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RESEARCH ARTICLE

THE BEHAVIOR OF TWO TUMOR MARKERS 'CEA' AND 'AFP' IN SMOKERS AND TOOMBAK USERS IN SUDAN BY USING IMMUNORADIOMETRIC ASSAYS

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ABSTRACT

Approximately 42% of the Sudanese population use toombak as smokeless tobacco. Tobacco specific N-nitroso compounds have been detected at high concentration in toombak. This study focused on the possible carcinogenicity of those compounds through the measurement of serum CEA and serum AFP in both toombak users and smokers. 160 subjects were involved with the average age of 32 years ranging from 20 to 60 years. These 160 subjects were categorized into 3 groups (control, smokers, and toombak users). Serum CEA and AFP were measured using an IRMA technique. The mean AFP level in the control, toombak users and smokers groups were 0.94, 1.98 and 2.34 Ku/l respectively. There is a significant difference between the means of serum AFP level in both smokers and toombak users and control group, $p = 0.0045$ and $p = 0.0049$ respectively. 13.2 % of the toombak users and 23.5 % of smokers have had serum AFP levels more than the upper limit of the normal range (0-3.28 Ku/l). Serum CEA level in control group ranged from 0 to 5.28 $\mu\text{g/l}$. The mean serum CEA levels of the toombak users and smokers was not differ significantly from that of the control group ($p > 0.05$). But 12.7% of the Toombak users and 14.7 of the smokers have had CEA level more than the upper limit of that of control. The relation between the duration of smoking and the level of CEA and AFP as well as the effect of age of subjects on the level of these analyte were studied. The results show no significant correlations where $p > 0.05$.

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INTRODUCTION

Habits associated with the use of smokeless tobacco are found worldwide, with countless variations in the nature of the product used, as well as with the customs associated with its use. The tobacco is often processed and treated with additives and flavoring agents. The majority of snuff used today has a relatively high moisture content, it is used orally and is placed between the buccal mucosa and gum. The alkaloid, nicotine, is the factor that creates dependence in the continued use of tobacco. Toombak is one of the varieties of tobacco, which is utilized without combustion (non-smoked tobacco). Tobacco-specific N-nitroso compounds have been detected at high concentrations in snuff and chewing tobacco. Thus, an evaluation of the possible carcinogenicity of those N-nitroso compounds was considered to be of importance. At least 2549 individual constituents have been identified in tobacco (1). This number includes tobacco constituents themselves as well as chemicals that are applied

to tobacco during cultivation, harvesting and processing. Processed tobacco contains 27 volatile amines, 11 aromatic amines and more than 50 N-heterocyclic compound (2). Of special relevance to carcinogenesis are secondary amines, which can give rise to N-nitrosamines during curing, fermentation and aging. Tobacco contains 0.5-5% of alkaloids (2). At least 85% of the total nicotiana alkaloids are nicotine (3). A large number of studies have shown that, during the aging, curing, fermentation and processing of tobacco, nicotine and other alkaloids give rise to carcinogenic tobacco-specific N-nitrosamines (4). The concentration of these N-nitrosoamines in tobacco exceeds by at least 100 fold the concentration found so far in other consumer products. There is a close relationship between the oral use of snuff and the development of oral carcinoma (5, 6, 7). The finding that 81% of patients with oral carcinoma indulged in oral use of toombak strongly suggests a close relationship between the habit and the disease. Although most of the studies of the relationship between the use of chewing tobacco or snuff and cancer have focussed on risk to the oral cavity and pharynx, some evidence is also available concerning cancer at other anatomical sites. In many of the studies reported, chewing tobacco or snuff use was often

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only one of many potential risk factors considered. In the majority of studies carried out in British coal miners, Danish patients and Swedes on oral precancerous lesions, which are a generalized, state associated with a significantly increased risk of cancer, the prevalence of oral leukoplakia among the total population examined was 3.6% (8). The concept of leukoplakia as a precancerous lesion is based on the findings: 1) of a significant number of oral carcinomas associated with a pre-existing area of leukoplakia; and 2) that some leukoplakias appear to undergo malignant transformation.

Among women, use of chewing tobacco or snuff was associated with cervical cancer (9), and significantly lowers birth weight by an average of 395.3g (10). Delay in diagnosis of such cases, allows tumors to invade deep into local structures and spread to regional lymph nodes in the neck, resulting in this high mortality, so we should think in prevention by early detection to reduce morbidity and mortality. Studies revealed that, smoking is one of the causative agents of lung and other cancers. Some authors found a significantly increased ($p < 0.001$) CEA levels in smoker's sera as compared to the non-smokers (11)

Objective: The aim of this study is to evaluate the level of the CEA and AFP in Sudanese smokers and toombak users for early detection of oral cancer and other site cancers.

