation between Gingival Index and thiocyanate concentration among different study groups by using Pearson correlation test. In Group II GI and SCN were negatively correlated, i.e., if SCN increases then GI decreases or if GI increases SCN decreases. Negative

correlation exists between GI and SCN of Group III and statistically non- significant. (Table 5)

Discussion

The study reveals that smokers showed a negative correlation between GI score and thiocyanate levels, i.e. in Group II r- 0.457, Group III r-0.123 which was in accordance with the study done by Kalburgi C.V et Al [7] in which correlation was Group II: r = −0.500, Group III: r = −0.123.

The present study revealed a significant increase in salivary SCN level in smokers as compared to non-smokers and healthy subjects i.e. mean score was 11.569±3.851 in smokers and very low in two other groups. Thus, it can be estimated more in smoked tobacco as compared to other groups which are in accordance with a study done by Aggarwal et al [8] in which they concluded a mean score of i.e.12.8± 3.19 in smokers and 3.6±1.26 in non-smokers. Also our result are in accordance with study done by Kalburgi V et al[7] in which they also found the mean score of salivary thiocyanate 7.54817±4.61955 in smokers, 2.3455±1.71345 in gutka chewers, 1.57132±1.41962 in non-smokers and 1.97453±1.58674 in healthy subjects. The result were also in accordance with study done by Baldawa P.S et al [9] in which they reported the mean score of salivary thiocyanate 5.31±0.51 in smokers and 1.17± 0.51 in control group.

It was also found that tobacco users may present with lower levels of gingival inflammation than non-users. In our study gingival mean was 1.7 ± 0.767 in smokers, 2.62 ± 0.490 in non-smokers and 0.00 ± 0.000 in healthy subjects. This distribution may point towards the fact that tobacco usage decrease gingivitis which is supported by a review conducted by Pejcic A et al [10] also in accordance with the study conducted by Sreedevi M [11] et al which showed that gingival bleeding was less in smokers as compared to non-smokers i.e. 0.62 ± 0.31 in smokers and 0.86 ± 0.41 in non-smokers. This was also similar to study done by Kalburgi et al [7] in which mean gingival score was 1.4±0.56 and 1.8±0.53 in smokers and gutka chewers, 1.9±0.51 in non-smokers with chronic periodontitis and 0.7±0.45 in healthy subjects. Another study done by Jogezai U et al [12] which showed that Gingival bleeding score was less in smokers i.e. 53.5% in non-smokers and 31.7% in smokers. The reduced bleeding in smokers has been attributed to gingival vasoconstriction induced by the actions of nicotine which also causes reduced blood flow, odema, decreased gingival inflammation, redness and bleeding because nicotine content found more in smoking tobacco 15.6mg/gm in cigarette smokers and 26.9mg/gm in beedi smokers [13].

Conclusion

It was concluded that there was a significant increase in salivary SCN levels in smokers as compared to non-smokers and healthy subjects. The study also reveals that tobacco users have a negative correlation towards gingival bleeding.

References

1. Glick M, Williams D. M, Kleinman D.V, Vujisic M, Watt R.G, Weyant R.J . F, “A New Definition for Oral Health Developed by the FDI World Dental Federation Opens the Door to a Universal Definition of Oral Health,” J Am Dent Assoc. vol. 147, no. 12 pp. 915-917, 2016
2. World Health Organization. Tobacco or Health: A Global Status Report. Geneva: WHO; 1997.
3. Reddy KS and Gupta PC. Report on Tobacco Control in India. Mumbai: Ministry of Health and Family Welfare; 2004.
4. WHO Framework Convention on Tobacco Control. 11th World Conference on Tobacco or Health 2000. Tobacco Fact Sheet: Tobacco in Developing World: The Economics of Tobacco Control. 2000.

