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Chronic atrophic oral candidiasis among patients with diabetes mellitus – role of secretor status

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SUMMARY

Non-diabetic individuals who are non-secretors of blood group antigens are prone to superficial infections by *Candida albicans*. In this study, 216 patients with diabetes mellitus who were denture wearers were examined for the presence or absence of denture stomatitis. There was an overall trend for non-secretors to be prone to denture stomatitis compared with secretors. Stepwise linear discriminant analysis was used to dissect the contribution of secretor status and other variables to the development of the disease. Secretor status was found to be a contributory factor among patients with non-insulin dependent diabetes but not among those with insulin-dependent diabetes. The possible reasons for this are discussed.

INTRODUCTION

Chronic atrophic oral candidiasis (denture stomatitis) is solely associated with the wearing of dentures. The studies of Butdz-Jörgensen [1] and Cawson [2] established the link between *Candida* sp. and denture stomatitis. Yeasts were recovered from over 90% of the lesions and antifungal treatment usually led to regression of the lesion. Subsequently, numerous studies have examined the prevalence of and predisposing factors to denture stomatitis [3]. Presence and continuous wearing of dentures at night, availability of sucrose in the oral environment through dietary intake, traumatic effects of the denture, antibiotics and corticosteroid treatment, diabetes mellitus and smoking have all been implicated as potential factors influencing the development of denture stomatitis.

The genetic inability to secrete ABO blood group antigens in body fluids has been associated with a variety of infectious diseases [4]. Non-diabetic individuals and pregnant women who are non-secretors are prone to superficial candida infections [5]. Non-secretors are also over-represented among carriers of *Candida albicans* in normal subjects and patients with non-insulin dependent diabetes mellitus (NIDDM) [6, 7].

The aims of the present study were:

(1) To assess the influence of secretor status in the development of denture stomatitis (DS) among patients with insulin-dependent diabetes (IDDM) and those with non-insulin dependent diabetes (NIDDM).

(2) To assess the contributions of the following variables to the development of DS: age; sex; duration of diabetic state; type of diabetes; control of diabetes measured by glycosylated haemoglobin (HBA_1); diabetic complications – retinopathy, neuropathy and nephropathy; antibiotics; corticosteroid treatment; smoking; alcohol; presence of dentures at night; type of denture; denture fit, extension, occlusion, age and hygiene; presence of persistent glycosuria and albuminuria; and history of superficial candida infections.

MATERIALS AND METHODS

Subjects

A total of 439 subjects attending for routine follow up examination at the Diabetic Outpatient Department (DOPD), Royal Infirmary Edinburgh were sampled. An initial pilot study examined 80 individuals and was followed by a study that sampled 359 individuals between September 1988 and March 1989. The method of selection was stratified random selection according to sex and type of diabetes.

Clinical history

Each subject was classified as insulin dependent (IDDM) or non-insulin dependent (NIDDM) according to the clinical history of onset, requirement for insulin and progression of the disease. Of the 439 subjects sampled, three could not be classified.

A full medical history including the presence of diabetic complications (retinopathy, neuropathy and nephropathy) was obtained during interview and from the patients' records. A history of medications, with particular reference to antibiotics or corticosteroid-containing preparations, within the past 6 months was noted. A social history of alcohol consumption and smoking was recorded. Subjects were questioned about history of superficial infections due to candida. Glycosuria and albuminuria were recorded as persistent if subjects had positive urine samples on more than two consecutive appointments at the DOPD. None of the subjects used any oral preparations containing antiseptics within the previous 6 months.

Clinical examination

A thorough oral examination of both soft and hard tissues was carried out by the same examiner (FZA). Any of the following abnormalities were noted: gingivitis, periodontitis, angular cheilitis, leukoplakia, median rhomboid glossitis; fissured, geographic or hairy tongue; and denture stomatitis. Denture stomatis has the characteristic appearance of chronic erythema of the portion of the palate underlying the denture. The inflammation is generally diffuse, but may be associated with fibrous hyperplasia of the palate giving it a granular appearance. The occlusion, fit, extension and hygiene of a denture where present was recorded

as 'good' or 'poor'. The age of the denture was recorded as well as whether it was left out of the mouth at night.

Samples

(1) Venous blood was obtained for ABO blood grouping and Lewis antigen determination. Analysis for glycosylated haemoglobin (HBA_1) and random plasma glucose carried out routinely were recorded.

(2) Swabs were obtained from five sites of the mouth including the palate and inoculated immediately into malt broth.

