Toxic thiocyanate levels in saliva of healthy adult smokers and non smokers from Baghdad city [+]

مستويات تركيز ايون الثايوسيانات في عينات لعاب الاشخاص الاصحاء من المدخنيين وغير المدخنيين من مدينة بغداد

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Abstract:

The use of tobacco is a world wide problem and the traditional method of tobacco use is smoking cigarettes. The aim of this study is to investigate the effect of cigarettes smoking on toxic thiocynate levels in saliva.

Thiocynate levels were determined by spectrophotometic method, for salivary samples collected from 44 healthy adult volunteers salivary thiocyanate (mean ± standard deviation: 2800 ± 1210µM) levels were significantly higher in the group of 29 tobacco smokers than in the group of 15 non smokers (3530 ± 1410, 1070 ± 950 µM). Its concluded that the numbers of cigarettes smoking per day have the real effect on salivary thiocyanate level

Key words:Thiocyanate, tobacco smoking, saliva and spectrophotometric method.

المستخلص:

يعد التدخين مشكلة عالمية، ويمثل تدخين السكائر الطريقة التجارية الاكثر شيوعا.قدرت في هذه الدراسة مستويات تركيز آيون الثايوسيانات (SCN^-) في عينات لعاب الاشخاص الاصحاء من المدخنين وغير المدخنين باستخدام طريقة قياس الامتصاصية في الطول الموجي المرئي. وكانت نتائج عينات (٤٤) متطوع: (المعدل ± الانحراف القياسي = ٢٨٠٠ ± ١٢١٠ µ M)

وسجلت نتائج المدخنين منهم ارتفاعا ملحوظا (P< ٠,٠٠١) مقارنة بغير المدخنين، اذ كانت النتائج لـــ ٢٩ شخصا من المدخنين (٣٥٣٠ ± ١٤١٠ µ M) و ١٥ شخص من غير المدخنين (١٠٧٠ ± ٩٥٠ µ M).

ثبت نتيجة البحث ان عدد السكائر المدخنة في اليوم كمثل العامل الرئيسي المؤثرعلى تركيز ايون الثايوسيانات في لعاب الاشخاص المدخنين.

Introduction

Tobacco smoke contains HCN gas. The ingestion of this smoke leads to the formation of the thiocyanate [SCN^-] in the liver [1].

Blood thiocyanate is mainly distributed in serum and its presence is regarded as evidence of cyanide detoxification. High levels of thiocyanate are also found in saliva, and the physiological role of salivary thiocyanate may be the antibacterial effect of hypo thiocyanate which is produced by the action of salivary peroxidases from thiocyanate [2].

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Saliva, like other bodily fluids, has been used to monitor human health and disease [3]. Saliva is no a passive ultra filtrate of serum [4], but contains a distinctive composition of enzyme, hormones, antibodies, and other molecules.

The use of saliva as a diagnostic fluid has been successfully applied in diagnostics and for predicating populations at risk for a variety of conditions [5,6,7,8,9].

Several studies had been searched on smoking and its relation to caries experience [10], dental status in different years - old [11], duration and salivary secretion [12], and toxic thiocyanate levels in saliva [1].

This study in tends to, determined thiocyanate levels in order to a scertain whether or not these levels are suitable for predicting tobacco smoking habit.

Material and methods:

The subjects of this study were randomly selected from different regions of Baghdad city, and at the sampling collection time they were all in good health.

All the subjects were asked about their smoking habits, sporting habits and their oral health. Age ranged from 18 to 53 years. The subjects were classified into four groups as follows: (1) non smoker group 0 (n= 15), (2) smoker group I (n=6, smoking 1-5 cigarttes per day) and (3) smoker group II (n=10, smoking 11-20 cigarettes per day (4) smoker group III (n=13, smoking more than 20 cigarettes per day).

Collection of stimulated salivary sample were done by using chewing gum, samples were centrifuged at 1500xg for 15 min at room temperature. The supernatant was then poured into a separate tube and stored on freezer.

The visible spectrophotometer is used to measure the absorbance of colored solutions at a variety of wave lengths. The darker the color is the higher the absorbance value is. In this study, the absorbance of several solutions consisting of varying concentrations of $[FeSCN]^{+2}$ complexes are measured at 430 nm to construe a standard curve. The unknown concentrations of $[FeSCN]^{+2}$ ion in saliva samples are determined using the linear relationship between absorption and concentration as established by the standard curve.

In order to measure the thiocyanate levels in the sample, the final solutions are obtained by mixing 0.5ml of the resulting clear solution with 0.0019M $Fe(NO_3)_3$, to give a total volume of 5ml if thiocyanate ion is present the clear solution becomes orange tinted. After all samples are prepared, the absorbance are measured at 430 nm, (by spectrophotometer 20, BAUSCH and LOMB) against 0.0019M Fe $(NO)_3$, which is used as a blank.

All the results were expressed as mean ± standard deviation. Statistical differences between the levels in the two groups were determined according to student's test.

Result and discussion:

The spectophotometric method using the reaction between thiocyanate ion and Fe^{+3} ion, which forms the complex $[FeSCN]^{+2}$. This complex exhibits a red – orange color. The deeper the color, the more concentrated the solution is. This color difference allows for the analysis by visible spectroscopy [13]. samples. The results of this study show that the greater the exposure to the smoke is the higher the concentration ion is in saliva. Since smokers are exposed to more tobacco smoke than non smokers, it seems that they should have higher levels of salivary thiocyanate.

The results in table (1) are fairly consistent with other laboratory data (2) which shows that there is a linear relationship between exposure to tobacco smoke and concentration of the thiocyanate ion in the body.

