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DENTURE STOMATITIS V CANDIDA AGGLUTININS IN HUMAN SERA

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A quantitative estimate of the titer of agglutinins to *Candida albicans* was made in the serum of 303 persons wearing complete dentures. Ninety-nine persons had a clinically normal palatal mucosa, whereas 204 persons showed inflammatory changes (48 with localized simple inflammation, 80 with generalized simple and 76 with granular inflammation). *Candida*, harboured on the palatal mucosa, was diagnosed both by culture and by smears. Mycelium was found almost exclusively in smears from patients with the generalized simple and the granular type of denture stomatitis, and these groups of patients showed significantly higher titers as compared with the controls and the patients with the trauma-induced localized simple inflammation. A relationship was established between the severity of the involvement and the level of the antibody titer. Zero-titers, however, occurred so frequently in infected persons that the agglutinin reaction must be considered as an invaluable aid in establishing evidence of palatal candidiasis. It is noteworthy that high agglutinin titers occurring in patients who respond poorly to topical antifungal therapy may indicate systemic candidiasis, a serious condition that requires immediate systemic antifungal therapy.

Agglutinins to *Candida albicans* have been demonstrated frequently in low titers in sera from normal subjects (*Andersen*, 1968; *Drake*, 1945; *Norris & Rawson*, 1947; *Todd*, 1937; *Winner*, 1955). According to *Winner* (1955) the detection of a high titer of agglutinins is of little or no diagnostic importance in cases of suspected superficial candidiasis as high titers may be present in the absence of overt infection. However, *Comaish et al.* (1963) demonstrated a clear relationship between candida infection of the skin and the level of serum agglutinins.

Lehner (1966), using the highly sensitive fluorescent antibody technique, found significantly higher titers to *C. albicans* in patients suffering from oral candidiasis than in control subjects and carriers of *C. albicans*.

In previous studies the etiological significance of candida infection for the generalized simple and the granular type of denture stomatitis has been established (*Budtz-Jørgensen & Bertram, 1970 a, b*). The present study was carried out to ascertain whether serum agglutinins to *C. albicans* occurred in higher titers in patients with denture stomatitis as compared with denture wearers with clinically normal palatal mucosa. Furthermore, it was the aim to see whether a possible relationship existed between the titer value and the degree of involvement of the oral mucous membranes.

MATERIAL AND METHODS

A total of 303 patients wearing complete dentures were examined. One hundred and fifty denture-wearers were attending the Department of Prosthetic Dentistry, The Royal Dental College, Aarhus, for construction of new dentures. The remaining 153 patients were a randomized sample from 294 denture-wearers who had been provided with complete dentures at the dental college during the previous two years. The patients were divided into two groups, namely:

- 1) 99 controls who were free from any signs of inflammation of the palatal mucosa,
- 2) 204 patients with inflammatory changes of varying intensity of the palatal mucosa. These patients were subdivided using the classification of *Budtz-Jørgensen & Bertram (1970 a)* into 48 patients with localized simple inflammation, 80 with generalized simple inflammation, and 76 with granular inflammation. The distribution of the patients according to sex and age is recorded in Table I. Of the participants in the study 60 % were women, and 40 % men. Women constituted 44.5 % of the control group, men 55.5 %. In the group of patients with denture stomatitis 66 % were women, 34 % men. The incidence of denture stomatitis was 75 % among the women, and 55 % among the men.

Examination. A clinical history was taken with special emphasis on conditions which might predispose to denture stomatitis, i.e. traumatic factors or factors which increase the susceptibility to candidiasis: antibiotic or corticosteroid therapy, systemic diseases, edentulous age, age of dentures, and denture usage. All patients were submitted to a thorough clinical examination of the oral mucosa. Inflammatory changes of the palatal mucosa, the tongue, or the angles of the mouth were recorded photographically. The

Table I.
Age and sex distribution for controls and patients with denture stomatitis

Groups of subjects		Age						Total	Mean age
		25—34	35—44	45—54	55—64	65—74	≥ 75		
Control group	Women	1	1	4	20	15	4	45	62.1
	Men		3	5	17	18	11	54	64.4
Localized simple inflammation	Women	1	2	7	9	12	2	33	59.8
	Men	1		1	6	1	6	15	65.2
Generalized simple inflammation	Women	3	3	13	16	14	5	54	58.6
	Men		2	3	6	14	1	26	63.7
Granular inflammation	Women	4	6	14	14	11	2	51	54.5
	Men			6	8	10	1	25	61.3

intensity of the inflammatory reaction in the palate was assessed using the criteria of *Budtz-Jørgensen & Bertram* (1970 a).

