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Research Article

Quantitative Analysis of Serum Levels of Trace Elements in Sudanese Snuff Dippers

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Abstract

Background: Oral cancer in Sudan has high incidence rate due to the use of *Toombak*, a home- made smokeless tobacco, rich in tobacco specific nitrosamines.There have been calls for continuous monitoring on toombak users to discover very early the carcinogenic changes to avoid mortality, and morbidity.

Objective: The purpose of this study was to assess technically, and financially affordable methodologies, that are reliable, reproducible, sensitive, specific and cost effective valid for use in mass screening amongst the high risk groups. (The toombak users). The present study was done to evaluate the levels of copper, zinc and magnesium in serum of toombak dippers.

Materials and Methods: The levels of copper, zinc, and magnesium were estimated in the serum of 150 toombak dippers. The values were compared with 50 normal age matched, healthy control subjects and 50 patients with oral squamous cell carcinoma, using Atomic absorption spectrophotometry.

Results: There was significant difference of the mean serum copper, zinc, and magnesium levels of toombak dippers when compared to the normal controls. In oral cancer patients there was significant difference in the copper, zinc, and magnesium levels. Copper level was found increased gradually starting from control through toombak dippers to oral squamous cell carcinoma patients, while the level of zinc and magnesium was found decreased in the same sequence.

Conclusion: Serum may be used as a potential diagnostic tool, which can be efficiently employed to evaluate the copper, zinc and magnesium levels in Sudanese snuff dippers for population based mass screening.Serum trace elements levels could be used as potential diagnostic markers for early changes caused by toombak dipping.

Keywords: Atomic absorption spectrophotometry, copper, magnesiumm, zinc, oral squamous cell carcinoma, tobacco specific nitrosamines, serum, toombak.

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1. Introduction

Oral Cancer is the 6^{th} most common Cancer in the world, and the most common cancer in head and neck.500,000 new cases are added annually worldwide, with increasing

rate of incidence in under developed countries [1]. The world cancer report of 2008 ranked oral cancer as the fifth most common cancer type among males in the less developed countries [2]. Oral cancer is the 5^{th} most common cancer in the Sudan with incidence rate of (920/year) comprising 9 % of the cases reported annually in Africa. This is strongly attributed to the use of local type of snuff known as *Toombak,* a very popular material in the Sudanese community [3]. toombak, is home-made from finely ground leaves of *Nicotiana rustica*, a tobacco species with a particularly high content of nicotine and minor alkaloids. This tobacco is mixed with Natron or atron (sodium bicarbonate) (about 4:1), to raise the PH for rapid absorption of nicotine then water is added to the mixture, and after a period of about 2 hours or longer the mixture is ready for use [4]. Snuff is habitually used orally by insertion between lower gum and cheek or lip (dipping) [5]. Nicotine ranges (8-102) mg/g. dry weight. Tobacco specific nitrosamines. TSNA ranges (420-550) µg/g.for N-nitroso nor nicotine.NNN, and (620-780) µg/gm. for 4- (N –Nitrosomethylamino)- 1- (3- pyridyl)- 1- butanone.NNK, dry weight respectively, the highest ever reported TSNA concentration in a tobacco product [6]. The toombak habituate is exposed minimally to (0.12-0.44 mg) of NNK per day, the highest documented uptake of a non occupational carcinogen [7]. The World Health Organization WHO. recommended less than (2.0 µg /g) dry weight combined NNN and NNK,in smokeless tobacco [8]. Toombak contains 100-fold higher levels of tobacco specific nitrosamines TSNA than commercial snuff in the U.S. and Sweden [9]. The TSNA content in the saliva of toombak dippers is at least ten times higher than that reported in the saliva of dippers of commercial snuff in USA or Sweden [10]. The abnormally high concentration of Tobacco specific nitrosamines in toombak is attributed first to the use of species Nicotiana - Rustica that contains Nicotine (8–10) times the nicotine in the nowadays universally used species Nicotiana – Tabaccum, as well as a high level of alkaloids [11]. Curing of snuff by fermentation is another major factor as it accelerates production of TSNA out of the tobacco leaves alkaloids, as well as the high temperature during storing [12] in addition to contamination, and poor hygiene environment during, processing, and the highly alkaline PH [13]. Toomback trade is legal in the Sudan, without any sort of control, or supervision on manufacturing process by health care authorities. Attempts should be made to reduce exposure to TSNA in oral snuff users in Sudan [14]. Toombak dipping is considered social stigma in Sudan for females, 95% of toombak users are males, [15] Although oral cancer is associated with documented risk factors, yet no comprehensive screening program [16, 17]. There is need for screening and continuous monitoring for toomback users, as a high risk group [14]. The short in budget hinders the National cancer control plans for oral