(3) Subjects were requested to rinse with 10 ml of sterile phosphate-buffered saline (PBS) for 1 min and to return the contents to a sterile container.

Laboratory analysis

All samples were collected between 09.00 and 12.00 h and processed within 1–2 h. ABO blood group was determined by slide agglutination with monoclonal anti-A and anti-B antibodies (Scottish National Blood Transfusion Service). Secretor status was determined by the presence of Lewis antigen on red blood cells by tube agglutination with monoclonal anti-Lewis^a and anti-Lewis^b antibodies (Scottish National Blood Transfusion Service). The haemagglutination inhibition method with boiled saliva [8] was used to confirm the Lewis antigen results for 159 individuals.

The swabs were incubated at 37 °C for 36–48 h, plated onto malt agar and incubated for a further 36–48 h.

The mouth rinse was concentrated by centrifugation at 2000 ***g*** and resuspended in 1 ml of PBS; 20 μl of the suspension were inoculated onto malt agar plates and incubated at 37 °C for 36–48 h. The number of colonies per sample were recorded.

Pure colonies were subcultured and identified with the API 20C AUX. Additionally all were identified by the conventional methods [9] of germ-tube production in horse serum, urease test and hyphae production on corn-meal agar following incubation at 28 °C for 48 h.

Statistical methods

All results were coded and a computerized database was set up to facilitate analysis using SPSSX. The relationship between prevalence of DS and other factors was tested by χ^2 (with Yates' correction) or Wilcoxon rank sum tests. Stepwise linear discriminant analysis was used to identify which combinations of factors best predicted the presence or absence of DS.

RESULTS

Oral conditions among the patients examined are summarized in Table 1. Of the 216 denture wearers, 76 (35%) had DS (Table 2). In 18% (14/76) the rinsing technique failed to isolate any yeasts. Similar results were obtained with the swab taken from the palate. The species most frequently isolated from the palate of patients with DS were *C. albicans* (33/76, 43%), followed by *Torulopsis glabrata* (6/76, 8%) and *C. tropicalis* (3/76, 4%). In 9%, the isolate could not be identified to a species level. Other species were isolated in 15% (11/76) of DS cases (Table 3).

Table 1. *Prevalence of oral conditions among diabetic individuals*

	Prevalence in total population screened	Prevalence among denture wearers
Gingivitis	114 (28%)	39 (42%)
Angular cheilitis	42 (10%)	25 (12%)
Denture stomatitis	76 (35%)	76 (35%)
Fissured tongue	25 (6%)	15 (7%)
Dry mouth	15 (3%)	13 (6%)
Geographic tongue	11 (3%)	7 (3%)
Median rhomboid glossitis	10 (2%)	7 (3%)
Hairy tongue	8 (2%)	5 (2%)
Glossitis	7 (2%)	5 (2·3%)
Leukoplakia	2 (0·5%)	2 (1%)

Table 2. *Prevalence of DS among patients with IDDM or NIDDM*

	+DS	−DS	Total
IDDM	32 (41%)	47 (59%)	79 (100%)
NIDDM	43 (32%)	91 (68%)	134 (100%)
	75	138	213

Missing, 3; $\chi^2 = 1{\cdot}20$; $P = 0{\cdot}27$.

Table 3. *Mycological profile of diabetic individuals (Palatal swab)*

Isolate	Denture wearers no. (%)	DS cases no. (%)
C. albicans	67 (31)	33 (43)
T. glabrata	13 (6)	6 (8)
T. beigelli	5 (2)	3 (4)
C. tropicalis	4 (2)	3 (4)
C. paratropicalis	3 (1)	2 (3)
S. cereviciae	2 (1)	0 (0)
C. stellatoidea	1 (0·5)	1 (1)
T. inconspicua	2 (1)	1 (1)
C. luscitaniae	1 (0·5)	0 (0)
C. humicola	1 (0·5)	0 (0·5)
C. pseudotropicalis	1 (0·5)	1 (1)
Unidentified	24 (11)	7 (9)
Missing	5 (2)	2 (3)
Other	4 (2)	3 (4)
No isolate	83 (38)	14 (18)
Total	216	76

Secretor status

There was a trend for non-secretors to be prone to DS (Table 4). Among individuals of blood group O with DS, non-secretors (13/27, 48%) appeared to be more prone to DS than secretors (19/68, 28%); $\chi^2 = 2{\cdot}69$, D.F. = 1, $P = 0{\cdot}1$. This was not seen among the 73 blood group A individuals. There were 10 (45%) A non-secretors compared with 15 (29%) A secretors with DS ($\chi^2 = 1{\cdot}1$, D.F. = 1, $P = 0{\cdot}3$).

