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STUDIES ON POSSIBLE FACTORS IN SALIVA IN THE ETIOLOGY OF ORAL CANCER

By

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دراسات على العوامل الموجودة في اللعاب والتي لها دور محتمل في حدوث وتكوين سرطان الفم

سميرة درويش و نادية زخاري
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أُجريت الدراسة الحالية على مجموعة مكونة من ١٩٥ شخص قُسمت طبقاً للحالة الصحية للفم إلى أربعة مجاميع : **المجموعة الأولى** : واشتملت على الأشخاص الاصحاء وهي المجموعة الضابطة ، **المجموعة الثانية** : وهي مجموعة المرضى الذين يعانون من Periodontitis حديثة أو متوسطة ، **المجموعة الثالثة** : وأشتملت على المرضى المصابون بـ Periodonbitis في الحالات المتقدمة ، **والمجموعة الرابعة** : وتضمنت المرضى بسرطان الفم. وأوضح الكشف الاكلينيكي المشتمل على تعيين معامل راسيل وفحص سقف الفم بأشعة أكس أن هناك زيادة في معامل راسيل والتي أختلفت حسب درجة أو وحدة الاصابة بـ Periodontitis . وقد إتضح أن سرطان الفم كان أساساً من نوع Squamous cell وأنه وُجد في قاع الفم وكذلك على جانبي وقاعدة اللسان . وعند إجراء بعض القياسات البيوكيمائية على عينات اللعاب التي تم الحصول عليها بطريقة غير تأثيريه من المجموعة الضابطة والمرضى فقد تبين أن هناك إرتفاع ملحوظ في كل من انزيم بيتا – جلكورنيداز (سواء كان مصدره أنسجة المريض أو البكتريا) في كل من الحالات التي تعاني من Periodontitis أو من سرطان الفم عندما قورنت بالمجموعة الضابطة . وبصرف النظر عن مستوى الثيوسيانات فلم يُلاحظ أي تغييرات عند مقارنة المرض بالمجموعة الضابطة . وعلى الجانب الآخر فقد وُجدت زيادة واضحة في هذا الانزيم عند مقارنة المدخنين بغير المدخنين بغض النظر عن اختلاف المجاميع . وقد ازداد محتوى النيتريت في لعاب المرضى الذين يعانون من Periodontitis المتوسط بينما لم تحدث أي تغييرات واضحة في لعاب مرضى سرطان الفم . وعلى الجانب الآخر لم توجد أي تغييرات في الأس الهيدروجيني (pH) في أي من المجموعات المختلفة التي تم دراستها .

Key Words: Saliva, Oral cancer.

ABSTRACT

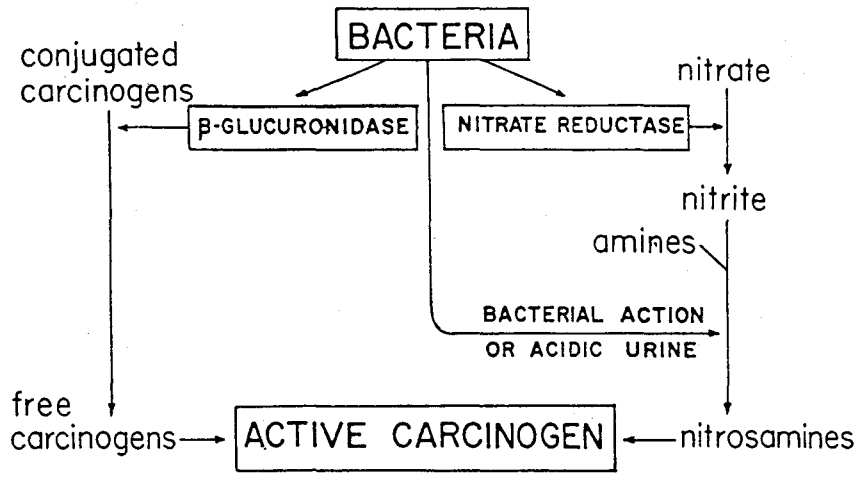
The present study was performed on 195 individuals divided according to their oral hygiene into the following four groups; Group I: Control healthy individuals; Group II: Patients suffering of early to moderate periodontitis; Group III: Patients suffering of advanced periodontitis; Group IV: Oral cancer patients. Clinical examinations including periodontal Russell index (R.I) and periapical X-ray revealed a gradual increase in R. I. according to the severity of the oral periodontal condition. Oral cancer was mainly of the squamous cell carcinoma type and localized in the floor of the mouth or the lateral and the base of the tongue. Unstimulated saliva of the controls and patients were subjected to different biochemical investigations, which revealed that: β-glucuronidase (BG), whether of tissue origin or bacterial origin was significantly elevated in cases suffering of oral periodontitis as well as oral cancer patients, as compared to their corresponding controls. Regarding the thiocyanate (SCN^-) level, no significant changes were observed when comparing the patient groups to the controls. On the other hand it was highly elevated when comparing the patient groups to the controls. On the other hand it was highly elevated when comparing smokers to non-smokers, regardless the group investigated. Nitrite (NO_2^-) content was significantly increased in the saliva of patients suffering of early to moderate periodontitis, whereas its level was nonsignificantly elevated in saliva of oral cancer patients. On the other hand no changes were observed concerning the salivary pH among the different groups.

