

Denture-related stomatitis: identification of aetiological and predisposing factors – a large cohort

M.H. FIGUEIRAL*, A. AZUL[†], E. PINTO[‡], P.A. FONSECA*, F.M. BRANCO* & C. SCULLY[§]
*Faculty of Dentistry, Porto University, [†]ISAVE, Braga, [‡]Faculty of Pharmacy of Porto University, Portugal and, [§]Eastman Dental Institute, London, UK

SUMMARY The aim of this study was to identify and characterize aetiological and predisposing factors in denture-related stomatitis (DRS), by means of a case-control, transversal study, in a large cohort of 140 persons wearing removable maxillary polymethylmethacrylate prostheses. Data were obtained by (1) a questionnaire that included the identification of the subject, demographic and social data, medical history and behaviour; (2) intra-oral examination; (3) evaluation of the prosthesis; (4) microbiological examination; (5) yeast identification and analyses using Epi-info and the chi-square test. Results showed significant associations between DRS and yeasts, gender, age and alcohol consumption. We also found a significant relationship between the

presence of yeasts and hyposalivation and decreased salivary pH. We found a highly significant difference between groups with and without DRS concerning the presence or absence of yeasts, regardless of the sample origin. Most cases of DRS showed the presence of *Candida albicans*. The results confirm a highly significant difference between groups with and without DRS concerning the presence or absence of yeasts.

KEYWORDS: denture-related stomatitis, aetiological factors, *Candida albicans*, removable dental prosthesis, saliva

Accepted for publication 1 October 2006

Introduction

Denture-related stomatitis (DRS) is defined as an inflammatory process of the mucosa underlying a removable, partial or total, dental prosthesis or appliance (1, 2). This definition can be interpreted widely but, in general, the international consensus excludes lesions with known aetiologies, such as allergies to denture materials, and burns related to direct relining of the prosthesis in the mouth.

Denture-related stomatitis is characterized by a lesion which is almost invariably asymptomatic (1, 3), usually affects the hard palate (2) and occasionally is associated with angular cheilitis (1, 4–9) and/or median rhomboid glossitis (1, 5, 9–11). In population-based series of randomly selected patients with removable prostheses, the mean prevalence of DRS was 50% (12), women being most commonly afflicted (9, 13–22).

Aetiological factors in DRS may be divided in two major groups – those related to the prosthesis and those that are infective. Those factors related to the prosthesis (3, 8, 14, 17, 23–27) include the trauma caused by an ill-fitting denture, lack of oral and prosthesis hygiene and a favourable environment for proliferation of microorganisms mainly between the supporting mucosa and the fitting surface of the denture. The infective causes include (17, 18, 27–43) some bacteria (28–31, 42, 43), but *Candida* spp., mainly *Candida albicans* are most frequently associated (18, 38, 44, 45).

In recent years, the emergence of HIV-infection and the use of immunosuppressive chemotherapy have increased the interest in infections caused by *Candida* spp. (46) since these are factors allow the change of a commensal microorganism into a pathogenic one (47). Others factors predisposing to infection by *Candida* spp. include endocrine dysfunction (48–50), nutritional

deficiency (23, 49, 51), cancer (48, 49, 52), some drugs (23, 48, 49), tobacco and alcohol (53, 54), and hyposalivation and decrease of saliva pH (55, 56). As *Candida* is the most important infective factor in DRS it is probable that some of these factors play a significant role in the predisposition to this pathology.

We have carried out a case-control, transversal study, in a large population of 140 persons wearing removable maxillary acrylic prostheses to further identify and characterize the aetiological and predisposing factors of DRS and to quantify and to identify potentially pathogenic yeasts.

Materials and methods

Patients from the Faculty of Dentistry of Porto University (FMDUP) Removable Prosthodontics Department, Portugal, were the population studied, with the consent of the local research ethics committee.