Table 4. *Secretor status and prevalence of DS among patients with diabetes*

	Denture stomatitis			
	Present no. (%)	Absent no. (%)	χ^2	P
Patient category				
Total (n = 199)				
Secretor	45 (31)	100 (69)	2·56	0·11
Non-secretor	24 (44)	30 (56)		
IDDM				
Secretor	18 (40)	27 (60)	0·03	0·87
Non-secretor	13 (45)	16 (55)		
NIDDM				
Secretor	27 (27)	72 (73)	1·90	0·17
Non-secretor	11 (44)	14 (56)		

Table 5. *Relationship between DS and presence of denture in the mouth at night*

		Denture stomatitis			
Patient category	Presence of denture	Present No. (%)	Absent No. (%)	χ^2	P
Total*	Yes	45 (52)	42 (48)	23·2	< 0·00001
	No	7 (12)	53 (88)		
IDDM	Yes	18 (49)	19 (51)	3·50	0·062
	No	3 (18)	14 (82)		
NIDDM	Yes	27 (54)	23 (46)	18·82	< 0·00001
	No	4 (9)	39 (91)		

* Of a total of 216 denture wearers, 43 were recruited during the pilot study and were not questioned about their denture wearing habits. For 26 individuals this information was not recorded during the main study period.

Significant associations were found between DS and the following:

Presence of dentures in the mouth at night. Significantly more individuals who did not remove their dentures at night (45) had DS compared with those who removed their dentures at night (7) ($P < 0·00001$). This was found particularly among NIDDM individuals (Table 5).

Number of colonies isolated by the mouth rinse technique. Subjects who did not have DS had significantly fewer colony forming units (median = 10 c.f.u./ml) than subjects with DS (median = 1850 c.f.u./ml, $P < 0·0001$). This was seen particularly for patients with NIDDM (median = 1500 c.f.u./ml with DS and 25 c.f.u./ml without DS, $P = 0·0003$). Those with IDDM did not show this relationship (median = 2400 c.f.u./ml with DS, 502·5 c.f.u./ml without DS, $P = 0·07$).

Random plasma glucose. Lower values for random plasma glucose were observed among the 132 patients without DS (median = 9·55 mmol/l) than those observed for the 72 patients with DS (median = 10·95 mmol/l, Mann–Whitney $P = 0·02$). This was seen particularly among individuals with NIDDM (median = 10·75 mmol/l with DS and 9·5 mmol/l without DS, $P = 0·04$). Individuals with IDDM

Table 6. *Stepwise discriminant analysis (Wilks)*

Sample	Cases predicted (%)	Variables isolated
IDDM ($n = 52$)	62·90%	Glycosuria ($P < 0·05$)
NIDDM ($n = 88$)	81·82%	Denture in at night ($P < 0·01$) No. of yeast colonies ($P < 0·01$) Non-secretion ($P < 0·01$)

did not show this relationship (median 11·250 mmol/l with DS and 10·15 mmol/l without DS, $P = 0·24$).

History of candidiasis. Among individuals with a history of superficial candidiasis 18/33 (55%) had DS compared with 58/182 (32%) with a negative history of candidiasis ($\chi^2 = 5·33$, D.F. = 1, $P = 0·02$). Separate analysis with respect to insulin dependency revealed a trend for individuals with a history of candidiasis to be more prone to DS: for IDDM individuals $\chi^2 = 1·2$, D.F. = 1, $P = 0·3$; for individuals with NIDDM, $\chi^2 = 3·26$, D.F. = 1, $P = 0·07$.

No significant association was found between DS and the following variables: sex; age; type of diabetes; control of diabetes (HBA_1); duration of diabetic state; ABO blood group; smoking; alcohol consumption; persistent glycosuria or albuminuria; denture fit, hygiene, occlusion, age or type (partial or full); presence of diabetic complications (neuropathy, nephropathy, retinopathy); contraceptive pill; antibiotics; and corticosteroids (systemic or topical).