INTRODUCTION

Oral cancer and pharynx in Egypt constitutes 7.62% of total cases of the Egyptian National Cancer Institute[1]. The etiology of oral cancer in man is still not clearly understood. However several pre-existing conditions have been frequently traced in patients with oral cancer and may be considered at least as contributing factors.

Epidemiological studies proved that cigarette, pipe and cigar smoking increase the incidence of oral cancer. This was attributed to the heat and its drying effect, as well as the cyanic compounds present in smoke and converted in saliva to thiocyanate which catalyse the formation of nitrosamines[2]. Carcinogenic polycyclic hydrocarbons present in the cigarettes may contribute also in the incidence of oral cancer[3].

Recently the problem of oral cancer etiology have been developed based on some biochemical background as well as the hygienic conditions of the oral cavity. The theoretical background of this approach is based mainly on the fact that bad oral hygiene is positively correlated with bacterial flora which have two main biochemical functions (see figure 1). Tawfik[4] also reported that bacterial infection may lead to bacterial enzymes, including nitrate reductase, capable of synthesizing nitrite which is a nitrosamine precursor, and β-glucouronidase (BG) enzyme, capable of hydrolysing detoxicated carcinogens, at pH 7.



The possible role of bacteria in bladder carcinogenesis.

The aim of the present work is to investigate the activity level of salivary BG which is of tissue origin; with optimal activity at pH 5, as well as that of bacterial origin; with optimal activity at pH 7. The NO_2^- as well as the pH of the saliva of Egyptian subjects with periodontal lesions and oral cancer, were also studied in an attempt to find out the possible biochemical factors that might be involve in the etiology of oral cancer among Egyptian subjects. The ultimate goal of the present study is to find out if there is any relationship between negligence of oral hygiene, the prevalence of periodontal diseases and the high incidence of oral cancer.

MATERIAL AND METHODS

This study was performed on 195 subjects divided into the following groups:

Group I Control group comparing 47 subjects with no oral lesions, aged from 30 to 56 years old.

Group II Comprising 53 subjects, characterized by early to moderate periodontitis, aged from 30 to 57 years old.

Group III Comprising 38 subjects suffering of advanced periodontitis, aged from 36 to 58 years old.

Group IV Comprising 57 patients, suffering of oral cancer and aged from 35-63 years old.

A case history was taken for each individual, comprising name, sex, age, occupation, residence and smoking habit.

Clinical Examination

Clinical examination for oral cavity was carried out including the periodontal index according to Russel[5] by summing all of the tooth scores and dividing it by the number of the teeth present. The criteria of this index depends on the condition of gingival inflammation and the degree of bony destruction. Periapical X-ray were also taken to confirm the periodontal condition.

