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Salivary Immunoglobulins and Lactoferrin in Dental Caries

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Abstract: The levels of serum IgG, IgA, salivary IgA and salivary lactoferrin were determined in high and low caries subjects. The levels of immunoglobulin and lactoferrin were found to be elevated in high caries subjects.

1. Introduction.

Numerous studies have shown variations in serum and salivary immunoglobulins in dental caries,^{4,7,11,15} some reports suggesting a protective function for salivary immunoglobulins.^{3,4,5,9,13}

Lactoferrin, the iron and copper binding protein in secretions,⁸ is present in saliva and has been described as a bacteriostatic agent.² As bacteria play a significant role in the establishment and progress of dental caries, it is important to know whether lactoferrin levels show any relation to the severity of disease.

In this communication, we summarise our preliminary data on serum immunoglobulins, salivary immunoglobulins and lactoferrin in dental caries in man.

2. Materials and Methods.

2.1. Subjects

The series consisted of 121 patients selected from those attending an 'Out Patients' clinic, at the General Hospital, Kandy and 28 caries free first year medical students from Peradeniya. The medical students were freshmen and were studied within one month of their arrival in the campus. Their family income was used to assess the socio-economic backgrounds and 28 students of socio-economic status comparable to that of the test group were selected. All subjects were examined by the same dental surgeon and those patients with evidence of other oral diseases, especially gingivitis, were excluded. The degree of dental caries was measured by the number of decayed, missing and filled teeth (DMF Index). The subjects were classified according to the DMF Index.

2.2. Collection of saliva and serum.

About 5 to 10 ml of saliva were collected under paraffin stimulation from each subject. The time of saliva collection was between 10 am and 12 noon. The saliva samples were clarified by centrifugation (200 g) and stored at -20°C . Blood was collected into Haematocrit tubes by the finger prick method, serum separated and stored at -20°C .

2.3. Determination of immunoglobulin and lactoferrin levels.

Unconcentrated saliva was used in all determinations. SIgA levels in saliva were determined by Single Radial immunodiffusion (SRID) in 1.5% Agarose using a rabbit antiserum (specific) to human S IgA (Nordic Immunological Laboratories, the Netherlands). The IIS IgA standard was prepared in this laboratory. Serum IgG and IgA levels were determined by SRID in 2% Agarose using class specific antisera (Nordic Immunological Laboratories) with reference to the WHO Standard Serum 67/97.

IIS IgA (as determined by gel filtration on a calibrated Sephadex G-200 column) was isolated from human colostrum. Gamma-globulins in human colostrum were isolated by repeated precipitation with 33% ammonium sulphate in phosphate buffer, pH 7.4. IIS IgA was obtained by gel filtration in the calibrated Sephadex G-200 column and further purified by affinity chromatography on CNBr-Sepharose 4B insolubilized rabbit anti-human SIgA (from Nordic immunological Laboratories, the Netherlands). The IIS IgA thus prepared showed one precipitation line on immunoelectrophoresis against rabbit anti-human Fab and rabbit anti-human SIgA (Nordic Immunological Laboratories). Calculation of standard concentrations was based on the assumed extinction coefficient for SIgA of $E_{280\text{ nm}}^{1\text{ cm}} 1\% = 13.5$.¹⁰ This preparation was also calibrated against the WHO Standard Serum 67/97 with a correction factor applied for differences in rates of diffusion.¹⁴

2.4 Determination of salivary lactoferrin levels.

Determination of salivary lactoferrin levels was performed by solid phase radio-immunoassay exactly as described previously.¹

3. Results.

The number of subjects in each caries group, the mean \pm S. D. of their age, DMF Index, levels of serum IgG, IgA, salivary IgA, and lactoferrin are shown in Table 1. In Table 2 are shown the statistical correlations (by linear regression analysis) between salivary IgA, lactoferrin and the DMF Index.

When the DMF Index was greater than 15, the mean serum IgG levels were significantly greater than that of the control group with a DMF Index=0. The salivary IgA and lactoferrin levels showed a gradual increase with the increase of DMF Index. In high caries subjects (DMF Index = 11-15 and ≥ 15) the mean levels of salivary IgA and lactoferrin were significantly higher than the corresponding values for the control group with DMF Index = 0.

