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Dental caries related to plasma IgG and α_1-acid glycoprotein

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Abstract

This study was aimed at determining whether dental caries is associated with induction of the systemic immune system or cytokine response.

Materials and methods:
For this purpose, 85 children from Den Pasar, Bali, Indonesia, aged 6–7 years, were examined clinically and blood plasma was obtained via finger puncture. The concentrations of the acute-phase protein alpha(1)-acid glycoprotein (AGP), total IgG and the specific IgG and IgM immunoglobulins against *Streptococcus mutans* were determined. Immunoelectrophoresis was used for the determination of the AGP concentration and ELISA for IgG and IgM detection.

Results:
The mean dmft of the whole group was 8.8 (±2.9), the mean number of infected pulps was 3.9 (±2.2) and the mean number of abscesses was 0.5 (±0.8). The plasma concentration of AGP ranged between 0.13 and 1.6 mg/ml serum (mean 0.86 ±0.26 mg/ml). Stepwise regression analysis revealed that the concentration of IgG against *S. mutans* (log-transformed) was significantly correlated with dmft (adjusted r^2 = 0.083, standardized ß coefficient=0.31, p=0.008). When the concentration AGP was included in the model the correlation improved significantly (for IgG: adjusted r^2=0.157, standardised ß coefficient=0.36, p=0.002; for AGP: ß coefficient=–0.30, p=0.009).

Conclusions:
The results suggest a relationship between caries and systemic parameters of inflammation. On the basis of this, severe caries might have consequences on the general health of the subject.

Introduction

Dental caries in young children can be a severe problem, especially in less-developed countries where dental health care is scarce. Also, dental education with respect to oral hygiene and use of dietary sugars is often completely absent. Indeed, in countries such as Thailand, Indonesia, and Surinam, the dmft of young children is high [Holm, 1990; Kreulen et al., 1997b; Sekiguchi and Machida, 1999]. Treatment is most often restricted to extraction of teeth in cases of severe tooth-pain. Beside the fact that this treatment is not pleasant for the patient, extraction of teeth might cause other problems at a later age, such as orthodontic problems. The current standard in the western societies is that infected teeth should be either restored or extracted, with the aim of preventing progression of the caries lesion either within the infected tooth, or within the adjacent teeth. Besides the effect of carious tissue on other teeth, the infection may affect the general health of the child. Such an effect has been described earlier. Children with rampant caries have a retarded growth and retarded weight gain when not treated. When the caries is treated, this retardation is lost, possibly due to the fact that the children are able to eat properly again [Ayhan et al., 1996; Acs et al., 1998, 1999]. A higher level of local acute-phase proteins was found in inflamed pulps than in healthy pulps [Proctor et al., 1991]. It is possible that severe caries also interferes with systemic factors, thereby negatively influencing the general health. Endocarditis is only one example related to oral health.

The present study was aimed at investigating whether systemic factors can be found which correlate with dental caries in caries-active children. We determined the concentrations of immunoglobulin IgG, total and specific for *Streptococcus mutans.* Antibody levels against *S. mutans* will normally not correlate to general health. However, in severe caries patients, antibody concentrations against *S. mutans* are increased and therefore indicative for a systemic immune response to the infection. We wondered whether this infection might affect the general health. For this, we tested another systemic factor, the acute-phase protein alpha(1)-acid glycoprotein (AGP). AGP is elevated in patients with severe acute and chronic inflammations [de Graaf et al., 1994; Eap and Baumann, 1993; Havenaar et al., 1997]. Increased levels of AGP are a negative predictor in the clearance of parasites in visceral leishmaniasis, a disease often found in tropical countries [Wasunna et al., 1995]. Elevated AGP levels in situations with a local infection such as dental caries may indicate that this
infection can influence the general health when the inflammation is not treated. Chronic inflammatory conditions will continuously stimulate the liver to synthesize increased amounts of plasma acute-phase proteins, one of which is AGP [de Graaf et al., 1994; Eap and Baumann, 1993; Havenaar et al., 1997; Koj et al., 1993]. Under acute inflammatory conditions such type of stimulation is considered to assist in damping down local inflammatory reactions. However, under chronic inflammatory conditions the hepatic reaction instead appears to sustain inflammation. In

this study, AGP was chosen as a marker for a systemic inflammatory reaction, not only because it is one of the major acute-phase proteins, but also because it is an important drug-binding protein [Israili and Dayton, 2001]. The latter property has been shown to decrease the free plasma concentration of a great number of drugs and consequently their activity [Israili and Dayton, 2001].