Collection of Unstimulated Saliva

The collection of saliva was performed according to Harvey and Panse[6] after a fasting period of 6-8 hours. Samples were collected after 10-15 minutes of rinsing mouth with water.

Biochemical Investigations

Saliva was subjected to the following investigations:

1. β-glucuronidase (BG) activity was assayed according the method of Fishman[7] using p-nitrophenyl-β-glucuronide as substrate. The activities were measured at both pH 5 and pH 7 to identify enzyme of tissue origin or bacterial origin, respectively.
2. Protein estimation was carried out using Lowry's method[8].
3. Salivary nitrite was determined according to the method of Montogomery and Dymock[9].
4. Thiocyanate was determined using the method of Powell[10].

RESULTS

Table 1 represents the R. I. of the different investigated groups with respect to the sex of the patients. The R. I. significantly increased parallel to the periodontal condition of each group. On the other hand, oral cancer patients had RI similar to patients of advanced periodontitis. No significant changes were observed between the R. I. of males and females within the same group.

Table 2 represents the R. I. of patients within different groups with respect to their smoking habit. Smokers of the control group as well as early to moderate periodontitis group showed significantly elevated R. I. when compared to their corresponding non-smokers.

Table 3 represents the BG activity at pH 5 in saliva. The enzyme was significantly elevated in the three diseased groups as compared to controls.

Table 4 represents the BG activity at pH 7 in saliva of the investigated groups. The enzyme was significantly elevated in parallel to the periodontal condition of patients, reaching its maximum in oral cancer patients.

Table 5 represents the nitrite level in saliva of the investigated groups. The nitrite level was significantly elevated in cases of early to moderate periodontitis. It was nonsignificantly elevated in oral cancer patients.

Table 6 represents the thiocyanate level in saliva. Thiocyanate was significantly elevated in saliva of smokers when compared to non-smokers regardless the investigated groups.

No significant changes were observed concerning the pH value of saliva among the different groups.

DISCUSSION

Data on the etiological factors of oral cancer in Egyptians are very sparse. Therefore it became necessary to investigate some biochemical factors that could lead to the development of this type of cancer.

Our approach to this problem was based on the fact that bad oral hygiene prevailing among Egyptians and their subsequent stages of periodontitis is accompanied by increase in quantity and quality of bacterial oral flora which possess high BG activity which acts on conjugated carcinogens yielding free carcinogens[11]. Also some bacteria posses nitrate reductase activity, which reduces nitrate to nitrite, which in turn reacts with secondary amines yielding nitorsamines[12].

Gentile et al [13], reported that BG plays an important role in altering chemical mutagenic activity. It hydrolyse the glucuronide conjugates, thereby reversing one of the main detoxification and excretion pathways. The level of this enzyme significantly influence the ratio of unconjugated to glucuronidated toxins or carcinogens[14]. The permeability of lysosomal membrane could be determined by BG activity and its release from the cell[15].

In the present study a gradual increase in BG activity, of both tissue or bacterial origin, was observed parallel to the degree of periodontitis as well as in patients with oral cancer. This is in accordance with Bang et al.[16], who reported a positive correlation between the activity of BG and the depth of the periodontal pockets as well as the amount of bone loss. Harvey and Panse[6] reported increased BG in oral cancer patients, such increase could have a possible role in the pathogenesis of oral cancer. Nakagawa et al.[17] reported elevated BG in patients with meningeal carcinomatosis. Rokicki et al.[18] and Ho et al. [19] reported that quantitation of BG is essential for studying the pathophysiological role in carcinogenesis, and it can be a valuable clinical indicator for tissue damage or malignancy, or the assessment of the result of tumour therapy. Wellman et al.[20] also reported its elevated levels in lymphoma cases. Its high activity in tumour cell indicates that some genetic alteration leading to a new gene or alteration in repressor of an existing gene in the cancer cell that leads to continuous synthesis of BG[21]. Ishikawa et al.[22] suggested that lysosomal enzymes resulting from the breakdown of epithelial and connective tissue cells, could play a damaging role in the periodontal tissue. Bernacki et al.[23] reported that the mechanism of cellular release of this hydrolytic enzyme probably

involves tumour lysosomal exocytosis. Increased tumour glycosidase level may promote increased cell shedding from primary tumours, local invasion and structural changes in tumour cell surface glycoconjugates. These cell surface changes could facilitate tumour cell thrombus formation, secondary site implantation and attachment in microcirculation to endothelial cells and/or subendothelial basement membrane components. They also reported that metastatic tumour cell variants generally have been found to be more invasive and capable of degrading proteoglycan basement membrane components in part due to these increased level of degradative enzyme.