cancer screening [18]. Taking biopsy is not suitable for screening purposes of high risk individuals for early oral cancer detection due to its invasive nature, high cost, need for specially trained medical person, and equipment [19]. Biopsy for detection of early oral cancerous signs, suffers from the reliability for the appropriate site for the obtainment of specimen to be conclusive, that leads to multiple follow up biopsies while repeated surgical biopsies can discourage the habituate from agreeing to further diagnostic biopsies as it causes fear, and stress, pain, and damage to healthy tissues in addition to risk of infection, and temporary disability, and discomfort [19]. considering that the habituate is not motivated to co operate with the test idea as he believes no need for it as long as there is no pain or swelling.Toludine blue, Rose Bengal, Iodine stain, 6 tolonium chloride stain have been used as detectors for early carcinogenic changes, but inexperienced practitoners can not use these techniques to diagnose such changes, besides low specificity [20–23]. The imaging diagnostic techniques such as radiographic techniques, nuclear medicine, magnetic resonance, or ultrasonography, need equipments, and trained staff, which is hard to provide by the health care system, suffering already from limited resources, and escalating costs. The WHO guidelines for screening programs in developing countries, put the cost of any test as a very important consideration.The national screening programs should avoid imposing the high technology of the developed world on countries that lack the infrastructure, and resources to use the technology appropriately, or to achieve adequate coverage of the population [18]. The trace elements have been extensively studied in recent years, to assess whether they had any role in the aetiology of cancer. Magnesium and zinc are the elements which have essential roles in the regulation of the cell growth, division and differentiation. High levels of copper have been observed to protect against a chemical induction of tumours [24]. The present study was done to evaluate the levels of copper, zinc and magnesium in serum of toombak dippers.

2. Materials and Methods

Randomized cross sectional study was conducted involving 250 subjects (all males) from Khartoum state,Sudan.This study was approved by concerned institution's ethics Committees. Study subjects were divided into 3 groups. first group 150 toombak dippers in an age range 27 to 79 years mean age (50 $\pm$ 11.85).Years with history of toomback use for more than 10 years, non smokers or alcohol drinkers. with no diagnosable change in oral mucousa. The second group 50 healthy non tobacco users control with the same age range.The third group oral squamous cell carcinoma OSCC. Patients with

age ranges between (30 to 82 years), with history of toombak use, and no previous history of malignancy. The study protocol was explained for the subjects involved, and a consent was obtained.5 ml of venous blood were collected from each subject in a plain polyethylene tube, the tubes were left for 30 minutes at room temperature. samples were centrifuged at 3,000 rpm for10 minutes to separate serum.2 ml of serum from each sample was put in a labeled cryo- tube kept in liquid nitrogen tank (-196 c), till the time of estimation.

2.1. Methodology for trace Elements estimation

Trace Elements were estimated using full automatic device. (Buck Scientific 210 /211 VGP atomic absorption spectrophotometer.USA) at National center for research. Khartoum, Sudan. The estimation of copper was done according to the method of weinstock, and Uhleman, 1981 [25]. The estimation of Zinc was done according to the method of Butrimovtz, and Purdy, 1977 [26]. Estimation of magnesium was done according to the method of Amino, 1964 [27].

2.2. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 21) software. Values are expressed as (means ± SD) and *P* (probability) values of 0.05 were considered to be significant with a 95% confidence interval. Serum levels of different parameters were statistically analyzed in all the three groups included in the study using analysis of variance (ANOVA) test, and intergroup relationship was analyzed using t- test. Relationships between variables were evaluated by Pearson correlation coefficient. *P* value of 0.05was considered to be statistically significant.

3. Results

The trace elements in the present study were found to be related to toomback dipping, as well as to duration of habit, frequency of dipping/day, and consumption of toombak in grams/day. The present study showed that the mean serum copper level in control group was:0.78 0 ± 0.27 mg/L. (mean ± SD).The serum copper level in toombak dippers group was 0.83 ±0. 22 mg / L (mean ± SD), while it was found to be 0.69 ± 0.23 mg / L. (mean ± SD) in the OSCC patients group. (Table 2). The difference between serum copper level in the toombak dippers group, and the level in the control group was significant (p

< 0.002).The mean serum copper level increased in toombak dippers group. toombak in gram/day (r=0.221 p=0.006) (Figure 1). The mean serum zinc level in control group was 0.83 ±0.33 mg / L (mean ± SD).The serum zinc level in toombak dippers group was 0.79 ± 0.27 (mean ± SD), while it was found to be 0.69 ± 0.25 mg / L. (mean ± SD) in the OSCC patients group. (Table 3). The difference between serum zinc level in the toombak dippers group, and the level in the control group was significant (p 039).The mean serum zinc level showed a decreasing trend from control group through toombak dippers group to OSCC patients group. the mean serum magnesium level in control group was 26.1 ± 5.10 mg / L (mean ± SD). The serum magnesium level in toombak dippers group was 24.8 ± 4.56 mg / L. (mean ± SD), while it was found to be 21.7 ± 5.60 mg / L. (mean ± SD) in the OSCCpatients group. (Table 4). The difference between serum magnesium level in the toombak dippers group, and the level in the control group was highly significant (p < 0001). The mean serum magnesium level showed a decreasing trend from control group through toombak dippers group to OSCC patients group.

Table 1: General characteristics of toombak users.