Materials and methods

Subjects

The subjects in this study were 6-year-old children from the first 2 groups of 3 different primary schools in Den Pasar, Bali, Indonesia. The schools were all located around the University of Den Pasar. In order to be included in the study, the children had to be present at school at the time of measurement and they had to provide a letter of informed consent, signed by their parents. Ethical approval of the study was obtained from the University of Den Pasar, Indonesia.

Clinical procedures

Two dentists examined the children. The examination was performed in the classroom, using a headlamp, a mirror and a probe. The child was lying on a table. dmft was determined by using the WHO standards [WHO, 1997]. Caries in both primary and secondary teeth was determined, but in the present study, only dmft was taken into account.

Caries lesions that had progressed to the extent that pulpal exposure was to be expected were recorded as 'pulpal involvement present'. Furthermore, the presence of root remnants, abscesses and fistulae was recorded. From all subjects approximately 75µl venous blood was isolated by finger puncture and was obtained in heparincoated capillary tubes. After standing for 4h at room temperature, the blood samples were centrifuged and the resulting plasma was collected and stored at –15°C and finally transported frozen to Amsterdam.

After the clinical procedures, the detailed dental status and treatment plans were handed over to the dental school in Den Pasar, thereby initiating treatment of the children by the local dentists.

Determination IgG and IgM specific for S. mutans

The ELISA was essentially performed as described previously [de Soet et al., 1987]. Briefly, a bacterial cell suspension was made of overnight-grown *S. mutans* cells (HG 982), diluted in coating mixture (6.75ml 0.2 *M* NaCO3 and 12ml 0.2 *M* NaHCO3 in 100ml water) to an A650 of 0.1. This suspension was incubated in 96-well microtiter plates at 4°C for 16h. Free binding sites were blocked by incubation with 1% bovine serum albumin in PBST (phosphate-buffered saline supplemented with 0.05% Tween 80) at room temperature for 30min. In between the incubations, the plates were washed three times with PBST. Plasma (2µl) diluted in PBST was added. The

plates were incubated for 2h at room temperature. After washing, 100 µl peroxidase-conjugated goat anti-human immunoglobulin IgG, HRP labelled (American Qualex, La Mirada, Calif., USA: 1:1,000 diluted in PBST supplemented with 1:200 normal goat serum) was added and incubated for 2h at room temperature. Peroxidase activity was measured by adding 100µl staining buffer [15ml Na2HPO4 (4.5g/100ml water), 300 µl TMB stock (25 m*M* tetramethylbenzidine (Sigma Chemical Co, St. Louis, Mo., USA), 87.3mg TMB.2 HCl in 10ml DMSO) with 60µl H2O2 (30%) in 15 ml of citric acid buffer (1.55g/100 ml water)]. The reaction was stopped after 20 min by the addition of 50µl H2SO4 (10%) followed by measuring the A450 absorbance of each well using a MR 7000 Micro plate reader (Dynatech Torrance, Calif., USA).

The determination of IgM specific for *S. mutans* was performed similarly, but by using peroxidase-conjugated goat anti-human immunoglobulin IgM, HRP labelled (American Qualex, La Mirada, Calif., USA: 1:1000 diluted in PBST). A batch of pooled human serum was used as a reference standard, which in the case of the negative control was absorbed with *S. mutans* prior to analysis.

The titer was defined as the dilution of the standard or samples where an A450 value was reached that was twice as high as the value of the negative control. The specificity of the determination of the concentration of antibodies to *S. mutans* was checked by absorption of the reference serum with *S. mutans* cells. This resulted in a complete loss of ELISA signal after 3 absorption steps, which indicates a specific test. The ELISA for the determination of the total IgG content was performed essentially as described above, with an affinity-purified non-conjugated goat anti-human IgG-Fc (KPL, Guildford, UK: 1:1,000 diluted in PBS) instead of the incubation with bacterial cells. All other incubations were identical.

AGP determination

The concentration of AGP was determined by rocket immunoelectrophoresis using polyclonal goat anti-human AGP antibodies (kindly provided by Dr. T. Stefaniak; Department of Veterinary Prevention and Immunology, Wrocław Agriculture Academy) for precipitation, as described by Laurell [1966]. Electrophoresis was carried out in 1% agarose M (Bio-Rad, Richmond, Calif., USA) in veronal-buffered saline (pH 8.6). Human Serum Protein Calibrator (Dakopats, Glostrup, Denmark), consisting of pooled human sera from healthy blood donors, was used as a standard for the determination of the AGP concentration.