In order to determine the relative contributions of variables in the development of DS, a stepwise discriminant analysis (SPSSX) was used. Initially, all variables were screened at an F value of 4 ($P < 0·05$). Analysis was then confined to the variables isolated as significant to increase the number of cases examined. Table 6 summarizes the contributory variables and the significance of their contribution in predicting infection. For patients with IDDM, the only variable identified by the analysis was persistent glycosuria ($P < 0·05$). For patients with NIDDM three factors were identified: denture present at night ($P < 0·01$), number of yeasts isolated ($P < 0·01$) and non-secretion ($P < 0·01$).

DISCUSSION

All forms of the oral conditions listed in Table 1 are more prevalent among the patients with dentures than those without dentures. Fissured tongue was encountered in 6% of the diabetic individuals. This figure is similar to that reported for 2478 dental patients [10]. The prevalence of geographic tongue and median rhomboid glossitis was slightly higher among diabetics than among dental and dermatology patients [11]. All the above conditions were much lower in prevalence than those reported for diabetic individuals by Farman who examined coloured South Africans [12]. These differences might be due to genetic and/or environmental factors between the predominantly Northern European population sampled here and that sampled by Farman. Dry mouth was a spontaneous complaint in only 3% of the samples compared to 34% reported by Sharon and colleagues [13].

The univariate analysis showed that prevalence of DS was similar among patients with IDDM or NIDDM. The prevalence of DS reported (35·2%) is within the range (24–60%) reported for non-diabetic individuals [3].

C. albicans was isolated from only 43% of the patients with DS. Isolation of *C. glabrata* (8%), and other yeasts (28%) from patients with DS indicate that, among diabetic individuals, species other than *C. albicans* are an important cause of disease.

Sex, denture trauma and hygiene [14–19], treatment with corticosteroids [21] and antibiotics [3], have variably been reported to be associated with DS among non-diabetic individuals. In this study, both the discriminant and univariate analyses did not reveal any association between these factors and DS.

Among patients with candida leukoplakia (CL), smoking is a significant factor in the pathogenesis of the lesion [20] which develops in sites away from the denture bearing area. In DS, smoking was not a factor in patients with either IDDM or NIDDM. This is consistent with the fact that the palate is protected from the effect of the smoke by the denture.

Control of diabetes as measured by glycosylated haemoglobin (HBA_1) was not associated with DS. Although this was unexpected at first sight, it is well established that blood glucose levels and salivary glucose levels among diabetic individuals do not correlate [13].

Discriminant analysis indicates that factors contributing to development of DS among patients with NIDDM are clearly different from those with IDDM. Patients with NIDDM who do not remove their denture at night, harbour a large number of yeasts and non-secretors of blood group antigens are particularly at risk of developing DS. In contrast, none of these factors influence the development of DS among individuals with IDDM. This is further supported by the results of the univariate analysis in which the P value for these factors among IDDM individuals are consistently higher than the P value among NIDDM individuals.

Although secretor status did not show a significant univariate association with DS among individuals with NIDDM, it was found to be significant in the multivariate analysis when adjusted for denture wearing habits and density of yeast colonization. This suggests that the univariate relationship might have been obscured by the other two contributory factors.

Persistent glycosuria was the only predictor of DS development among IDDM individuals. The presence of 11·1 mmol/l or more of glucose in the arterial blood results in the appearance of glucose in the urine. It was unexpected that neither HBA_1 nor random plasma glucose were implicated as both are more precise indicators of glucose availability than glycosuria. The possibility than glycosuria was a chance isolation cannot be dismissed especially since the significance of this variable was marginal. It appears that the discriminant analysis was not effective in identifying variables important to the development of DS among IDDM individuals.

Non-secretion of blood group antigens was a significant contributory factor among NIDDM but not among IDDM individuals. Non-secretion has been associated with carriage of yeasts among non-diabetic individuals [6] and patients with NIDDM [7]. This complements the results relating to the development of DS in these patients. It has been suggested that patients with IDDM are more

immunocompromized than NIDDM so that any protective effect of secretion of blood group antigens does not make a significant contribution to prevention of colonization or disease. A previous study [22] did not find an association between secretor status and DS among diabetics; however, the number of patients examined was much smaller, there was no differentiation between IDDM and NIDDM individuals and multivariate analysis was not applied to the data.

Identification of factors contributing to the development of DS among individuals with IDDM and NIDDM might have implications for treatment of this condition. Treatment of DS among patients with NIDDM might be similar to that in non-diabetic individuals [23], i.e. removal of denture, especially at night, which also reduces the density of colonization by yeasts. Among patients with IDDM, treatment of DS seems likely to depend on improvement of poor diabetic control reflected in persistent glycosuria.

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