

## Factors influencing oral carriage of yeasts among individuals with diabetes mellitus

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### SUMMARY

A total of 439 individuals with diabetes mellitus were examined for carriage of yeasts by the oral rinse and palatal swab techniques. Eighteen genetic or environment variables were assessed for their contribution to carriage of yeasts. The factor contributing to palatal and oral carriage of yeasts among individuals with insulin dependent diabetes mellitus (IDDM) was age ( $P < 0.01$ ). The factor contributing to palatal carriage of yeasts among individuals with non-insulin dependent diabetes mellitus (NIDDM) was poor glycaemic control (glycosuria  $P < 0.01$ ); carriage in the oral cavity as a whole was influenced additionally by non-secretion of ABH blood group antigens ( $P < 0.05$ ). Introduction of a denture altered the above risk factors. For individuals with IDDM, oral carriage was associated with the presence of retinopathy ( $P < 0.05$ ); palatal carriage was influenced by poor glycaemic control (HbA<sub>1c</sub>  $P < 0.01$ , plasma glucose levels  $P < 0.05$ ) and age ( $P < 0.05$ ). For those with NIDDM, palatal carriage was associated with continuous presence of the denture in the mouth ( $P < 0.01$ ); oral carriage was associated with plasma glucose levels ( $P < 0.05$ ).

### INTRODUCTION

Oral candidiasis is most prevalent as chronic atrophic candidiasis [1]. Individuals with diabetes are prone to infection; and chronic atrophic candidiasis has been reported to be more prevalent among diabetic individuals compared with non-diabetic controls [2]. Since disease is frequently preceded by colonization, it is important to identify factors which predispose to colonization. Few studies have examined the factors which predispose to colonization of diabetic patients by *Candida albicans*; and none of these specifically analysed patients with insulin dependent diabetes mellitus (IDDM) separately from those with non-insulin dependent diabetes mellitus (NIDDM) [2–6].

Both genetic [7, 8] and environmental factors [9] have been reported to affect carriage of *Candida* species. Group A  $\beta$ -haemolytic *Streptococcus pyogenes* are isolated more frequently from the pharynx of individuals who are non-secretors of ABH antigens [10] and non-secretors were significantly over-represented among carriers of meningococci [11]. Among non-diabetic individuals and individuals

with NIDDM non-secretors of blood group O are over-represented among carriers of *C. albicans* compared with secretors [7, 8].

Environmental factors variably reported to be associated with carriage of yeasts include: presence of a denture; continuous wearing of a denture; denture fit, occlusion, trauma, hygiene and age; smoking; and control of diabetes [3, 12].

This was the first study designed to compare the factors contributing to carriage of yeasts in individuals with IDDM or NIDDM; its aims were:

- (1) To compare the oral rinse technique with the palate swab method for isolating yeasts.
- (2) To compare the species of yeasts isolated from patients with IDDM or NIDDM.
- (3) To assess the association between secretor status and carriage taking into account denture status.
- (4) To dissect the contributions of the following variables to the carriage of yeasts and, specifically, *C. albicans*: age; sex; type of diabetes; control of diabetes as measured by glycosylated haemoglobin A<sub>1</sub> (HbA<sub>1</sub>), random plasma glucose levels, persistent glycosuria and albuminuria; diabetic complications – retinopathy, neuropathy, nephropathy; antibiotic usage; corticosteroid treatment; smoking; alcohol consumption; presence of the denture in the mouth at night; denture fit, extension, occlusion hygiene and age; presence of denture stomatitis; and history of superficial candida infections.

#### MATERIALS AND METHODS

##### *Subjects*

A total of 439 subjects attending for routine follow up examination at the diabetic out-patient clinic, 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 family history of diabetes, clinical history of onset, requirement for insulin, progression of the disease. Three of the 439 subjects sampled 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 clinic. 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. 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*

Venous blood was obtained for ABO blood grouping and Lewis antigen determination. Routine analyses for glycosylated haemoglobin (HbA<sub>1</sub>) and random plasma glucose were recorded. Swabs were obtained from five sites (palate, tongue, floor, right and left angles of the mouth) and inoculated immediately into malt broth. Each subject provided a fresh, unstimulated sample of saliva which was collected in a sterile Universal container.