Isolation and identification of yeasts. *Candida* harboured on the palatal mucosa were isolated and quantitated using the impression technique previously described (*Budtz-Jørgensen & Bertram*, 1970 a). *C. albicans* was identified using the «filamentation test» (*Stenderup & Brown-Thomsen*, 1964), while other fungi were classified according to *Lodder & Kreger-van Rij*, 1952, using fermentation tests.

Classifications of the *candida* species were performed at the Institute of Medical Microbiology, Department of Bacteriology, (Head: A. Stenderup), University of Aarhus.

Scrapings. With the end of a standard microscope slide scrapings were taken from the palate, tongue, and from the fitting surface of the denture. Scrapings from the angles of the mouth were taken in cases of angular cheilitis. The material was spread on slides, fixed in a solution of ether/alcohol 1:1, and stained according to the periodic acid Schiff-method with hematoxylin as counterstain. The palatal smears comprised material from both the mucosa and the denture. A measure of the concentration of *candida* in the smears was obtained using *Davenport's* index (*Davenport*, 1970).

Tube-agglutination reaction. Each patient had 10 ml blood taken and the serum was separated and stored at -20°C . The sera were studied for agglutinating antibodies using both live and formol-killed organisms as antigen.

Sera were examined first for agglutinins using formol-killed organisms, thereafter the procedure was repeated with living-cell suspensions as antigen. The living-cell suspension was prepared according to *Andersen* (1968), the formol-killed suspension according to *Winner* (1955) by harvesting the cells in a mixture of 0.4 % formaldehyde in saline. Both cell-suspensions were prepared with *Candida albicans* type A and standardized to a strength of 70 million organisms by a haemocytometer count. In Widal glasses 0.1 ml volumes of serially halved serum dilutions were produced using a 0.9 % NaCl-phosphate buffer (pH 7.2). Antigen suspension (0.1 ml) was then added to these and to a control tube with 0.1 ml NaCl-phosphate buffer. The tubes were covered with tin foil, incubated for 3 hours at 37°C, and kept at 4°C overnight. The next day the result was read using a curved mirror under a strong light. The tubes were examined for agglutination independently by two persons. In cases of discrepancies in the read titer the test was repeated.

Statistical methods. As statistical evaluation of the samples by means of a Chi square test revealed a non-normal distribution it was necessary to apply a non-parametric statistical test. The two-sample rank test (*Mann & Whitney*, 1947) for a sample larger than 20 was used to establish the significance level (*Goldstein*, 1964).

$$U = \frac{NN^1}{2}$$

$$t = \frac{U - \frac{NN^1}{2}}{\sqrt{\frac{NN^1(N + N^1 + 1)}{12}}}$$

This statistical method has the advantage of testing the difference between ranked categories.

Values of $P \leq 0.05$ were accepted as statistically significant.

RESULTS

Clinical features

Involvement of the angles of the mouth and the tongue were seen primarily in patients with generalized simple (45 %) and granular (36 %) inflammation compared to controls (4 %) and patients with localized simple inflammation (10 %) (Table II). It is notable that angular cheilitis and rhomboidean glossitis were found almost exclusively among patients with granular or generalized simple inflammation.

Table II

The occurrence of accompanying signs (cheilitis, glossitis) among controls and patients with denture stomatitis. The number of smears giving a corresponding positive yield for mycelium are given in the parentheses

Groups of subjects	Cheilitis	Glossitis		Accompanying signs		Total
		rhomboidean	diffuse	number	%	
Control group	1		3 (2)	4	4	99
Localized simple inflammation		1 (1)	4 (3)	5	10	48
Generalized simple inflammation	17 (17)	11 (10)	17 (16)	36	45	80
Granular inflammation	6 (6)	10 (10)	9 (7)	21	36	76

Culture of yeast-like fungi

Yeasts were isolated in 100 %, of the patients with generalized simple inflammation, in 95 % of the patients with the granular inflammation, in 58 % of the patients with localized simple inflammation, and in 42 % of the controls. *C. albicans* was found in 195 (88 %) of the persons with yeasts isolated from the palatal mucosa. In 27 (12 %) persons other yeast-like fungi were isolated whereas in 26 persons *C. albicans* as well as other yeast-like fungi were isolated. Other yeast-like fungi comprised the following species:

- Candida tropicalis* (8)
- Candida krusei* (6)
- Candida parapsilosis* (5)
- Candida macedoniensis* (2)
- Candida mycoderma* (2)
- Candida catenulata* (1)
- Torulopsis glabrata* (30)

The number of persons which yielded the candida species in question is in the parenthesis.