Table 1
Classification of cases according to Russell Index (R. I.).

Groups	Russel Index					
	Total		Male		Female	
	Mean ± S. E.	%	Mean ± S. E.	%	Mean ± S. E.	%
Control	0.68 ± 0.05	100	0.74 ± 0.05	100	0.56 ± 0.09	100
Early to Moderate Periodontitis	2.18 ± 0.09***	320	2.34 ± 0.12***	316	1.814 ± 0.13***	325
Advanced Periodontitis	6.26 ± 0.14***	920	6.22 ± 0.14***	841	6.67 ± 0.32***	1191
Oral Cancer	6.21 ± 0.09	913	6.20 ± 0.01***	838	6.2 ± 0.16***	1107

Percentage was calculated taking control as 100%
*** P<0.005

Table 2
Russel Index with respect to smoking habit.

Groups	Russel Index			
	Non-Smokers		Smokers	
	Mean ± S. E.	%	Mean ± S. E.	%
Control	0.56 ± 0.06	100	0.91 ± 0.05	135
Early to Moderate Periodontitis	1.90 ± 0.09	100	2.56 ± 0.147***	134
Advanced Periodontitis	6.39 ± 0.13	100	6.12 ± 0.240***	95
Oral Cancer	6.07 ± 0.12	100	6.34 ± 0.110***	104

*** P<0.005

Table 3
β-Glucuronidase activity at pH 5 in saliva of the different investigated groups

Groups	β-Glucuronidase activity					
	Total		Male		Female	
	Mean ± S. E.	%	Mean ± S. E.	%	Mean ± S. E.	%
Control	397.8 ± 33.1	100	433.2 ± 40.8	100	322.0 ± 53.1	100
Early to Moderate Periodontitis	934.3 ± 78.3***	235	951.5 ± 96.01***	210	898.2 ± 137.9***	277
Advanced Periodontitis	924.5 ±108.1***	232	929.6 ±114.4***	215	447865.49 ± 376.9**	268
Oral Cancer	854.7 ±116.76***	215	756.3 ± 93.7***	175	967.4 ± 227.3*	300

βG. activity was calculated as μg p-nitrophenol/hour/mg protein.
Percentage was calculated assuming the control as 100 percent.
* P < 0.025
** P < 0.01
***P < 0.005

Table 4
β-Glucuronidase activity at pH 7 in saliva of the different investigated groups

Groups	β-Glucuronidase activity					
	Total		Male		Female	
	Mean ± S. E.	%	Mean ± S. E.	%	Mean ± S. E.	%
Control	200.1 ± 16.77	100	202.0 ± 20.7	100	206.1 ± 32.01	100
Early to Moderate Periodontitis	363.4 ± 29.7***	182	373.4 ± 38.92***	185	337.1 ± 38.65***	164
Advanced Periodontitis	443.7 ± 63.4***	222	443.3 ± 68.6***	219	447.9 ± 91.67**	217
Oral Cancer	506.4 ± 78.76***	253	466.3 ± 83.8***	231	552.5 ± 153.66*	268

Legends as table (3).

Table 5
Nitrite content in saliva of the different investigated groups

Groups	Nitrite Level					
	Total		Male		Female	
	Mean ± S. E.	%	Mean ± S. E.	%	Mean ± S. E.	%
Control	70.31 ± 10.41	100	76.14 ± 13.32	100	56.68 ± 16.14	100
Early to Moderate Periodontitis	113.39 ± 18.12*	161	99.83 ± 14.9	131	144.76 ± 49.39	255
Advanced Periodontitis	82.44 ± 14.21	117	83.24 ± 14.65	111	61.85 ± 46.24	109
Oral Cancer	87. 73 ± 20.43	125	77.39 ± 23.15	102	99.22 ± 34.97	175

Results are expressed as μg nitrite/mg protein.
* P< 0.025.