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 Universal container.

### *Laboratory analysis*

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-Le<sup>a</sup> and anti-Le<sup>b</sup> antibodies (Scottish National Blood Transfusion Service). The haemagglutination inhibition method with boiled saliva was used to confirm the Lewis antigen results for 159 individuals [13].

The Corning electrophoresis method was used for measuring HbA<sub>1</sub> (normal range 4.5–8.0%, coefficient of variance = 4%).

The swabs in malt broth were incubated at 37 °C for 36–48 h to enrich for *C. albicans*, plated onto malt agar and incubated for a further 36–48 h.

The mouth rinse was concentrated by centrifugation 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 Auxanogram (API Systems S.A., France). All were also identified by the conventional methods of germ-tube production in horse serum, urease test and hyphae production on corn-meal agar following incubation at 28 °C for 48 h [14].

### *Statistical analysis*

All results were coded and a computerized database was set up to facilitate analysis by the SPSSX. Univariate analysis was by the  $\chi^2$  (with Yates' correction) or Wilcoxon rank sum tests. MacNemar's test for paired alternatives was used to compare the results of the oral rinse and the palate swab.

Stepwise linear discriminant analysis (Wilk's method) was used to identify which combinations of factors best predicted yeast carriage. During the analysis, predictor variables can be added or removed in a stepwise manner, each variable being selected on the basis of an *F* level of 4 for entry or removal, corresponding to *P* = 0.05.

## RESULTS

Table 1 summarizes the characteristics of the population sampled with respect to their diabetic status. Individuals with IDDM were younger, had a higher mean value of HbA<sub>1c</sub>; and they had a higher proportion of non-secretors (36.4%) compared with individuals with NIDDM (22%) ( $\chi^2 = 10.15$ ;  $P < 0.005$ ). The mean number of cigarettes per day smoked by subjects with IDDM or NIDDM were similar.

*Mycological profile of the sample population*

Only 29% (128/439) of the individuals examined had no yeasts in any of the five sites swabbed; this compared well with the oral rinse technique in which 34% of the individuals were culture negative. The concordance between the results obtained by these methods was 85%. By MacNemar's test for paired alternatives, there was no significant difference between the discordant pairs obtained by the two sampling methods ( $P > 0.1$ ). Table 2 compares the species of yeasts cultured from swabs of the five sites of the mouth with those obtained from the oral rinse from individuals with IDDM or NIDDM.

*Univariate analysis of factors affecting carriage*

Tables 3–8 present analysis of factors examined for their association with carriage of yeasts; similar results were obtained when carriage of *C. albicans* was analysed separately.

Isolation of yeasts from the palate or from the oral rinse was not associated with secretor status when the results were analysed by type of diabetes and/or denture status.

Blood group was not associated with palatal carriage. When carriage was assessed by the oral rinse, however, individuals with NIDDM who wore dentures and who were of O blood group were more likely to be carriers than those of blood group A ( $\chi^2 = 7.93$ ,  $P = 0.05$ ) (Table 3). Comparisons with B and AB were not done because of the small numbers of individuals with these blood groups. Analysis of secretor status with reference to blood group revealed a significant association between non-secretion and increased frequency of carriage of yeasts among individuals with IDDM who were blood group A and who wore dentures; there were 9/19 A-secretor carriers compared with 12/12 A-non-secretor carriers ( $\chi^2 = 7.07$ ,  $P = 0.008$ ).

Yeasts were isolated significantly more frequently from the palate of patients with IDDM with or without dentures (Table 4). Similar results were obtained by the oral rinse method.

Increase in age was significantly associated with a decreased frequency of isolation of yeasts from the palate of patients with IDDM but not those with NIDDM (Table 5). Similar results were obtained by the oral rinse technique.

The HbA<sub>1c</sub> values of palatal carriers were higher compared with non-carriers; particularly among individuals with IDDM (Table 6). Results from the oral rinse technique showed a similar trend but did not attain significance at  $P < 0.05$  for individuals with IDDM or NIDDM.