Table III

The results of the agglutination reaction of 303 sera given in percentage with both living cell-suspension and formol-killed cell-suspension of C. albicans as antigen

Group of subjects		Reciprocal of antibody titre								Number of subjects
		nega- tive	≥ 2	≥ 4	≥ 8	≥ 16	≥ 32	≥ 64	≥ 128	
Control group	live	28.3	71.7	61.6	36.3	15.1	9	3		99
	formol-killed	58.6	41.4	38.4	36.4	22.2	14.1	6.1	1.0	
Localized simple inflammation	live	45.8	54.2	45.9	27.1	12.5	2.1			48
	formol-killed	62.5	37.5	35.4	29.1	10.4	6.0			
Generalized simple inflammation	live	22.5	77.5	73.7	61.2	46.2	28.7	11.2	1.2	80
	formol-killed	33.7	66.3	66.3	63.8	56.3	31.2	15	3.8	
Granular inflammation	live	30.3	69.7	68.4	60.5	36.8	18.4	5.6	5.3	76
	formol-killed	36.8	63.2	56.6	55.3	42.1	23.7	7.9	6.6	
Formol-killed antigen	controls/generalized simple: P < 0.001				Live antigen	controls/generalized simple: P < 0.001				
	controls/granular: 0.001 < P < 0.01					controls/granular: 0.01 < P < 0.02				
	localized simple/generalized simple: P < 0.001					localized simple/generalized simple: P < 0.001				
	localized simple/granular: P < 0.001					localized simple/granular: 0.001 < P < 0.01				

Direct smear

Mycelium was present in smears from the palate in 2 of the 99 controls (2 %), in 3 of the 48 patients with localized simple inflammation (6 %), in 73 of the 80 patients with generalized simple inflammation (91 %), and in 55 of the 76 patients with the granular inflammation (72 %). Glossal smears yielding mycelium were seen in 9 controls (9 %), in 4 patients with localized simple

inflammation (8 %), in 41 patients with generalized simple inflammation (51 %), and in 33 patients with granular inflammation (43 %). The number of smears from glossal and angular affections with mycelium is recorded in Table II. It is seen that there is a pronounced relationship between glossal and angular inflammation and candida infection.

Agglutinating antibody titer in serum

With formol-killed cells agglutinins to *C. albicans* were detected in 41 % of the controls, in 38 % of the patients with localized simple inflammation, in 66 % of the patients with generalized simple inflammation, and in 63 % of the patients with granular inflammation (Table III). However, when using live candida cells in the antibody titrations agglutinins to *C. albicans* were found in 72 % of the controls, in 54 % of the patients with localized simple inflammation, in 77.5 % of the patients with generalized simple inflammation, and in 70 % of the patients with granular inflammation (Table III). It is seen that the living cell-suspensions revealed a higher proportion of sera with agglutinins to *C. albicans* both in controls and in patients with denture stomatitis, whereas slightly higher titers were demonstrated with the formol-killed cell-suspension than with the living cell-suspension. There was a fairly good correlation between the titer found with the two antigen-suspensions for the individual sera. However, some of the sera which showed titers ≥ 8 when tested with live antigen showed 2–4 fold higher titers in the preceding test with formol-killed antigen. On the other hand, a large part of the sera which were negative when tested with formol-killed antigen were positive to a living cell-suspension at 2–4 fold dilutions of the sera.

A significant higher antibody titer was found in the group of patients with generalized simple and granular inflammation as compared with controls and the group of patients with localized simple inflammation when using both live and formol-killed candida cells as antigen (Table III).

As live antigen revealed more positive sera than formol-killed antigen agglutinin titers found with the former antigen suspension will be used in the following when comparing the antibody titer with related factors.