Study variable	Minimum	Maximum	Mean	Std. Deviation
Duration of habit (year)	10.0	55.0	26.63	10.98
Consumption in grams/day	30.00	70.00	46.50	9.65
Frequency of dipping/day	10.0	30.0	14.18	3.065
Time the quid kept in the mouth (minute)	5.00	25.0	9.373	2.539
Age (year)	27.0	79.0	50.90	11.86

Table 2: Shows copper results of subjects with OSCC, and toombak dippers as compared to healthy control.

Parameter	Group	Mean S±D	P-Value
Serum Copper mg / L	1-Toombak Dippers	0.83 ± 0.22	0.002
	2-Healthy control	0.78 ± 0.27	
	3-OSCC Patients	0.69 ± 0.23	

Table 3: Shows serum Zinc results of subjects with OSCC., and toombak dippers as compared to healthy control.

Parameter	Group	Mean ± SD	P-Value
Serum Zinc mg / L	1-Toombak Dippers	0.79 ± 0.27	0.039
	2-Healthy control	0.83 ± 0.33	
	2-OSCC Patients	0.69 ± 0.25	

Table 4: Shows Magnesium results of subjects with OSCC., and toombak dippers as compared to healthy control.

Parameter	Group	Mean ± SD	P-Value
Serum Magnesium mg / L	1-Toombak Dippers	24.8 ± 4.56	0.000
	2-Healthy control	26.1 ± 5.10	
	3-OSCC Patients	21.7 ± 5.60	

Table 5: Descriptive statistics of serum Copper level in toombak dippers compared to healthy control.

Serum copper Mg/L	Minimum	Maximum	Mean	Std. Deviation
1-Toombk dippers	0.10	1.65	0.87	0.22
2-Healthy Control	0.35	1.38	0.78	0.27

Table 6: Descriptive statistics of serum zinc level in toombak dippers compared to healthy control.

Serum Zinc Mg/L	Minimum	Maximum	Mean	Std. Deviation
1-Toombk dippers	0.22	1.70	0.79	0.28
2-Healthy Control	0.39	1.61	0.83	0.33

Table 7: Descriptive statistics of Magnesium level in toombak dippers compared to healthy control.

Serum Magnesium Mg/L	Minimum	Maximum	Mean	Std. Deviation
1-Toombk dippers	15.3	37.5	24.8	4.56
2-Healthy Control	15.8	35.5	26.1	5.10

4. Discussion

serum copper level of toombak dippers group in the present study was high compared to the level of control group serum copper (Table 2) the difference was statistically significant. The serum copper level of squamous cell carcinoma was found to be high compared to healthy control.The results agreed with the findings of Tudek, et al [28] Darna, et al [29] and with Sreelatha, et al [30] who reported increased level of serum copper in oral squamous cell carcinoma patients group compared to control group. Varhgese et al. [31], studied the serum copper level in leukoplakia, they found high level of copper in leukoplakia group compared to control group with a statistical significance Silverman, and Thompson. [32] reported significant increase in serum copper in head, and neck squamous cell carcinoma. Serum Zinc in the present study had shown lower level in toombak dippers, and squamous cell carcinoma patients compared to control group. (Table 3). There was statistically significant difference. Darna, etal [29] reported statistically significant difference between low serum zinc of oral squamous cell carcinoma patients group, and the higher level of zinc of the control group Sreelatha,et al. [30] reported similar results. The results of the present study disagreed with the study of Boliniarz, et al [33], who had found higher zinc level in

oral squamous cell carcinoma patients group compared to healthy control group, and also contradicted with the results of Silver,and Thompson [32] who had found higher level of zinc in cancer patients compared with controls. Magnesium levels in serum of the groups of the present study had shown statistically significant variation (Table 4). The level in toombak dippers group was found to be (21.6 ± 4.56 mg/l),while it was (26. ± 15.1 mg /l), in healthy control group.These results agreed with what was reported by (Deheinzelin, et al [34] who had found hypo magnesemia in oral cancer patients confirming the earlier results of (Chernow, et al (1995), and (Guern, et al (1996) who both found low magnesium level in oral squamous cell carcinoma patients compared to healthy control group. Sreelatha, et al [30] found decreased serum magnesium level in oral squamous cell carcinoma patients group compared to healthy control group. Taysi S, etal [34] also reported lower serum magnesium level in cancer patients compared to controls. Vyas RK et al [36] concluded that the zinc, and serum magnesium levels were found to be low in the patients with malignancies. The results of the present study disagreed with the findings of Akinmoladunm, etal [37] as they reported higher serum magnesium level in cancer patients compared to controls. Trace elements assessment in serum, is a highly appreciated parameter in diagnosis, and prognosis processes concerning oral squamous cell carcinoma.

5. Conclusion

The present study is a trial to assess the effects of toombak on dippers depending on biochemical markers, with the advantage of detecting the anticipated changes before being displayed by the affected cells. The results of the three groups studied (toombak dippers, control group, OSCC group) had shown clear variation between the 3 groups members. The change in the level of these elements can be used as marker that reflects susceptibility towards cancer.

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