Table 1 - The Mean, \pm S. D. of Age, DMF Index Serum IgG, Serum IgA, Salivary IgA and Salivary Lactoferrin.

Subject Group	Age (years)	DMF Index	Serum IgG (IU/ml)	Serum IgA (IU/ml)	S. IgA (μ g/ml)	Lactoferrin (μ g/ml)
DMF Index = 0 (n = 28)	20 \pm 1.2	0	57 \pm 27	51 \pm 27	67 \pm 20 (46-113)	5 \pm 7 (0.2-26)
DMF Index = 1-5 (n = 41)	21 \pm 2	2.6 \pm 1.2	51 \pm 21	44 \pm 26	76 \pm 76 (42-176)	8 \pm 11 (0.2-41)
DMF Index 6-10 (n = 24)	21 \pm 1	7.5 \pm 1.3	52 \pm 20	52 \pm 29	82 \pm 45 (46-126)	17 \pm 17 (0.6-42)
DMF Index 11-15 (n = 32)	23 \pm 4	12.4 \pm 1.4	54 \pm 15	64 \pm 29*	95 \pm 92* (46-151)	20 \pm 21* (2-84)
DMF Index \geq 15 (n = 24)	27 \pm 6	20.9 \pm 4.1	73 \pm 26**	57 \pm 22	116 \pm 113** (54-319)	27 \pm 20** (5-58)

* : difference from DMF Index = 0 , significant at 20% level.

** : difference from DMF Index = 0 , significant at 0.05% level.

Table 2 - Correlation between Salivary IgA, Lactoferrin and the DMF Index

Subject Group	Salivary IgA and DMF Index	Lactoferrin and DMF Index	Lactoferrin and IgA
DMF Index = 1-5	correlation ,, -0.163 , NS	correlation ,, 0.063 NS	correlation ,, 0.062 NS
DMF Index = 6-10	,, 0.348 , p = 0.01	,, 0.036 NS	,, 0.035 NS
DMF Index = 11-15	,, 0.281 , p = 0.01	,, 0.4564 , p = 0.001	,, 0.4722 , p = 0.001
DMF Index = \geq 15	,, 0.204 , p = 0.01	,, 0.501 , p = 0.001	,, 0.503 , p = 0.001

NS: not significant.

4. Discussion.

Subjects with a DMF Index greater than 15 exhibited a significantly elevated level of serum IgG; the group with a DMF Index = 11 to 15 (but not above 15) exhibited a significant elevation of serum IgA. These findings are in general agreement with other reports.^{4,7} In contrast, salivary IgA and lactoferrin demonstrated a gradual increase with the increase of the DMF Index. The standard deviations were so high, however, that only in the group with DMF Index greater than 15 (greater than 10 for lactoferrin) was the difference from the control group sufficient to be statistically significant. To our knowledge, this is the first report on elevated levels of lactoferrin in dental caries.

The concentration of salivary proteins are known to be affected by the rate of saliva secretion.^{3, 10} However, it is not known to what extent these experimentally determined differences reflect true differences in the glandular secretion of salivary proteins. In the present investigation, we were not able to measure the rates of saliva secretion and therefore, it was not possible to correct for differences in rates of flow.

The absolute levels of salivary IgA observed in the present series are higher than the values reported by others.^{4,6,10,12,15} Such variations are to be expected for reasons of ethnic, social and economic differences. However, technical errors due to differences in Standard SIgA preparations cannot of course be excluded. Unfortunately an international standard for SIgA is not available and therefore accurate standardizations could not be made.

The pattern of variation of salivary IgA in relation to the DMF Index probably reflects the stimulation of salivary IgA production by antigens of cariogenic organisms. A deficiency of secretory immunoglobulins in saliva precipitating dental caries seems unlikely. The significance of the observed elevation of salivary lactoferrin in high caries subjects is not clear. Further studies to elucidate the role of lactoferrin in dental caries and also the antibody specificity of salivary IgA are in progress.

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