Antibody titer of carriers and non-carriers of candida

In the control group a relationship could be established between a positive yield of candida and the antibody titer as higher titers were found among carriers of candida than among non-carriers, $0.001 < P < 0.01$ (Table IV).

Table IV

The distribution of agglutinin titers in carriers and non-carriers of candida

Groups of subjects	Agglutinin titer								Total
	negative	≥ 2	≥ 4	≥ 8	≥ 16	≥ 32	≥ 64	≥ 128	
Carriers of candida	9	2	8	13	5	4	1		42
Non-carriers of candida	19	8	16	9	3	2			57

Table V

Relationship between agglutinin titer and the degree of inflammation in patients with granular inflammation

Groups of patients	Agglutinin titer								Total
	negative	≥ 2	≥ 4	≥ 8	≥ 16	≥ 32	≥ 64	≥ 128	
Slight inflammation	12		4	4	3	2			25
Moderately-severe inflammation	11	1	2	14	11	7	1	4	51

Table VI

Relationship between agglutinin titer and the degree of inflammatory involvement in patients with generalized simple inflammation

Groups of patients	Agglutinin titer								Total
	negative	≥ 2	≥ 4	≥ 8	≥ 16	≥ 32	≥ 64	≥ 128	
No accompanying signs	14	2	6	6	5	6	5		44
Cheilitis and/or glossitis	4	1	4	6	9	8	3	1	36

It was not possible to find a corresponding relationship for the group of patients with the localized simple inflammation.

Table VII

Relationship between agglutinin titer and age in patients with generalized simple inflammation

Groups of patients	Agglutinin titer								Total
	negative	≥ 2	≥ 4	≥ 8	≥ 16	≥ 32	≥ 64	≥ 128	
25—64 years	13	3	4	8	10	5	2		45
65—84 years	5	1	5	4	4	9	6	1	35

Table VIII

Relationship between agglutinin titer and age in patients with granular inflammation

Groups of patients	Agglutinin titer								Total
	negative	≥ 2	≥ 4	≥ 8	≥ 16	≥ 32	≥ 64	≥ 128	
25—64 years	20	1	3	13	9	6	0	2	54
65—84 years	3	0	3	5	5	3	1	2	22

Relationship between antibody titer and the severity of the involvement

It was not possible to find any distinct relationship between antibody titer and the severity of the infection for the groups of patients with generalized simple and granular inflammation. High titers were not found more frequently among the patients who yielded the largest quantity of fungi by cultivation or in smears. Nevertheless, for the group of patients with granular inflammation higher titers were found among patients with a moderately or severely inflamed palatal mucosa as compared with those showing only slight inflammation, $0.001 < P < 0.01$ (Table V). A corresponding relationship between the degree of inflammation and antibody titer was lacking for the group of patients with generalized simple inflammation. However, in this group higher titers were found in patients with involvement of the angles of the mouth and/or the tongue, $0.01 < P < 0.05$ (Table VI).

Relationship between antibody titer and sex and age

Sera with agglutinins to *C. albicans* occurred with the same frequency and with the same titers among men and women, both in controls and in patients with denture stomatitis. With regard to age high agglutination titers were

equally distributed above and below the age of 65 in the control group and in the group of patients with localized simple inflammation. On the other hand, among the patients with generalized simple inflammation high titers were seen more frequently in the age group 65–84, $0.01 > P > 0.02$ (Table VII). The same was true for the patients with granular inflammation, however, in this group the relationship between age and agglutinin titer did not reach the level of significance, $0.05 > P > 0.1$ (Table VIII).

DISCUSSION

In the present study the etiological significance of infection with *C. albicans* for the generalized simple and the granular type of denture stomatitis has been further confirmed. Thus, there was a high occurrence of mycelium in smears from these lesions, and significantly higher agglutinin titers to *C. albicans* were demonstrated in sera from these patients as compared with sera both from controls and patients with the localized simple inflammation.

According to *Comaish et al.* (1963) and *Lehner* (1966) agglutinin titers ≥ 16 are significant for candida infection, whereas a titer of 8 merely indicates that candida is present. These findings were confirmed in the present study, although, not so decidedly. Thus, in the control group, 55 % of the carriers of candida in the palatal mucosa showed a titer of ≥ 8 against 33 % of those without a positive culture for candida. In the groups with generalized simple and granular inflammation, titers ≥ 16 occurred 2–4 times more frequently than in the control group or the group with localized simple inflammation.