Table 1: Thiocyanate levels in salivary samples taken from healthy adults volunteers**.**

Subject number	Age (yr.)	Gender (a)	Smoking frequency	Thiocyanate in saliva ($x10^{-3}$ M)	Number of years smoking
1	19	F	S0	0.0	-
2	40	F	S0	0.93	-
3	29	F	S0	1.15	-
4	36	F	S0	1.04	-
5	30	M	S0	0.0	-
6	23	M	S0	2.36	-
7	20	M	S0	0.0	-
8	38	M	S0	2.36	-
9	33	M	S0	1.92	-
10	19	M	S0	1.04	-
11	19	M	S0	1.04	-
12	18	M	S0	0.0	-
13	53	F	S0	1.48	-
14	20	F	S0	0.0	-
15	47	M	S0	2.69	-
16	20	M	S1	2.03	2
17	45	M	S1	1.26	10
18	43	F	S1	1.04	4
19	36	F	S1	2.14	4
20	44	F	S1	2.58	10
21	32	M	S1	1.37	5
22	43	M	S2	4.56	13
23	42	M	S2	3.24	15
24	28	M	S2	3.68	15
25	38	M	S2	4.23	25
26	29	M	S2	5.11	10
27	22	M	S2	3.31	7
28	23	M	S2	3.9	7
29	26	F	S2	1.26	4
30	22	M	S2	1.92	6
31	21	M	S2	4.56	4
32	43	M	S3	5.5	30
33	25	M	S3	4.01	6
34	24	M	S3	3.9	6
35	21	M	S3	6.1	6
36	53	M	S3	4.45	33
37	23	M	S3	5.44	8
38	25	M	S3	4.23	7
39	28	M	S3	4.89	17
40	47	M	S3	4.78	31
41	34	M	S3	4.23	9
42	46	M	S3	3.9	20
43	37	M	S3	4.56	18
44	47	M	S3	3.9	25

a) M: male, F: female. b) Number of cigarettes per day, S0:0, S1: 1 – 5, S2 : 11 – 20, S3: more than 21.

Table (1) shows the age of the subject and the number of years smoking and thiocyanate levels in salivary samples. There is no relation between the age of the subject and salivary thiocyanate determined level in total subjects (n=44) was obtained. The determined levels ranged from 0.0 to 6100 µM in total subjects from 0.0 to 2690µm in non smoker subjects and from 1040 to 6100µM in smoking persons. As shown in table (2), salivary thiocyanate

(p<0.001) levels were significantly higher in the smoker group than in the non smoker group. Within the smoker group, only light smokers (having smoked 1 – 5 cigarettes per day) gave no significant difference (P>0.1) levels compared to the non smoker group, while both moderate smoker group (having smoked 11 – 20 cigarettes per day) and heavy smoker group (having smoked more than 21 cigarettes pre day) gave significantly higher in the smoker group than in the non smoker group. Within the smoker group, only light smokers (having smoked 1 – 5 cigarettes per day) gave no significant difference (P>0.1) levels compared to the non smoker group, while both moderate smoker group (having smoked 11 – 20 cigarettes per day) and heavy smoker group (having smoked more than 21 cigarettes pre day) gave significantly higher (P<0.001) salivary thiocyanate levels.

Table (2): Average thiocyanate levels (μM) in salvia samples taken from healthy volunteers**.**

	Total	Non smoker	Smoker	1 – 5	11 - 20	More than 21
Number of subject	44	15	29	6	10	13
Salivary thiocyanate μM (mean ± SD)	2800 ± 1210	1070 ± 950	3530 ± 1410 **	1740 ± 600	3600 ± 1210 **	4610 ± 710 **

**** Higher significant P<0.001**

As the literature show that the tendency for persons smoking more cigarettes to give higher salivary thiocyanate [2,14] levels, our results follow the same trend. The reported salivary thiocyanate levels also show considerable variability among the literature. Considering the circadian pattern in salivary thiocyanate level and the considerable variation in the saliva collection procedures [15], it is inadequate to compare the literature data unless saliva collection procedures are standardized. Nevertheless, the literatures indicate higher levels in smokers than non smokers. Our data for the non smokers (1070 ± 950 μM) and the smoker (3530 ± 1410 μM) is similar to the Krebs (942 – 4340 μM)(1) and differ from the other (non – smokers, 542 ± 406 μM smokers: 1655 ± 891μM) [2].

The reasons for this discrepancy are not clear, but it may be attributed to the racial difference in salivary thiocyanate levels.

Table (3): Average thiocyanate levels (μM) in saliva samples related to sample related to number of years smoking.

	Total	Non smoker	Smoker	No. of years smoking		
				2-9	10-7	18-33
Number of subject	44	15	29	15	7	7
Salivary thiocyanate μM (mean ± SD)	2800 ± 1210	1070 ± 950	3530 ± 1410	3300 ± 1600 **	3620 ± 1390 **	4470 ± 650 ***

****Higher significant p<0.001, ***p<<0.0001**

Table (3) shows the number of years smoking for the subject and the salivary thiocyanate levels. No relationship was observed between these levels and specific period of smoking. All the three groups: 2 – 9 years smoking, 10 – 17 years smoking and 18 – 17 years smoking and 18 – 33 years of smoking gave significantly higher levels compared to the non – smoker group (P<0.001). While, the longest period of smoking gave the highest levels (mean ± SD = 4470 ± 560μM).

In conclusion long term use of tobacco smoking have no effect on salivary thiocyanate levels in compare to the number of cigarettes smoking per day which is the more effect factor on salivary thiocyanate levels. Also results showed that age of the subject have no relation to salivary thiocyanate levels.

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