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Comparison of Secretory IgA and IgG Isotype Levels in Palatal Secretions of Denture Stomatitis Patients with Denture Wearers having Clinically Healthy Palates.

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Abstract - *Denture stomatitis is an inflammatory condition affecting palatal mucosa covered by a denture. Aetiology is multifactorial involving poor hygiene, constant denture wearing, trauma and colonisation by Candida species. In inflamed tissues raised levels of albumin, IgG isotypes and secretory IgA (sIgA) would be expected. SIgA is a major component of the protective immunological response to mucosal infection. Palatal secretions of 21 patients with denture stomatitis and 22 denture wearing patients with clinically healthy palates were analysed for albumin, IgG isotypes and sIgA levels. Albumin and IgG isotype levels were found to be higher in denture stomatitis patients compared with healthy palate patients. Unexpectedly, SIgA levels were found to be significantly ($p<0.05$) lower in denture stomatitis patients compared with healthy palate patients.*

KEY WORDS: denture stomatitis, sIgA, IgG isotypes

INTRODUCTION

Denture stomatitis ('DS') is a common inflammatory disease in patients wearing palatal dentures. In random population samples, the prevalence is estimated at about 50%[1]. DS presents clinically as an erythematous, oedematous palatal mucosa confined to the area covered by the denture. The aetiology of this disease is multifactorial, involving poor oral and denture hygiene, *Candida albicans*, microbial denture plaque, constant denture wearing and trauma[2]. DS is not found in palatal mucosa not covered by a denture and is hardly ever found beneath mandibular dentures. *C. albicans* adheres well to both denture acrylic and the palatal mucosa[3] by a complex set of ligand interactions with acrylic, epithelia, salivary pellicle and extracellular matrix proteins[4]. A depressed host defence may also predispose towards DS as the prevalence is increased in patients with both Type 1 and Type 2 diabetes[5].

A major component of protection against mucosal infection of the oral cavity is the adaptive secretory immunoglobulin A (sIgA) response against oral pathogens[6]. SIgA is produced by both major and minor salivary glands. It has been shown that minor salivary glands account for 50% of sIgA in saliva[7]. Minor salivary glands are of particular interest in protection against palatal infection as the palate contains sIgA producing glands in the sub-mucosa adjacent to the maxillary ridge. This is especially pertinent to palatal mucosa covered by a denture, since sIgA produced by the major glands is unlikely to be present in sufficient quantity between the denture and the palate.

If DS is an inflammatory reaction of the palatal tissues to *C. albicans* and denture plaque bacteria, an elevation of sIgA levels in palatal secretions would be expected. Extra-vascular albumin is a marker for the inflammatory response. If the inflammatory lesion contains permeable blood vessels, then the presence of albumin[8] and IgG[9] in the inflammatory exudate, at levels above those normally produced by the minor salivary glands, would also be expected. This study compared levels of albumin, sIgA and IgG isotypes (1,2,3,4) in palatal secretions from DS patients with denture wearers having clinically 'healthy' (i.e. no signs of inflammation) palates.

MATERIALS AND METHODS

Following Ethical Committee approval for this study, volunteers were recruited from edentulous patients attending the University Dental Hospital, Cardiff, Wales, U.K. Signed informed consent was obtained from each patient. After a thorough clinical examination, 43 patients in good general health were chosen for this study. 22 males and 21 females (mean age 70 years, median 72, range 35 to 93) participated. Diabetics and patients with clinical signs of xerostomia were excluded from this study. 21 patients had clinical signs of DS (Newton's Type II DS - generalised erythema involving all palatal mucosa covered by the denture, Figure 1), while 22 had palates with a clinically healthy tissue appearance.