The mean number of cigarettes per day was a highly significant factor

Table 1. Characteristics of the sample population

| Type of diabetes   | Age         | HbA <sub>1</sub> | Smokers (%) | Cigarettes per day | Non-secretors (%) | Secretors (%) |
|--------------------|-------------|------------------|-------------|--------------------|-------------------|---------------|
|                    | mean (s.d.) | mean (s.d.)      |             |                    |                   |               |
| IDDM<br>(n = 231)  | 40.3 (16.0) | 10.3 (2.1)       | 41          | 17.3 (11.1)        | 36.4              | 63.6          |
| NIDDM<br>(n = 205) | 58.1 (9.3)  | 9.8 (2.3)        | 34          | 17.3 (11.7)        | 22.0              | 78.0          |

Table 2. Species of yeast isolated from individuals with IDDM or NIDDM with or without dentures (percentages)

| Patient category   |                | Sample | None | <i>C. albicans</i> | <i>C. tropicalis</i> | Other |
|--------------------|----------------|--------|------|--------------------|----------------------|-------|
| No dentures        |                |        |      |                    |                      |       |
| IDDM<br>(n = 150)  | Oral rinse     | 33     | 47   | 2                  | 0                    | 18    |
|                    | Palate         | 44     | 48   | 1                  | 1                    | 6     |
|                    | Tongue         | 38     | 54   | 2                  | 0                    | 6     |
|                    | Floor of mouth | 41     | 54   | 1                  | 1                    | 3     |
|                    | Right angle    | 56     | 38   | 0                  | 0                    | 5     |
|                    | Left angle     | 60     | 35   | 0                  | 0                    | 5     |
| NIDDM<br>(n = 70)  | Oral rinse     | 40     | 28   | 2                  | 0                    | 31    |
|                    | Palate         | 63     | 28   | 0                  | 0                    | 9     |
|                    | Tongue         | 63     | 32   | 0                  | 0                    | 5     |
|                    | Floor of mouth | 70     | 27   | 0                  | 0                    | 3     |
|                    | Right angle    | 68     | 27   | 0                  | 0                    | 5     |
|                    | Left angle     | 75     | 24   | 0                  | 0                    | 2     |
| Dentures           |                |        |      |                    |                      |       |
| IDDM<br>(n = 79)   | Oral rinse     | 24     | 43   | 4                  | 0                    | 29    |
|                    | Palate         | 33     | 47   | 9                  | 2                    | 9     |
|                    | Tongue         | 37     | 49   | 5                  | 2                    | 8     |
|                    | Floor of mouth | 34     | 49   | 5                  | 3                    | 8     |
|                    | Right angle    | 50     | 29   | 9                  | 1                    | 10    |
|                    | Left angle     | 42     | 41   | 6                  | 2                    | 9     |
| NIDDM<br>(n = 135) | Oral rinse     | 41     | 24   | 6                  | 3                    | 27    |
|                    | Palate         | 51     | 29   | 6                  | 3                    | 12    |
|                    | Tongue         | 51     | 34   | 8                  | 2                    | 6     |
|                    | Floor of mouth | 53     | 31   | 7                  | 3                    | 7     |
|                    | Right angle    | 54     | 25   | 9                  | 3                    | 9     |
|                    | Left angle     | 58     | 24   | 10                 | 3                    | 6     |

Table 3. Blood groups and carriage of yeasts assessed by the oral rinse technique

| Category      | Blood group | Non-carriers | Carriers | $\chi^2$ | P     |
|---------------|-------------|--------------|----------|----------|-------|
|               |             | no. (%)      | no. (%)  |          |       |
| IDDM          | O           | 22 (39)      | 34 (61)  | 1.49     | 0.22  |
| No dentures   | A           | 11 (26)      | 32 (74)  |          |       |
| IDDM          | O           | 5 (16)       | 26 (84)  | 1.24     | 0.27  |
| With dentures | A           | 10 (31)      | 22 (69)  |          |       |
| NIDDM         | O           | 9 (33)       | 18 (67)  | 0.13     | 0.73  |
| No dentures   | A           | 7 (44)       | 9 (56)   |          |       |
| NIDDM         | O           | 17 (32)      | 36 (68)  | 7.93     | 0.005 |
| With dentures | A           | 20 (67)      | 10 (33)  |          |       |