From the 199 participating individuals examined there were 71 persons excluded. Inclusion criteria included: adults (over 18 years), of both sexes, with maxillary acrylic prosthesis [full/complete denture (FD) or removable partial denture (RPD)] having at least half palatal coverage, but who had not had a new or modified prosthesis within the previous 6 months. All patients under antifungal treatment were excluded. A final study group of 128 individuals was identified: 58 cases having DRS (diagnosed according to Newton's classification) (6) and 70 with no evidence of DRS.

We then enlisted 12 more patients with DRS, to make a total of 140 participants divided into two equal groups for a case-control, transversal study: 70 cases with DRS and 70 controls (no DRS).

All participants signed a declaration of informed assent according to the rules of the Declaration of Helsinki guaranteeing the necessary confidentiality of collected information.

Of the 140 individuals that constituted the sample, 87 (62.1%) were female and 53 (37.9%) male. Ages ranged 18–86 years; mean age 59.75 years.

Data were obtained as follows:

(1) By means of a questionnaire that included the identification of subject, demographic and social data (gender, age, degree of education and occupation), medical history (illnesses and medications taken during the last year) and behavioural data (tobacco, mg of nicotine/day; alcohol consumption, g of ethanol/week; diet, cariogenic or not; oral and prosthesis hygiene, habits and frequency).

(2) Clinical examination, including indices [oral hygiene – IHO-S index (57), dental health – CPO index (58), periodontal health – CPITN index (59, 60)] and presence/absence of oral pathology – WHO classification (61). To identify and characterize the different presentations of DRS, we used the Newton Classification, described by Budtz-Jørgensen and Bertram (62):

DRS type I – localised inflammation or hyperaemia points.

DRS type II – diffuse erythema.

DRS type III – papillary hyperplasia of the palate.

Salivary pH and whole stimulated flow rates were studied by mean of Dentobuff® Strips.*

(3) Evaluation of the dental prosthesis, by direct examination, as well as the patient's subjective opinion. The data analysed included: number of years wearing a prosthesis; type of denture (complete or partial); prosthesis hygiene (modification of the Tarbet index) (63, 64); vertical dimension (from aesthetics, phonetics and anthropometric criteria); occlusion; retention and stability (as described by Hoad-Reddick) (65); age of the prosthesis and whether continuously or discontinuously used.

(4) Microbiological samples were obtained from (i) the hard palate mucosa by swab; (ii) the prosthesis fitting surface by swab; (iii) the stimulated whole saliva by mastication of a paraffin block – Dentobuff® Strips* – 200 µL of stimulated saliva were collected; and (iv) mouth rinses with 10 mL of sterile deionised water for 30 s, then centrifugated at 2000 *g* for 5 min and collected 10 µL of the sediment.

(5) Microbiological samples to isolate and assess the development of yeast colonies were plated onto Sabouraud Dextrose Agar with Gentamicin, Chloramphenicol and TTC (SANOFI-Pasteur – ref. 63734) and incubated for 48 h at 37 °C. The resulting growths were quantified by counting the number of colony forming units (CFU) mL⁻¹, and isolated for identification.

(6) Identification of yeasts: (i) morphologically: production of germ tubes after incubation in human serum for 120 min at 37 °C; and production of chlamyospores when inoculated under a sterile cover slip for 72 h at 25 °C on Corn Meal Agar[†] containing Tween 80;[‡] and (ii) biochemically, for carbon assimilation, using ID32C identification kits.[§]

*Orion Diagnostica, Schaan, Liechtenstein.

†Oxoid Difco, Quilaban, Sintra, Portugal.

‡Sigma Difco, Quilaban, Sintra, Portugal.

§bioMérieux Portugal Lda., Carnaxide, Portugal.

Data were analysed with computer software Epi-info (version 6.04a), using the chi-square test (with Yate's correction) and Fisher's exact test, to compare the proportion of each variable between the groups with or without DRS and the ones where yeasts were identified or not.

Results

The results were analysed in three ways prevalence of the different variables in the study population; distribution of those variables in groups with or without DRS; distribution of the same variables in groups with or without yeasts. The relationship between these data was also studied.