Palatal secretion collection: the subject's palatal denture surface was first cleaned by brushing with soap and water to remove any adherent plaque, then thoroughly rinsed and dried. Two pieces of Whatman 3mm chromatography paper with a total surface area of 945mm^2 were placed either side of the midline on the palatal surface of the patient's denture (Figure 2). The denture was then inserted into the mouth for 5 minutes to collect palatal secretions. The denture was removed and the papers immediately placed inside the upper chamber of a modified centrifuge tube[10] and centrifuged at 500*g* for 5 minutes. The paper was

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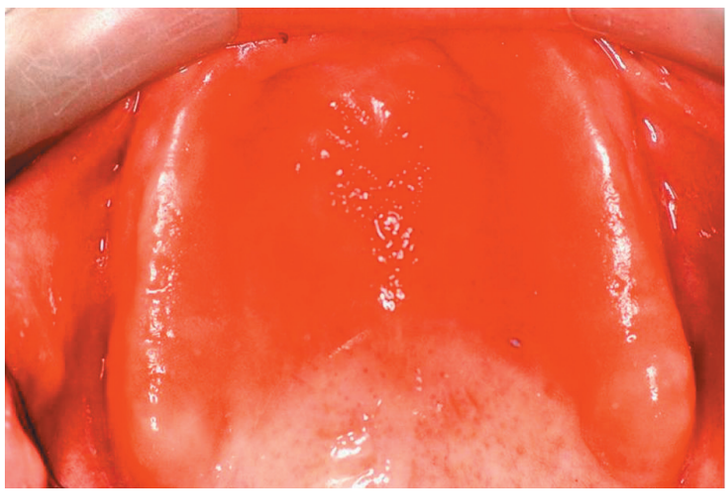

Figure 1. *Newton's Type II denture stomatitis.*

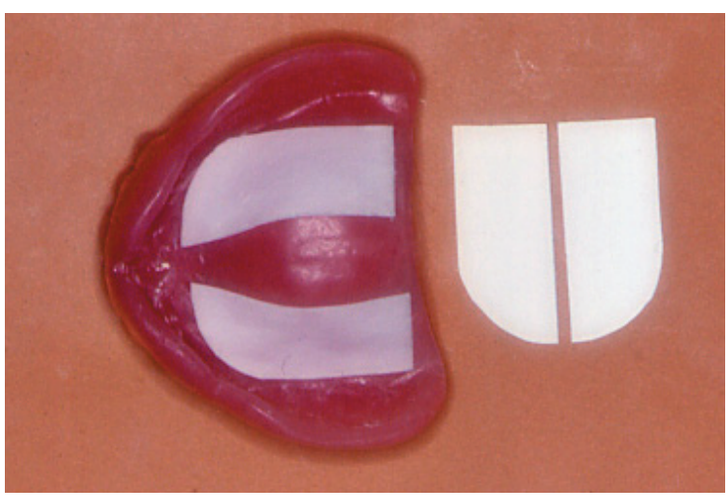

Figure 2. *Chromatography paper inserts for palatal denture.*

moistened with 250µl of 0.02% 'Tween 20' in phosphate buffered saline and re-centrifuged at 500*g* for 5min. The moistening and centrifugation were repeated twice to give a total of three elutions which were frozen at -20°C until analysed.

SIgA and albumin levels in palatal secretions were measured by radial immunodiffusion ('RID') using assay kits and standards according to the manufacturer's protocol. The results were expressed as mg/L. IgG_1, IgG_2, IgG_3 and IgG_4 levels in palatal secretions were estimated by enzyme linked immunosorbent assay ('ELISA') using prepared microtitre plates and standards according to the manufacturer's protocols. The results were expressed as mg/L.

RESULTS

Statistical analyses

For those analytes whose distribution was log-normal (albumin, IgG_1, IgG_2, IgG_3, IgG_4) geometric means were derived (with 95% confidence intervals 'CIs') and log-transformed (base *e*) for statistical analyses. For sIgA means (with 95% CIs) were calculated. Mean levels (log transformed where necessary) in DS patients were compared with those in healthy patients, using two-sample *t*-tests. Results for log-transformed analytes were exponentiated to give ratios of geometric means with 95% CIs. Analyses were performed using Stata version 7.0.

Table 1 compares levels of analytes in DS with healthy palate patients. Albumin levels were 2.49 times greater in the DS group (but the confidence interval was wide). Levels of all four IgG isotypes were elevated in the DS patients compared with healthy patients (Figure 3). Mean sIgA levels in the DS patients were significantly lower [mean difference -140.7 (95% CI), range -269.6 to 11.8] compared with those in the healthy patients (Figure 4).

Table 2 shows association between analyte levels and DS from logistic regression models. Results from a multivariable model including all the analytes were difficult to interpret because of co-linearity between the levels of the different analytes. In multivariable models including log albumin, sIgA and individual log IgG isotypes the patterns of negative associations with sIgA and positive associations with the IgG isotypes were maintained.