Table 4. *Type of diabetes and isolation of yeasts from the palatal swab*

| Category         | Non-carriers | Carriers* | $\chi^2$ | P†    |
|------------------|--------------|-----------|----------|-------|
|                  | no. (%)      | no. (%)   |          |       |
| All              |              |           |          |       |
| IDDM             | 70 (39)      | 108 (61)  | 8.5      | 0.004 |
| NIDDM            | 101 (55)     | 82 (45)   |          |       |
| Without dentures |              |           |          |       |
| IDDM             | 48 (44)      | 62 (56)   | 5.03     | 0.014 |
| NIDDM            | 40 (62)      | 24 (38)   |          |       |
| With dentures    |              |           |          |       |
| IDDM             | 22 (33)      | 44 (67)   | 4.81     | 0.013 |
| NIDDM            | 61 (51)      | 58 (49)   |          |       |

\* All yeasts including *C. albicans*.

† P value refers to comparisons between IDDM and NIDDM

Table 5. *Median age and carriage of yeasts determined by palate swab*

| Category         | Median age (years) |          | P*    |
|------------------|--------------------|----------|-------|
|                  | Non-carriers       | Carriers |       |
| IDDM             | 58.00              | 49.00    | 0.033 |
| with dentures    |                    |          |       |
| (n)              | (22)               | (44)     |       |
| IDDM             | 38.50              | 29.00    | 0.011 |
| without dentures |                    |          |       |
| (n)              | (48)               | (62)     |       |
| NIDDM            | 61.00              | 59.00    | 0.07  |
| with dentures    |                    |          |       |
| (n)              | (61)               | (58)     |       |
| NIDDM            | 56.00              | 50.50    | 0.09  |
| without dentures |                    |          |       |
| (n)              | (39)               | (24)     |       |

\* P determined by Mann-Whitney test.

Table 6. *Glycaemic control (HbA<sub>1</sub>) and carriage determined by palate swab*

| Category         | Median HbA <sub>1</sub> units |          | P*    |
|------------------|-------------------------------|----------|-------|
|                  | Non-carriers                  | Carriers |       |
| IDDM             | 9.55                          | 11.10    | 0.067 |
| with dentures    |                               |          |       |
| (n)              | (18)                          | (236)    |       |
| IDDM             | 9.30                          | 10.60    | 0.017 |
| without dentures |                               |          |       |
| (n)              | (45)                          | (55)     |       |
| NIDDM            | 9.30                          | 9.50     | 0.15  |
| with dentures    |                               |          |       |
| (n)              | (57)                          | (52)     |       |
| NIDDM            | 8.70                          | 9.90     | 0.26  |
| without dentures |                               |          |       |
| (n)              | (40)                          | (21)     |       |

\* P determined by Mann-Whitney test.

Table 7. Smoking and carriage of yeasts determined by palate swab

| Category               | Mean cigarettes/day |          | P*      |
|------------------------|---------------------|----------|---------|
|                        | Non-carriers        | Carriers |         |
| All                    | 4.54                | 7.83     | < 0.001 |
| (n)                    | (156)               | (181)    |         |
| IDDM with dentures     | 7.95                | 9.20     | 0.24    |
| (n)                    | (20)                | (44)     |         |
| IDDM without dentures  | 5.72                | 7.32     | 0.43    |
| (n)                    | (43)                | (59)     |         |
| NIDDM with dentures    | 2.89                | 6.87     | 0.051   |
| (n)                    | (57)                | (54)     |         |
| NIDDM without dentures | 4.28                | 9.09     | 0.13    |
| (n)                    | (36)                | (23)     |         |

\* P determined by Mann-Whitney test.