Subjects, material and methods: In this study 160, Sudanese male were involved with an average age of 32 years ranging from 20 to 60 years. This number was divided into 3 groups. These groups are smokers, toombak users and control. Three to Five ml of blood were collected from each person. The serum was separated and stored below $-20\text{ }^{\circ}\text{C}$.

Assay method: The assay method used in this study was the immunoradiometric assay, which uses a solid-phase antibody, and I^{125} radio-labeled antibody as tracer. The reagents for these assays were brought from NETRIA, London. The minimal detectable dose for CEA assay was $3\text{ }\mu\text{g/l}$ and for AFP was 1.0 ku/l . Computer software for statistic (Microcal.Org) was used for the analysis of data.

RESULTS

Table 1. Levels of AFP and CEA in different groups

	Control	Smokers	Toombak users
AFP			
Mean in ku/l	0.94	2.34	1.98
SD	1.17	2.91	2.159
Range	0-4.87	0-14.26	0-11.49
CEA			
Mean in $\mu\text{g/l}$	2.035	2.68	2.88
SD	1.6	2.2	2.6
Range	0-6.1	0-8.37	0-14.4
N	50	50	50

AFP: Calculation of the reference range was performed using the mean $\pm 2\text{SD}$ and it was found to be from 0 to 3.28 Ku/l . Comparing the mean serum AFP in the control group to that of smokers and toombak users revealed a significant difference, where $p = 0.0045$ and $p = 0.0049$ respectively. 13.2% of the toombak users and 23.5% of smokers have had AFP level more than the upper limit of the normal range ($0-3.28\text{ Ku/l}$). Fig. (1)

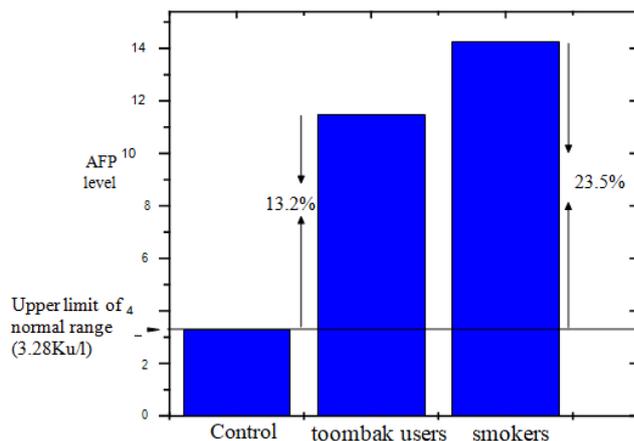


Fig. (1) Percentage of smokers and toombak users having AFP more than the upper limit of the normal range

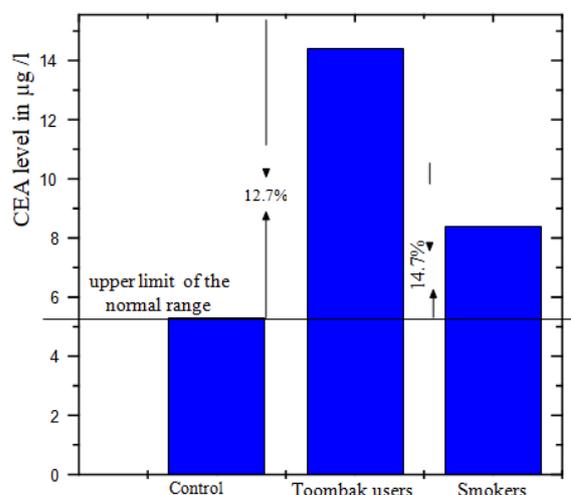


Fig. (2), Percentage of smokers and toombak users having CEA more than the upper limit of the normal range

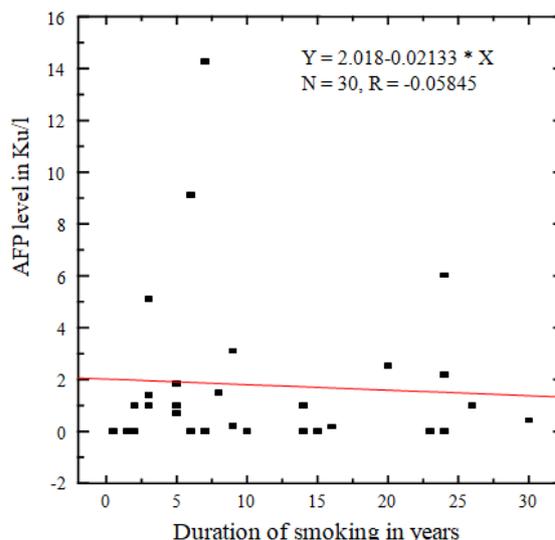


Fig. (3) The effect of smoking duration on serum AFP

CEA: Comparing serum CEA level of the toombak users and smokers to that of the control group, the means were not significantly different at 0.05 level, although 12.5% of the toombak users and 14.7% of the smokers had have CEA levels more than the upper limit of the reference value (Fig. 2).