Table 3 shows pair wise correlations in DS patients and healthy patients for the salivary analytes. In DS patients sIgA was highly correlated with albumin, IgG_1, IgG_3 and IgG_4. This was not found in healthy patients. IgG_1 was also highly correlated with albumin in DS patients, but not in healthy palate patients. All IgG isotypes were highly correlated with each other in DS patients. In healthy patients only IgG_2 and IgG_4 were correlated.

DISCUSSION

As expected, albumin levels and all four IgG isotype levels were elevated in palatal secretions in DS patients when compared with healthy patients. The higher levels found in the DS patients might suggest there is a local contribution, presumably from plasma cells, in the chronically inflamed palatal tissue. Stimulation of the palatal mucosa by a denture in patients with clinically healthy palates might induce a low grade inflammation and vascular permeability that would result in the appearance of the four IgG isotypes and albumin in palatal secretions[11].

The elevations of the IgG_2 and IgG_4 isotypes are of special interest. IgG_2 antibodies are primarily directed against carbohydrate antigens and IgG_4 is elevated where there is prolonged antigenic stimulation. As the principal carbohydrate antigens of *C. albicans* are mannans and the colonisation of palatal tissues and the palatal surface of the denture is chronic, it is feasible that our data suggest a chronic immune response to carbohydrate antigens. Only direct estimation of the antigenic specificity of the IgG_2 and IgG_4 isotypes would address this supposition, so further investigation is needed.

The finding that sIgA levels were significantly lower in DS patients was unexpected. The lower sIgA levels in the

Table 1. *Levels of analytes (mg/L) in DS and healthy patients*

Analyte	*Healthy palate patients (n=22)*	*DS patients (n=21)*	
	Geometric mean (95% CI)	*Geometric mean (95% CI)*	*Ratio of geometric means (95% CI)*
Albumin	52.4 (33.0 to 83.1)	130.7 (37.7 to 453.2)	2.49 (0.71 to 8.81), p=0.15
IgG_1	1.16 (0.77 to 1.76)	2.33 (1.49 to 3.64)	2.00 (1.11 to 3.60), p=0.022
IgG_2	0.15 (0.07 to 0.33)	1.47 (0.70 to 3.07)	9.96 (3.43 to 28.93), p<0.001
IgG_3	0.33 (0.19 to 0.57)	1.15 (0.48 to 2.77)	3.49 (1.29 to 9.41), p=0.015
IgG_4	0.29 (0.13 to 0.68)	2.25 (0.68 to 7.45)	7.62 (1.87 to 31.09), p=0.006
	Mean (95% CI)	*Mean (95% CI)*	*Difference in means (95% CI)*
sIgA	349.5 (249.0 to 449.9)	208.8 (122.6 to 294.9)	-140.7 (-269.6 to 11.8), p=0.033

Table 2. *Odds ratios per unit increase in analyte levels (with 95% CI and P values), comparing DS with healthy patients*

	Odds ratio (95% CI), p value
Log albumin	1.26 (0.92 to 1.72), p=0.16
Log IgG_1	2.26 (1.07 to 4.75), p=0.032
Log IgG_2	2.09 (1.33 to 3.29), p=0.002
Log IgG_3	1.63 (1.07 to 2.50), p=0.022
Log IgG_4	1.49 (1.09 to 2.04), p=0.012
sIgA (per 100 mg/ml)	0.71 (0.51 to 0.99), p=0.043

Table 3. *Pair-wise correlations (with corresponding P values) between the levels of different analytes, in healthy and DS patients*