Table 8. Continuous wearing of denture and isolation of yeasts from the palate

| Category | Denture presence | Non-carriers | Carriers | $\chi^2$ | P     |
|----------|------------------|--------------|----------|----------|-------|
|          |                  | no. (%)      | no. (%)  |          |       |
| All      | Yes              | 30 (40)      | 45 (60)  | 6.61     | 0.01  |
|          | No               | 32 (65)      | 17 (35)  |          |       |
| IDDM     | Yes              | 10 (32)      | 21 (68)  | 0.002    | 0.96  |
|          | No               | 5 (38)       | 8 (62)   |          |       |
| NIDDM    | Yes              | 20 (45)      | 24 (55)  | 5.97     | 0.015 |
|          | No               | 27 (75)      | 9 (25)   |          |       |

associated with carriage of the whole population sampled (Mann-Whitney  $P < 0.001$ ). When results were analysed with respect to types of diabetes and denture status, smoking was of marginal significance ( $P = 0.051$ ) only among denture wearers with NIDDM (Table 7).

Yeasts were isolated more often from the palate of subjects who left their dentures in the mouth at night; however, this was observed only among individuals with NIDDM (Table 8). This pattern was not found when carriage was assessed by the oral rinse method.

The following variables were not associated with frequency of carriage of yeasts by either isolation technique; sex; duration of diabetes; complications – neuropathy, nephropathy and retinopathy; alcohol consumption; contraceptive pill; systemic corticosteroid treatment; topical corticosteroid application; antibiotics; fit, occlusion or hygiene of denture; persistent albuminuria; or history of superficial candida infections. Denture status was not significantly associated with frequency or density of colonization by yeasts.

*Multivariate analysis*

Univariate analysis might incorporate dependent variables which need prior knowledge to control for their effect. The multivariate analysis identified factors

Table 9. *Palatal carriage of yeasts: variables isolated by multivariate analysis (Wilk's Method)*

| Diabetes                  | Dentures | Variables isolated   | % cases correctly predicted |
|---------------------------|----------|--|-----------------------------|
| IDDM<br>( <i>n</i> = 110) | No       | Age ( <i>P</i> < 0.05)   | 61                          |
| IDDM<br>( <i>n</i> = 51)  | Yes      | HbA <sub>1</sub> ( <i>P</i> < 0.01)<br>Age ( <i>P</i> < 0.05)<br>Plasma glucose ( <i>P</i> < 0.05) | 71                          |
| NIDDM<br>( <i>n</i> = 49) | No       | Glycosuria ( <i>P</i> < 0.01)  | 78                          |
| NIDDM<br>( <i>n</i> = 80) | Yes      | Continuous wear of denture<br>( <i>P</i> < 0.01)   | 64                          |

Table 10. *Oral carriage of yeasts: variables isolated by the multivariate analysis (Wilk's Method)*

| Diabetes                  | Dentures | Variables isolated   | % cases correctly predicted |
|---------------------------|----------|--|-----------------------------|
| IDDM<br>( <i>n</i> = 97)  | No       | Age ( <i>P</i> < 0.01)   | 68                          |
| IDDM<br>( <i>n</i> = 71)  | Yes      | Retinopathy ( <i>P</i> < 0.05)   | 76                          |
| NIDDM<br>( <i>n</i> = 43) | No       | Glycosuria ( <i>P</i> < 0.01)<br>plasma glucose ( <i>P</i> < 0.05)<br>Non-secretion ( <i>P</i> < 0.05) | 67                          |
| NIDDM<br>( <i>n</i> = 78) | Yes      | Plasma glucose ( <i>P</i> < 0.05)  | 48                          |

which contribute to carriage of yeasts among individuals with IDDM or NIDDM who wear dentures and those without dentures. Tables 9 and 10 summarize the results obtained from the palate swab and oral rinse techniques respectively. Similar results were obtained when carriage of *C. albicans* was analysed separately. The percentage of carriers correctly predicted by the isolated variables indicates their prognostic value.

Individuals with IDDM who did not wear dentures were best segregated on the basis of their age into non-carriers and carriers of yeasts; carriage decreased with increased age. This was true when carriage was assessed by either technique. The univariate analysis of this group of patients showed similar associations; younger individuals were more prone to carriage of yeasts.