Fig. (3) and Fig. (4) show the effect of the duration of smoking on AFP and CEA levels. The correlation coefficients were -0.058 and 0.0326 respectively. The relation between the age of smokers and the levels of AFP and CEA was studied (Fig. 5 & 6). The correlation coefficient was 0.07 and 0.01 respectively.

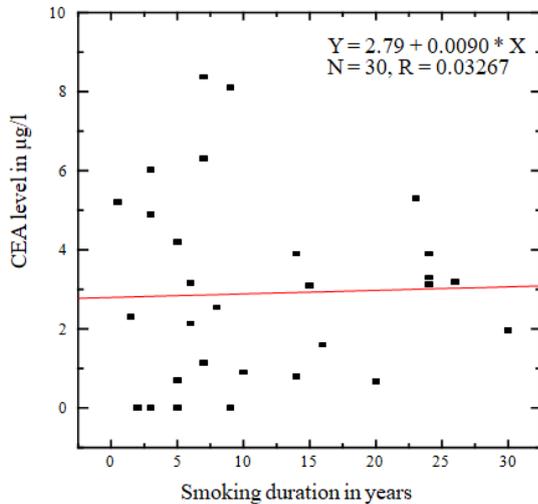


Fig. (4) Effect of smoking duration on serum CEA level

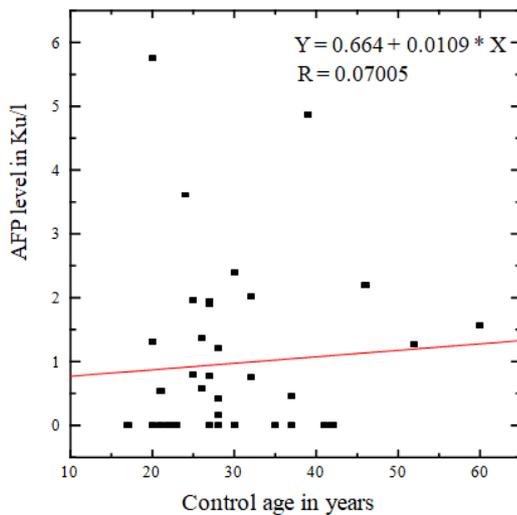


Fig. (5) The relation between the level of AFP and subject's age in control group

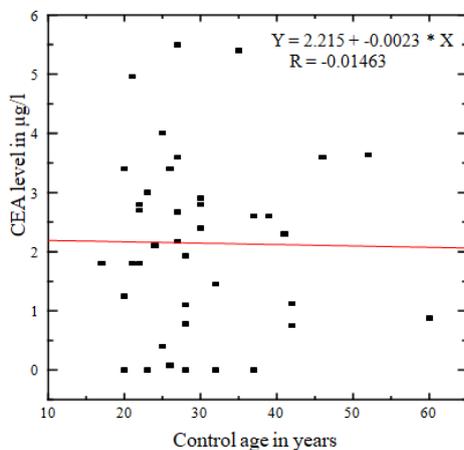


Fig. (6) Relation between the level of CEA and subject's age in control group

DISCUSSION

This study was performed in Sudanese population where the habit of using toombak is widely common among males. Toombak has been shown to contain carcinogens such as N-nitrosamines, which are derived from tobacco. Saliva of snuff dippers was also found to contain a significant amount of nitrosamines (Hoffman and Adams, 1981) therefore ensuring prolonged exposure of the oral mucosa to the carcinogens in the absence of snuff. The possibility of the relationship between toombak and oral carcinoma is strengthened by the close association of the tumor site with the area in which the snuff was habitually placed- notably the anterior oral region in males (Elbeshir et al., 1989). Since the CEA was known to be an associate marker for a variety of tumors and the AFP concentration is increased in different malignant tumors as well as in benign tumors, both CEA and AFP were measured to examine for the effect of smoking and using toombak.

Although smoking cigarette was known to cause significant elevation in the level of CEA, here it was observed that, there was a slight increase in the level of CEA. This may be due to that, smoking cigarette is not a common habit among Sudanese population. Sudanese are not heavily smokers, where most of them smoke 8 cigarette/day in average and even for short period of time ranged from 2 to 10 years except for some few cases who smoke for more than 30 years. Smoking and using toombak were found to be causative agents of increased AFP level in serum. The age and the duration of smoking had nothing to do with the level of both CEA and AFP in the control group. Putting in mind that, CEA and AFP are not sensitive markers for diagnosing both abnormalities i.e. diseases caused by the two habits. Some might find serious effects caused by the two habits (toombak and smoking) if investigated through other procedures, like cytological techniques.

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