	Log albumin	*Log sIgA*	*Log IgG_1*	*Log IgG_2*	*Log IgG_3*	*Log IgG_4*
(a) Healthy palate patients (n=22)						
Log albumin	1					
Log sIgA	0.41 (0.061)	1				
Log IgG_1	0.27 (0.23)	0.30 (0.18)	1			
Log IgG_2	0.41 (0.061)	-0.03 (0.80)	0.58 (0.005)	1		
Log IgG_3	0.63 (0.002)	0.10 (0.65)	0.28 (0.20)	0.39 (0.07)	1	
Log IgG_4	0.62 (0.002)	0.12 (0.61)	0.28 (0.20)	0.61 (0.003)	0.65 (0.001)	1
(b) DS patients (n=21)						
Log albumin	1					
Log sIgA	0.79 (<0.001)	1				
Log IgG_1	0.65 (0.001)	0.62 (0.003)	1			
Log IgG_2	0.57 (0.007)	0.39 (0.084)	0.65 (0.002)	1		
Log IgG_3	0.70 (<0.001)	0.61 (0.003)	0.55 (0.01)	0.63 (0.002)	1	
Log IgG_4	0.70 (<0.001)	0.71 (<0.001)	0.53 (0.013)	0.43 (0.051)	0.78 (<0.001)	1