The most significant results were

(1) The prevalence of DRS in the 128 patients who fulfilled the study inclusion criteria was 45.3% ($n = 58$). In the study group with DRS ($n = 70$), we found 29 patients with DRS type I (41.4%), 24 with DRS type II (34.3%) and 17 with DRS type III (24.3%). (2) In the groups with and without DRS, results showed significant differences in the following variables:

(i) gender – DRS was more prevalent in females (59.8%) than in males (34.0%) ($P = 0.005$) (Table 1);

(ii) age – DRS prevalence was lower in elderly subjects (85.7% for individuals <35 years, 50.4% for individuals between the ages of 35 and 75 years, and 31.3% for individuals >75 years), with the increase of age we found less DRS in the population studied;

(iii) alcohol consumption – DRS prevalence significantly decreased with increased alcoholic consumption ($P = 0.041$) (Table 2);

(iv) number of years wearing a prosthesis – the prevalence of DRS increased with increased numbers of years of wearing a prosthesis (31.4% for use <5 years, 48.4% for use from 5 to 15 years and 57.9% for use >15 years);

Table 1. Distribution of gender in the group with or without denture-related stomatitis (DRS)

Gender	<i>n</i> (%)	With DRS, <i>n</i> 1 (%)	Without DRS, <i>n</i> 2 (%)
Female	87 (62.1)	52 (59.8)	35 (40.2)
Male	53 (37.9)	18 (34.0)	35 (66.0)

$P = 0.005$; $n = 140$.

Table 2. Distribution of alcohol consumption (g of ethanol week⁻¹) in the group with or without denture-related stomatitis (DRS)

Alcohol (g of ethanol week ⁻¹)	<i>n</i> (%)	With DRS, <i>n</i> 1 (%)	Without DRS, <i>n</i> 2 (%)
0	72 (51.4)	42 (58.37)	30 (41.7)
1–250	29 (20.7)	15 (51.7)	14 (48.3)
>250	39 (27.9)	13 (33.3)	26 (66.7)

$P = 0.041$; $n = 140$.

Table 3. Distribution of prosthetic variables in the group with or without denture-related stomatitis (DRS)

Variables evaluated	<i>n</i> (%)	With DRS, <i>n</i> 1 (%)	Without DRS, <i>n</i> 2 (%)
Vertical dimension, $n = 135$			
Normal	101 (74.8)	46 (45.5)	55 (54.5)
Reduced	34 (25.2)	23 (67.6)	11 (32.4)
Occlusion, $n = 137$			
Satisfactory	100 (73.0)	45 (45.0)	55 (55.0)
Unsatisfactory	37 (27.0)	25 (67.6)	12 (32.4)
Age of the prosthesis, $n = 135$			
0–9 years	116 (85.9)	53 (45.7)	63 (54.3)
≥10 years	19 (14.1)	14 (73.7)	5 (26.3)
Continuous use, $n = 138$			
Yes	73 (52.9)	46 (63.0)	27 (37.0)
No	65 (47.1)	24 (36.9)	41 (63.1)

Vertical dimension, $P = 0.042$ and $n = 135$; occlusion, $P = 0.031$ and $n = 137$; age of the prosthesis, $P = 0.043$ and $n = 135$; continuous use, $P = 0.003$ and $n = 138$ (to a few individuals it was not possible to evaluate all the variables so we have different total number of patients studied – ' n ').

(v) vertical dimension – DRS was more significantly prevalent in subjects with a reduced vertical dimension ($P = 0.042$) (Table 3);

(vi) occlusion – DRS was significantly more prevalent in cases with a more unstable occlusion ($P = 0.031$) (Table 3);

(vii) age of the dental prosthesis – DRS was significantly more prevalent in subjects with older prosthetic appliances ($P = 0.043$) (Table 3);

(viii) continuous use of the prosthesis – DRS was significantly more prevalent in patients who wore their prosthesis continuously ($P = 0.003$) (Table 3).