Among individuals with IDDM who wore dentures, an increase in the frequency of palatal carriage of yeasts was associated with an increase in HbA<sub>1</sub> level (*P* < 0.01); younger individuals (*P* < 0.05) and increased random plasma glucose levels (*P* < 0.05). The variable which was the most efficient predictor of carriage assessed by the oral rinse technique was, however, the presence of retinopathy (*P* < 0.05). Univariate analysis of this group of patients showed age to be associated with palatal carriage.

Individuals with NIDDM who did not wear dentures were more likely to be palatal carriers of yeasts if they had persistent glycosuria (*P* < 0.05). Carriage



assessed by the oral rinse method was influenced by persistent glycosuria ( $P < 0.01$ ), higher random plasma glucose levels ( $P < 0.05$ ) and non-secretion of blood group antigens ( $P = 0.05$ ). Univariate analysis of this group revealed an association between persistent glycosuria and carriage among individuals with NIDDM ( $\chi^2 = 5.32$ ,  $P = 0.02$ ).

Individuals with NIDDM who wore dentures were at risk of palatal carriage of yeasts if they wore their dentures continuously ( $P = 0.01$ ). Carriage of yeasts assessed by the oral rinse technique was best predicted by an increased random plasma glucose levels ( $P < 0.05$ ). Comparison with univariate analysis showed that continuous wearing of dentures was a significant factor only when carriage was assessed by palatal swab.

#### DISCUSSION

The results are discussed in the context of the objectives of the study. The first objective of the study was to compare isolation of yeasts by the oral rinse technique with those obtained by the palate swab. The oral rinse technique compared well with the results of the swab technique; there was 85% concordance between the results obtained with the two methods.

*C. albicans* was the species most frequently isolated from the swabs while species other than *C. albicans* were isolated more often from the oral rinse. This might be due to incubation of swabs in the malt broth suppressing other species of yeasts. By the swab technique, yeasts were isolated most frequently from the tongue followed by the palate, floor of the mouth and the angles of the mouth. Similar results have been reported [3]. This supports the suggestion that the tongue might act as a reservoir for yeasts [15]. For future work, the technique appropriate for the objectives of the study should be employed. The oral rinse technique can be used where quantitative and overall carriage of yeasts are required; swabs can be used for examination of specific sites for carriage.

In this study, 66% of the diabetics were carriers of yeasts by the oral rinse technique, a figure slightly higher than those reported in previous studies of diabetics (41–62%) [2, 3, 5, 6, 16].

The second objective was to compare isolation of yeasts from patients with IDDM compared with those with NIDDM. *C. glabrata* and *C. tropicalis* were isolated more frequently from patients with either IDDM or NIDDM who wore dentures. There were significantly more carriers among individuals with IDDM compared with NIDDM subjects; no other study has found this association [2, 3, 16]. This might be due to the smaller sample sizes of other studies, differences in populations sampled and their distinction of IDDM from NIDDM based solely on treatment of the diabetic condition. In this study subjects were classified as IDDM or NIDDM according to family history of diabetes, clinical history of onset, requirement for insulin and progression of the disease.

By the API identification system, some studies have found 97% of isolates to be *C. albicans* [5, 17]. In this study only 54% of the isolates were *C. albicans* (Table 2); this figure includes the proportion that was not identified by API 20C Aux as *C. albicans* but found to be so by conventional methods. These results are similar to studies which found 60% of isolates from diabetics to be *C. albicans* [3, 16]. This

emphasizes the need for accurate identification as patients with diabetes are more likely to carry and to have disease due to species other than *C. albicans* [18] which might not be sensitive to routinely prescribed oral antifungal agents [19].

The third objective was to assess the association between secretor status and carriage of yeasts among individuals with IDDM separately from those with NIDDM. In most diseases the influence of blood group or secretor status is marginal; sample sizes need to be large enough to discern such relationships with confidence [20]. By univariate analysis, other studies found a relationship between non-secretion of blood group antigens and carriage of yeasts among diabetics [8, 17]. These studies did not take into account the denture status [8] or type of diabetes [17] of the sample population. Another study did not report an association between secretor status and carriage of yeasts or development of disease [2]; however, the number of subjects was much smaller, denture status and type of diabetes were not considered.