Table 6
Thiocyanate level in saliva of the different investigated groups

Groups	Thiocyanate Level			
	Non-Smokers		Smokers	
	Mean ± S. E.	%	Mean ± S. E.	%
Control	25.4 ± 2.9	100	95.09 ± 18.41***	374
Early to Moderate Periodontitis	28.1 ± 3.4	100	97.65 ± 15.29***	347
Advanced Periodontitis	16.7 ± 2.4	100	121.60 ± 20.45***	728
Oral Cancer	24.2 ± 1.89	100	106.50 ± 34.10***	440

Results are expressed as mg SCN^- per litre saliva.
Percentage were calculated assuming non smokers as 100 for each group investigated
*** P < 0.005.

Hence it is of considerable interest to develop inhibitors against these enzymes, as it was reported by Walaszek et al.[24] that some potent anticarcinogens may act by increasing clearance of carcinogens as glucuronides through the inhibition of BG. Pegram et al.[25] also reported that lowered BG activity was observed in lines resistant to aflatoxin carcinogenesis.

Concerning the pH of saliva, it was found that the buffering capacity of fasting unstimulated mixed saliva is strong enough to shift the pH toward the alkaline side in all the investigated groups. This was in agreement with Fosdick[26].

The present study revealed significantly increased nitrite level in saliva of patients with early to moderate periodontitis. Nevertheless, nonsignificant elevated NO_2^- level was observed in saliva of oral cancer patients.

Hornby[27] stated that the presence of nitrite in saliva should not be ignored as a risk factor to oral cancer, since it serves as a precursor for in vitro nitrosation capacity of saliva. Shapiro et. al.[28] and Tricker et al[29] also reported the hazard of nitrosation of secondary amines present in human saliva, in presence of nitrite. It is worth mentioning that strains of Candida albicans, with high nitrosation potential, were isolated from lesions with more advanced precancerous changes. These yeast 2 cells were present in the superficial part of epithelium of the lesions as branching mycelium, and in some cases extending from the mucosal surface to the deeper epithelium cell layers, forming fungal transportation system which could channel nitrosamine precursors in the saliva at the mucosal surface to deeper parts of the epithelium where the produced nitrosamines[30].

The present study showed significant increase in the SCN^- level in saliva of smokers as compared to non-smokers, irrespective of the group investigated. This elevation is attributed to the cyanides present in the smoke which are detoxicated in the form of SCN^-[31]. The biological significance of SCN^- is related to its capacity to act as catalyst for nitrosamine formation[31 and 32].

The R. I. which represents the gingival diseases were gradually increasing parallel to the degree of periodontitis. Oral cancer patients had a R. I. similar to that of advanced periodontitis group. The R. I. was also elevated in smokers of control group and patients suffering early to moderate periodontitis when compared to their corresponding non-smokers

The present results showed that most of the periodontal diseases started above the age of 30 years old, and the disease becomes more advanced at forties, which agrees with that reported by Helderman[33]. Also the peak of oral cancer was observed around the age of of fifty, which accords with El-Nidany[34]. Eighty eight percent of the oral cancer cases were of the squamous cell carcinoma type and 12% were sarcomas, which agrees with El-Nidany[34] and Mohamed[35], who reported higher incidence of squamous cell carcinoma among Egyptians due to the lack of oral hygiene, chronic irritation or nutritional problems such as Vit. A deficiency.

Considering the site of the oral cancer, it was mainly localised in the floor of the mouth, the lateral and the base of the tongue more than the dorsum of the tongue, and the roof of the mouth. This might be due to the mucous membrane at these regions, and the stasis of saliva that leads to stasis of bacteria and subsequently elevated BG, nitrate reductase and nitrite levles.

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