(3) There were significant differences in the following variables related to both – DRS and the presence of yeasts: gender (the presence of yeasts was significantly more prevalent in females – $P = 0.012$), age (yeasts

were present in 100% of individuals <35 years of age, in 72.6% of individuals aged between 35 and 75 years and in 43.8% of individuals >75 years) and alcohol consumption (the prevalence of yeasts significantly decreased with the increase of alcohol consumption).

(4) The presence of yeasts was not statistically significantly associated with the variables directly related to the prosthesis itself (number of years wearing a prosthesis; type of denture; prosthesis hygiene; vertical dimension; occlusion; retention; stability; age of the prosthesis; and continuously or discontinuously use).

(5) However, independently of the presence of DRS, we found a significant relationship between the presence of yeasts and two salivary characteristics: both hyposalivation ($P = 0.041$) and low salivary pH ($P = 0.007$).

(6) There was a highly significant difference between the groups with and without DRS concerning the presence or absence of yeasts, regardless of the origin of the sample ($P = 0.000002$ for samples from the palatal mucosa; $P = 0.0000001$ for samples from the fitting surface of the prosthesis; $P < 0.000000001$ for samples from mouth rinses with sterile water; and $P = 0.000003$ for samples from stimulated saliva).

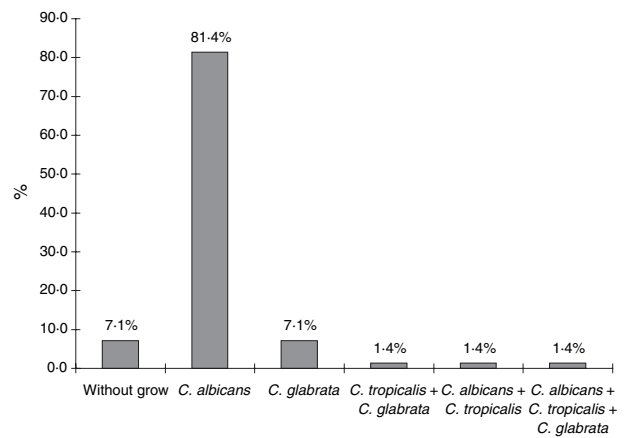


Fig. 1. Prevalence of isolated yeasts species in the group with denture-related stomatitis (DRS). *Candida albicans* is the single isolated strain in 57 of the 70 (81.4%) individuals with DRS. *Candida glabrata* was found in 5 (7.1%) and in other 5 (7.1%) no yeast growth was recorded. From the other three patients, we isolated *C. glabrata* and *C. tropicalis*, *C. albicans* and *C. tropicalis*, and finally *C. glabrata*, *C. albicans* and *C. tropicalis*.

(7) *Candida albicans* alone was isolated from most of the 70 cases of DRS (81.4%) which was significantly greater compared with the isolation in only 27.2% of the controls. All isolated species are showed in Figs 1 and 2.