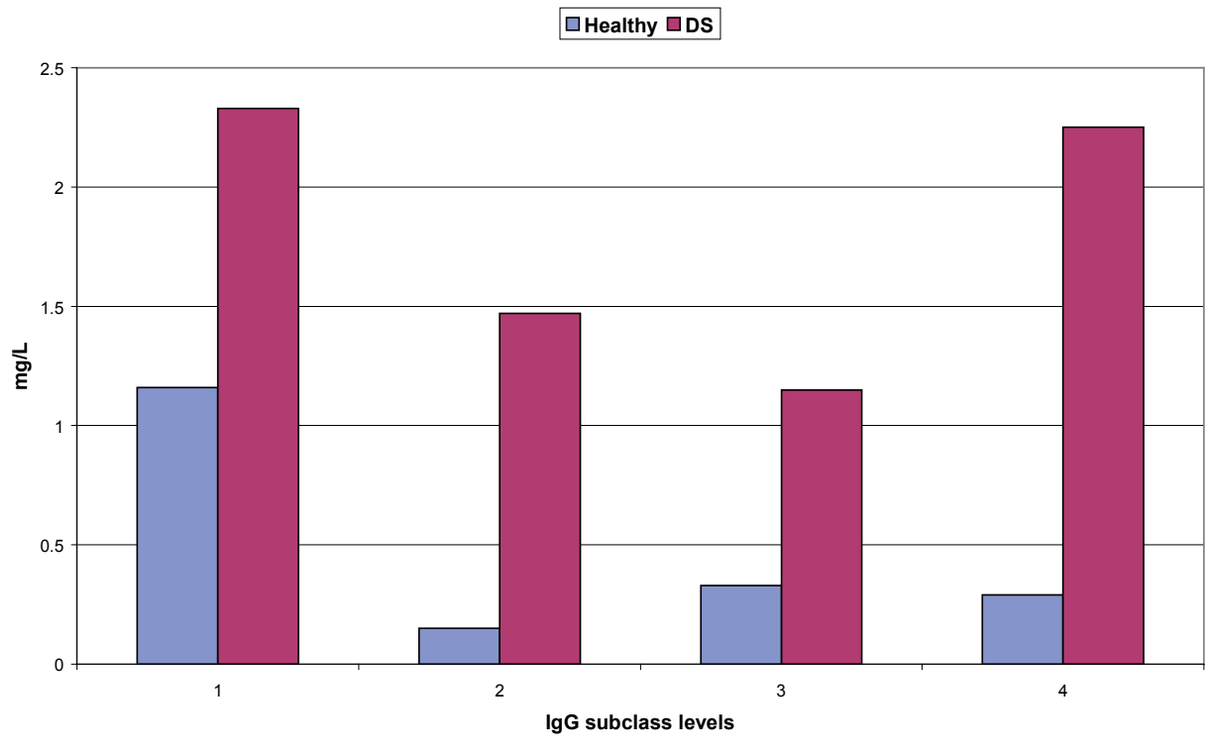

Figure 3. *IgG subclass levels comparing healthy with DS patients*

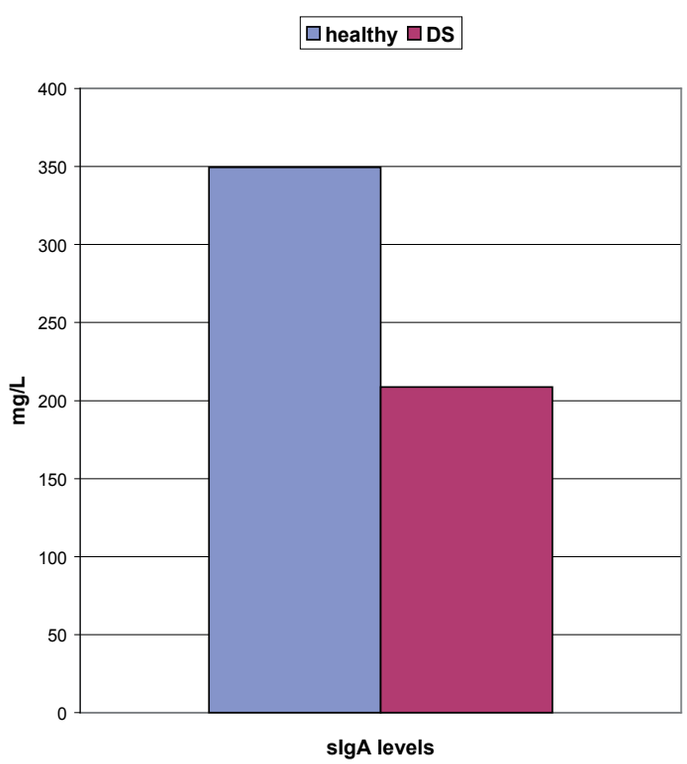

Figure 4. *sIgA levels comparing healthy with DS patients*

presence of elevated IgG isotype levels could possibly be due to a feedback inhibition of sIgA secretion, secondary to the inflammatory process. Other possibilities could be a reduced number of plasma cells producing IgA dimers or a reduced capacity of the ductal epithelium to transport IgA dimers into the glandular lumen[12]. Some studies that have examined palatal gland secretion rates and IgA levels have found reductions in both with increasing age[13]. However, other studies have not shown such age-related decline in flow rates[14-16].

It is possible that higher levels of sIgA in palatal secretions of healthy denture wearers would confer protection against mucosal disease induced by *C. albicans* and / or microbial plaque. The reduced levels found in DS patients would

predispose towards disease. This is an important finding and warrants further investigation.

Chewing in dentate subjects stimulates increased secretion of IgA by the parotid glands and the mechanism appears to be an increase in epithelial transcytosis of IgA[17]. Chewing also stimulates salivary flow from both labial minor salivary glands and the parotid gland[18]. It seems reasonable to assume that chewing might also stimulate flow from the palatal minor salivary glands but even more so in palatal denture wearers, as the masticatory forces would also be transmitted to the palate as well as the residual alveolar ridges.

Studies of minor salivary gland secretions have shown that each group of glands has different properties, including secretion rates and component levels[19]. This would suggest that the different groups of minor salivary glands are uniquely adapted to the performance of discrete local function(s) as well as contributing to the properties of whole saliva.

In the case of the palatal glands, one such function might be to minimise the colonisation and prevent development of opportunistic pathogenicity by salivary bacteria and fungi such as *C. albicans*. The placement of an upper denture, occluding the palatal tissues and transmitting mechanical pressures, might stimulate minor salivary gland flow and concomitantly increase sIgA secretion. A susceptible host who wears a denture continuously, together with poor denture hygiene, would constantly expose the palatal tissues to denture plaque and *C. albicans*. Feedback inhibition of glandular output reducing sIgA production and / or secretion would lower local host resistance, permit chronic infection and thus give rise to the clinical signs of inflammation resulting in denture stomatitis.

CONCLUSIONS

All IgG isotype levels were significantly raised in the palatal secretions of DS patients when compared with those from denture wearers with clinically health palates. Albumin levels were also higher in palatal secretions of DS patients.

However, sIgA levels were found to be significantly ($p<0.05$) lower in the palatal secretions from DS patients when compared with denture wearers with clinically normal palatal mucosa.

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MANUFACTURERS' DETAILS

- Chromatography paper from Whatman Ltd., Maidstone, England, U.K.
- Tween 20 from BDH Chemicals Ltd., Poole, England, U.K.
- RID kits from BIND A RID™, The Binding Site, Birmingham, England, U.K.
- ELISA kits from BINDAZYME™, The Binding Site, Birmingham, England, U.K.
- STATA version 7.0 statistical software from StataCorp LP, Texas, U.S.A.

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