Among individuals with NIDDM, the results of the multivariate analysis show that non-secretion of blood group antigens was a marginally significant factor influencing carriage in the oral cavity as a whole. Palatal carriage of yeasts was not dependent on secretor status. Density of colonization reflected in the quantitative carriage of yeasts assessed by the oral rinse is influenced by secretor status; carriage *per se* does not depend on secretor status.

The fourth objective of the study was to dissect the contribution of various other factors to carriage of yeasts. The following factors were not associated with carriage by either univariate or multivariate analyses: sex; duration of diabetes; diabetic complications of neuropathy or nephropathy; alcohol consumption; contraceptive pill; systemic corticosteroid treatment; topical corticosteroid application; antibiotic usage; fit, occlusion or hygiene of denture; persistent albuminuria; or history of superficial candida infections. Those for which significant associations were found are discussed below with reference to previous reports.

The prevalence of yeasts in the adult mouth has been shown to rise with age. The effects of age are not always easily separated from disease and medical treatment [21, 22]. Age was not related to isolation of yeasts from diabetics [2, 3, 17]. In a Canadian study [6], diabetics over 50 years of age had a higher density of yeasts compared with those less than 50 years old. Age was not isolated as a significant factor in the multivariate analysis, and the authors suggested that carriage in the older age group might be due to the higher number of denture wearers. In the study reported here, increase in age was associated with a decrease in the frequency of carriage especially among non-denture wearers with IDDM (Tables 5 and 9). This was unexpected; one explanation might be that decreased isolation of yeasts with increasing age reflects the efficiency of the mucosal immune defences against yeasts with increased frequency of challenge. Studies on vaginal carriage of yeasts found an inverse correlation between isolation of yeasts and levels of serum anti-candida IgA [23]. Secretory anti-candida IgA has been reported to correlate with levels of serum IgA [24]. Analysis of secretory and humoral immune responses of the study population to yeasts are needed to obtain evidence for this hypothesis.

Poor glycaemic control (HbA<sub>1c</sub> and plasma glucose) was a significant factor

associated with palatal carriage of yeasts among patients with IDDM who wore dentures. Carriage assessed by the oral rinse was not associated with HbA<sub>1c</sub> levels; similar results were reported in other studies [2, 5]. The single study in which multivariate analysis was used found an association between HbA<sub>1c</sub> level and colonization determined by palate or denture base swab; however, this study did not differentiate individuals with IDDM from those with NIDDM [6]. Further evidence that diabetic control is associated with carriage was the significant association between carriage and plasma glucose levels or persistent glycosuria among patients with NIDDM who did not wear dentures. Studies on other diabetic populations reported similar results [16], but the majority did not find this association [2, 3, 6, 17].

Retinopathy was of marginal significance as a predictor of carriage among denture wearers with IDDM. This complication results from microvascular changes associated with diabetes which might also impair immune response through inadequate diffusion of tissue mediators and indicates that systemic changes associated with diabetes predispose to colonization.

Studies in non-diabetic populations reported a positive correlation between smoking and carriage of bacteria [25, 26] and yeasts [27]. Among diabetics, smoking was a significant factor influencing carriage of yeasts; however, the type of diabetes and denture status were not taken into account [3]. Although similar association was found in this study, this association was not significant when results were analysed with respect to type of diabetes and denture status.

In contrast to other studies which reported a higher prevalence and/or density of yeasts among diabetics who wore dentures [2, 3, 5, 6], we found no correlation with frequency or quantity of yeasts isolated with either technique. Similar results were reported by Darwazeh and colleagues [17]. There is no obvious explanation for these discrepancies. Continuous presence of the denture might influence analysis of denture status results; however, we found this association only for palatal carriage among patients with NIDDM (Tables 8 and 9). Other studies found continuous presence of dentures associated with increased frequency and density of yeasts [3, 17].

The contribution of factors such as antibiotics and corticosteroids to carriage of yeasts is not easily differentiated from that of the underlying illness. Most of the studies in which associations between carriage and these treatments were found were carried out on hospitalized patients. The present study examined out-patients, the majority of whom were not on either of these treatments; therefore, no inferences can be made on the role of these therapies on oral carriage.

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