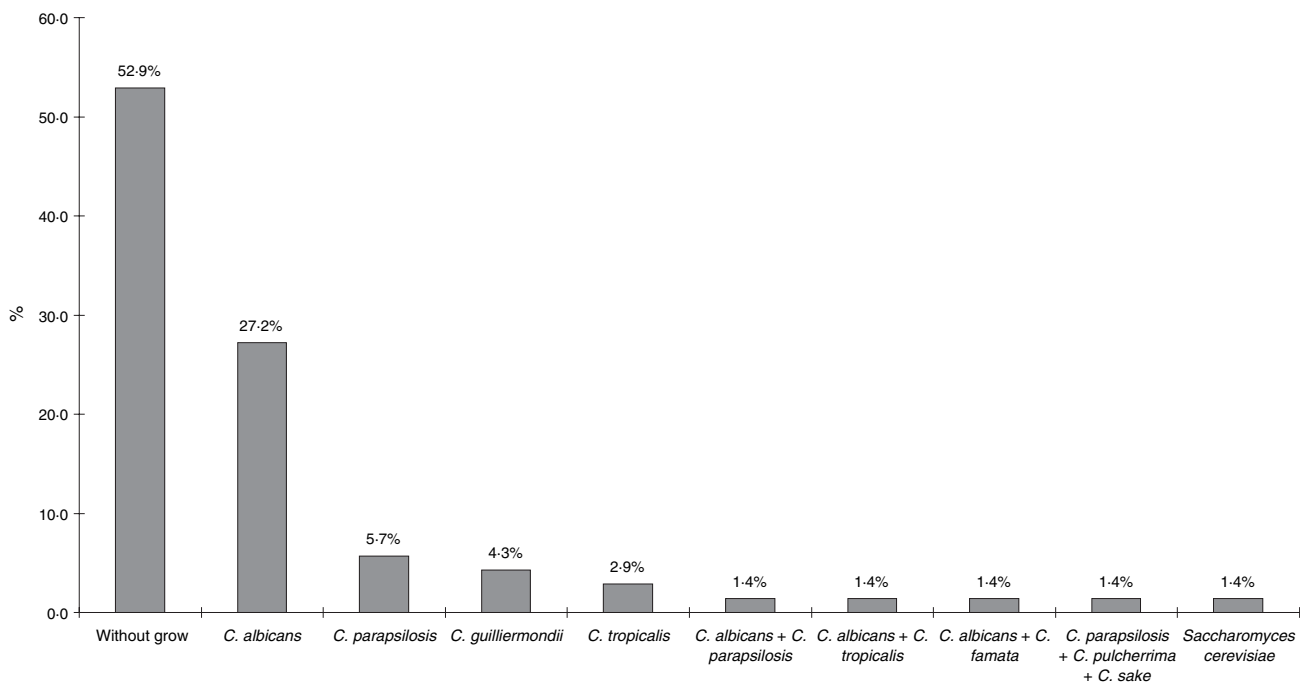


Fig. 2. Prevalence of isolated yeasts species in the group without denture-related stomatitis (DRS). From the 70 individuals without DRS it was not recorded any yeast growth in 37 (52.9%). *Candida albicans* was isolated as single strain in 19 (27.2%) individuals and together with others strain in 3 (5.2%) individuals. In this group, we have a greater variability of *Candida* strains.

Discussion

The prevalence of DRS varies from 10–75% (54) of prosthesis-wearers. The results of the present study showed a prevalence of 45.3% in accordance with previous reports (2, 13, 25, 66–70). In the present study, type I DRS was most common; in the group of 70, we found 29 patients with type I (41.4%), 24 with type II (34.3%) and 17 with type III (24.3%). Only Kulak-Ozkan *et al.* (29) and Pires *et al.* (13) showed a prevalence of type I higher than ours (51.7% and 53.8%). The prevalence of type III found in our study accords with previous reports (3, 23, 29, 62, 71). The variability between different investigations can be at least partially explained by the subjectivity of the classification, and thus several authors have suggested using different indices (17, 54, 64, 72).

The higher prevalence of DRS in females found in our study (59.8% *cf.* 34.0%) is also similar to previous reports (1, 9, 13, 14, 16–18, 21, 22, 73), but others (25, 74–77) have reported a prevalence similar in both genders. Nyquist (14) explains (with no evidence) the fact that women develop more DRS because of their hormonal changes. The fact that the majority of the women in our study were in a group age where menopause is likely to occur (mean age = 59.75 years) may have some influence on the higher prevalence of DRS found. We also found significantly more yeasts in females. With similar results in their study, Bastiaan and Reade (78) explain it by the fact that iron deficiency, frequent in apparently healthy women, may be an important predisposing factor to infections by *Candida*.

In our study, both the prevalence of DRS and yeasts decreased in older subjects, an observation made by others (14, 25, 79), but difficult to explain, unless these older surviving individuals have a greater immune defensive capacity. So, the lower clinical manifestations in older subjects are due to a smaller number of yeasts.