In *Lehner's* study on immunofluorescent antibodies in patients with generalized simple inflammation («chronic atrophic candidiasis», *Lehner*, 1965) high titers were demonstrated in patients with both angular cheilitis and involvement of the palatal mucosa. In the present study a definite relationship was established between the severity of the involvement and the antibody level. Thus, in the group of patients with generalized simple inflammation significantly higher titers were seen when the tongue and/or the angles of the mouth were infected. Among the patients with granular inflammation a significant relationship was established between the antibody level and the degree of inflammation.

The present group of patients is not a representative section of the population with regard to age and sex (*Statistisk Aarbog for Aarhus*, 1971). Therefore, the material probably does not represent the population distribution of denture wearers with regard to socio-economic position, age, and sex. However, an incidence of denture stomatitis in women and men of respectively 75 % and 55 % indicates that the true incidence of denture stomatitis

in the Danish population must be very high. Denture stomatitis, whether it was the localized simple, the generalized simple or the granular type, was seen more frequently in women than in men, a finding that corresponds well with other investigations (*Nyquist*, 1952; *Cawson*, 1966; *Love et al.*, 1968). Previous studies have shown that the incidence of agglutinin titers to *C. albicans* is higher in women than in men (*Andersen*, 1968; *Drake*, 1945; *Norris & Rawson*, 1947; *Todd*, 1937; *Winner*, 1955). In the present study no difference was seen in the relative distribution of high titers among men and women, neither for the control group nor for the patients with denture stomatitis. However, as the overall incidence of denture stomatitis is higher in women than in men this may explain why high titers are seen more frequently in women.

Andersen (1968) found higher titers against *C. albicans* among elderly than among young persons. This was not seen in the present study among the non-infected denture-wearers. The overall incidence of complete dentures is proportional with age (*Osborne et al.*, 1966), a fact that probably will result in a more frequent occurrence of denture stomatitis among the elderly. It was not stated in *Andersen's* study whether patients with dentures, especially with denture stomatitis, were excluded from the material. Therefore, the higher incidence of high titers among elderly may not be related to age as such, but rather to a more frequent occurrence of dentures and palatal candidiasis. In the present study patients with generalized simple and granular inflammation showed raised titers with age. *Esterly* (1968) found higher titers in patients with long standing candidiasis. Consequently, the raised titers seen among the elderly, infected denture-wearers may be caused by a long standing palatal candida infection which is more probable among the elderly than the young.

The way in which the antigen is prepared when determining the serum agglutinin titer to *C. albicans* seems to be important. Thus, a large quantity of the sera that were negative to formol-killed antigen turned out to be positive in low titers at the subsequent titration with a living cell-suspension. The explanation of this may be either that the formol-washing destroys some of the cell-wall antigens, or that enough formol remains on the surface of the candida cells — even after thorough washing in saline — to destroy small amounts of antibody. Therefore, for detection of agglutinins in low titers a suspension of live cells, should be used. Since candida antibody deteriorates upon repeated freezing and thawing (*Hipp & Murray*, 1971) this may explain why some of the sera showed slightly decreased antibody level with the living cell-suspension as compared with the titer found in the preceding titration with formol-killed antigen.

Even though the present study has demonstrated a clear correlation between oral candidiasis and the level of serum agglutinins the agglutinin reaction cannot be recommended as a diagnostic aid for detection of candida-induced denture stomatitis as zero — and low — titers occurred frequently in infected persons. A palatal smear is a convenient method to provide reliable evidence of oral candidiasis. However, the determination of serum antibody level to candida may give valuable prognostic information, as a high antibody titer indicates a breakdown of host defenses that normally prevent systemic candida invasion from cutaneous or mucocutaneous foci (*Taschdjian et al.*, 1970). It is controversial whether the detection of precipitating antibodies against candida is a diagnostic evidence of systemic candidiasis, (*Taschdjian, et al.*, 1967; *Chew & Theus*, 1967). However, *Wegman & Müller* (1971) found agglutinin titers ≤ 160 in patients with mucous candidiasis, whereas titers above 160 indicated systemic candidiasis. Therefore, in patients with palatal candidiasis who respond poorly to local antifungal therapy or show immediate relapse, the level of serum antibody against candida should be determined; accordingly, patients with high titers should be treated both with local and systemic antifungal therapy.

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