5. Xu Y, Szép S and Lu Z, "The Antioxidant Role of Thiocyanate in the Pathogenesis of Cystic Fibrosis and other Inflammation -Related Diseases," Proc Natl Acad Sci U.S.A, vol. 106 no. 48, pp.20515 -20519, 2009.
6. Muhammad I.I, Wasagu R S U and Abubakar M.K "Salivary Level of Thiocyanate in Smokers and Non-Smokers: A Case Study Taken at Usmanu Danfodiyo University, Sokoto Nigeria" A Journal of Life Sciences, vol 7 no. 1 pp. 1-5.
7. Kalburgi, C.V, Naik K.L, Kokatnur M.V, Warad S, " Estimation and Correlation of Salivary Thiocyanate Levels in Healthy and Different forms of Tobacco Users having Chronic Periodontitis: A Cross-Sectional Biochemical Study," Contemp Clin Dent vol.5, no. 2, pp. 182–186, 2014.
8. Aggarwal A, Keluskar V and Goyal R, Parveen V, "Salivary Thiocyanate: A biochemical Indicator of Cigarette Smoking in Adolescents," Oral Health Prev Dent vol. 11, no. 3, pp. 221-227, 2013.
9. Baldawa P.S, Kulkarni V.V, Koshy A.V, Sheikh S, Varur R. Srivastva G, " Levels of Salivary Thiocyanate and its Relation with Occurrence of Micronuclei Using Exfoliative Cytology in Smokers and Non-Smokers," Indian Journal of Dental Research; vol. 27, no. 6, pp. 568-573, 2016.
10. Pejcic A, Obradovic R and Kesic L, "Smoking and Periodontal Disease- A review," Facta Universities Series: Medicine and biology, vol. 14, no. 2, pp. 53-59, 2007.
11. Sreedevi M, Ramesh A, Dwarakanath C. Periodontal Status in Smokers and Non- Smokers: A Clinical, Microbiological and Histopathological Study. International Journal of Dentistry 2012; 1-10.
12. Jogezai U, Maxood A and Khan NA, "Comparison of Periodontal Health Status of Smokers and Non-Smokers," Journal of Ayub Medical College, Abbottabad vol. 25 no., pp. 183-186, 2013.
13. Reddy S, Pyder H.A.K. Estimation of Nicotine Content in Popular Indian Brands of Smoking and Chewing Tobacco Products. Indian J Dent Res; vol. 19, no. 2, pp.88-91, 2008.

Legends Tables

Table 1 Distribution of study subjects according to mean GI scores

Groups	N	Mean(± S.D)
Group I	50	0.00 (± 0.000)
Group II	50	2.62 (± 0.490)
Group III	50	1.30 (± 0.767)

Table 2 Comparison of GI scores among study subjects (using ANOVA test)

Groups	Mean Difference	Std. Error	p value
Grp I vs Grp II	-2.62000*	.07626	0.000
Grp I vs Grp III	-1.30000*	.07626	0.000
Grp II vs Grp III	1.32000*	.07626	0.001

$p \leq 0.05$ is significant

Table 3 Distribution of study subjects according to mean salivary scn concentration

Groups	Total	Mean(± S.D)
Group I	50	0.358(±0.299)
Group II	50	1.793(±0.908)
Group III	50	11.569(±3.851)

SCN- Thiocyanate, S.D- Standard Deviation

Table 4 Comparison of salivary scn concentration among study subjects

Group	Mean Difference	Std. Error	p value
Grp I vs Grp II	-1.43448*	.49050	.004
Grp I vs Grp III	-11.21034*	.49050	.000
Grp II vs Grp III	-9.77586*	.49050	.000

p value < 0.05 shows statistically significant difference, SCN- Salivary thiocyanate

Table 5 Correlation between GI and SCN concentration among different groups using pearson correlation test)

Groups	GI Scores	Thiocyanate Conc.	Pearson Correlation Coefficient	p value	Significance
Group I	0.00	0.358	-	-	-
Group II	2.62	1.793	-0.457	0.035	Significant
Group III	1.70	11.569	-0.015	0.919	Non-Significant