Our results uniquely showed the prevalence of DRS decreased with increased alcohol consumption ($P = 0.041$), and we found similar results as to the presence of yeasts. It is conceivable that alcohol directly reduces the oral yeast count and, consequently, reduces the likelihood of DRS. Previous authors have not found an association between alcohol consumption and DRS or the presence of yeasts (54, 80).

The most likely reason for the increased DRS prevalence related to the prosthesis was the denture instability (3, 8, 14, 17, 23–28), which leads to more trauma. In

our study, the prosthetic variables significantly associated with DRS are the number of years wearing a prosthesis, the vertical dimension, the occlusion, the age of the dental prosthesis and its continuous wearing. Other authors have found similar variables related with the prosthesis to be significant to the development of DRS (2, 3, 13, 14, 17, 23, 25, 54, 62, 79, 81–84). We found no association between these variables and the presence of yeasts, and it is likely therefore that prosthetic factors play mainly a traumatic role in DRS pathogenesis.

However, in contrast to most authors (17, 25, 27, 62, 79, 85–87) we found no significant differences in prosthesis hygiene between the DRS and control groups. Tarbet (63), whose index we used to evaluate the hygiene of the prosthesis, also did not find a correlation between plaque and *Candida*. This author who found that palatal erythema might be related to *Candida* or to bacterial plaque and interpreted these data as indicating that plaque and *Candida* are operating as independent factors in influencing tissue erythema scores.

We failed to show any association between DRS and saliva characteristics. However, we found a significant relationship between the increased presence of yeasts and hyposalivation and decreased salivary pH. These results are similar to those report by Sakki *et al.* (80) and Närhi *et al.* (88). Saliva inhibits yeast colonization (56, 80, 89, 90) and decreased salivary pH facilitates adhesion of *C. albicans* (55, 56, 86, 88, 91, 92).

We did not examine the bacteriology of DRS, but several authors (28, 42, 93–95) have highlighted the importance of the bacteria. However, Koopmans *et al.* (30) found 10 times more yeasts on the denture plaque in DRS than in controls. Budtz-Jorgensen *et al.* (28) also showed that the percentage of yeasts was statistical significantly higher in the DRS group. Radford *et al.* (96) reported the importance of the sequential development of denture plaque and its colonization by *Candida* organisms, but the plaque quantity rather than composition is likely more important for the development of DRS (31, 95, 97).

Our results show a highly significant difference between groups with and without DRS concerning the presence or absence of yeasts. Many authors confirm this association between DRS and yeasts infection (9, 17, 18, 27, 29, 34–41, 53, 62, 90). Like in these studies the differences that we find between groups remained, independent of the method of sample collection and the origin of the sample. We also confirmed that the main

isolated yeast strain was *C. albicans* (9, 13, 17, 18, 20, 29, 36, 37, 39–41, 45, 48, 53, 98–104). In DRS group, we just found *C. albicans*, *C. glabrata* and *C. tropicalis* (this one always in association of one of the others), while in the group without DRS we have a greater variability of *Candida* strain. It seems to be acceptable to think that these strains isolated only at non-DRS subjects could be less pathogenic to the human.

Conclusions

From the results of this study, we conclude that

- (1) the prevalence of DRS in our population is 45,3% (type I, 41.4%; type II, 34.3%; type III, 24.3%);
- (2) women are more susceptible to DRS, but elderly individuals and those with higher alcohol consumption are less susceptible;
- (3) prosthetic factors, which favour the development of DRS, include: duration of wearing a dental prosthesis, reduced vertical dimension, unstable occlusion, older prostheses and continuous wearing of a prosthesis;
- (4) prosthetic factors related to DRS appear to play mainly a traumatic role in its pathogenicity;
- (5) yeasts, particularly *C. albicans*, are associated with the development of DRS;
- (6) increased age, and alcohol consumption appeared protective of DRS.

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Correspondence: Maria Helena Figueiral, Faculty of Dentistry of Porto University, Rua Nova dos Paus, 202 4460-143 Guifões, Portugal.
E-mail: hfigueiral@netcabo.pt