Statistical analyses

Statistical analysis was performed using SPSS 10.0.7. The IgG data were log-transformed, and for some graphical representations categorized into 16 groups. Spearman correlations on the raw data as well as on categorized data were calculated. To test the relationship between 3 parameters, a stepwise regression analysis was performed to correlate the dmft value with IgG and AGP.

Results

Eighty-five children of 6–7 years were examined; 39 were boys and 46 were girls. No child was caries free and the mean dmft was high (8.8; Table 1). It is remarkable that most children had infected pulps, i.e. cavities to such an extent that pulpal exposure was visible or at least inevitably to be expected during excavation. Children with a high dmft had significantly more teeth with pulpal involvement (r=0.604, p<0.001). These children also had abscesses more often (r=0.356, p=0.001).

Table 1 Clinical data of 85 6-year-old children in Bali

	Mean	SD	Range
Age, years	6.4	0.03	5.7–7.0
dmft	8.8	2.9	1–17
Number of teeth with infected pulpas	3.9	2.2	0–12
Number of residual roots	0.9	1.1	0–5
Number of abscesses	0.5	0.8	0–3

The distributions of the concentrations of AGP and IgG specific for *S. mutans* are shown in figure 1. These data show a normal distribution, but with a relatively wide range for AGP (mean 0.86 ±0.26 mg/ml), suggesting that both healthy and less-healthy children were present in the group. It was concluded that all data were normally distributed. The concentration of AGP was significantly negatively correlated with dmft (r=–0.25 p=0.036, Spearman; Fig. 2). The IgG specific for *S. mutans* was correlated with both dmft (r=0.31, p=0.008, Spearman) and AGP (r=0.26, p=0.04, Spearman). Because of the normal distribution of the data, a stepwise regression analysis could be performed on the dmft, IgG and AGP. This analysis revealed that dmft was significantly correlated with the log-transformed concentration of IgG against *S. mutans* (adjusted r^2=0.083, standardized ß coefficient=0.31, p=0.008). When the concentration of AGP was included in the model, the prediction improved significantly (for IgG: adjusted r^2=0.157, standardized ß coefficient=0.36, p=0.002; for AGP: ß coefficient=–0.30, p=0.009). Other factors, such as total IgG and IgM against *S. mutans* were not significantly correlated with dmft, AGP or IgG against *S. mutans*.

Discussion

It has been found that cytokine production may be influenced by the caries process, especially due to *S. mutans* and *Lactobacillus casei* infection [Hahn and Falkler,1992; Hahn et al., 2000; Plitnick et al., 1998]. In the present study, no bacterial counts were performed, since we expected that

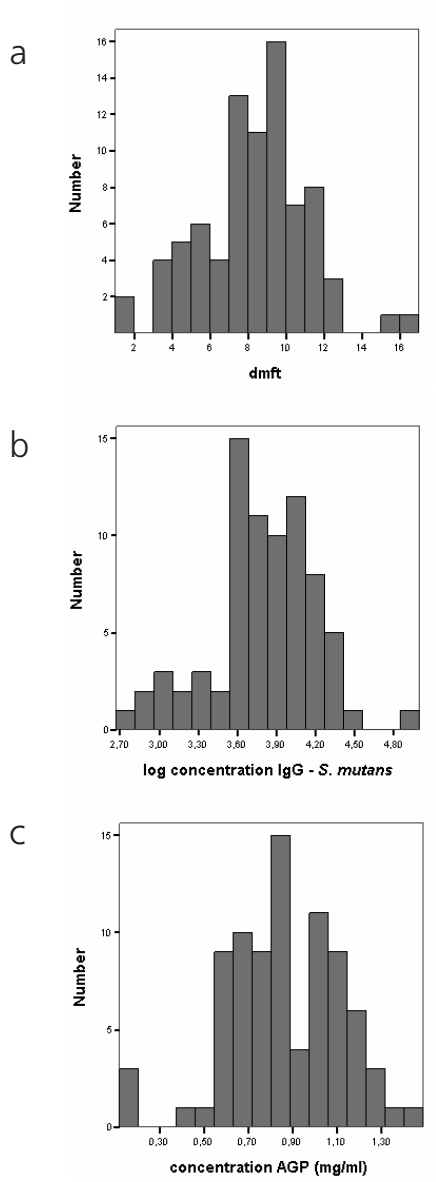

Figure 1 The frequency distributions of dmft and plasma concentrations of AGP and IgG against *S. mutans*. Analyses of the data indicated normal distributions.

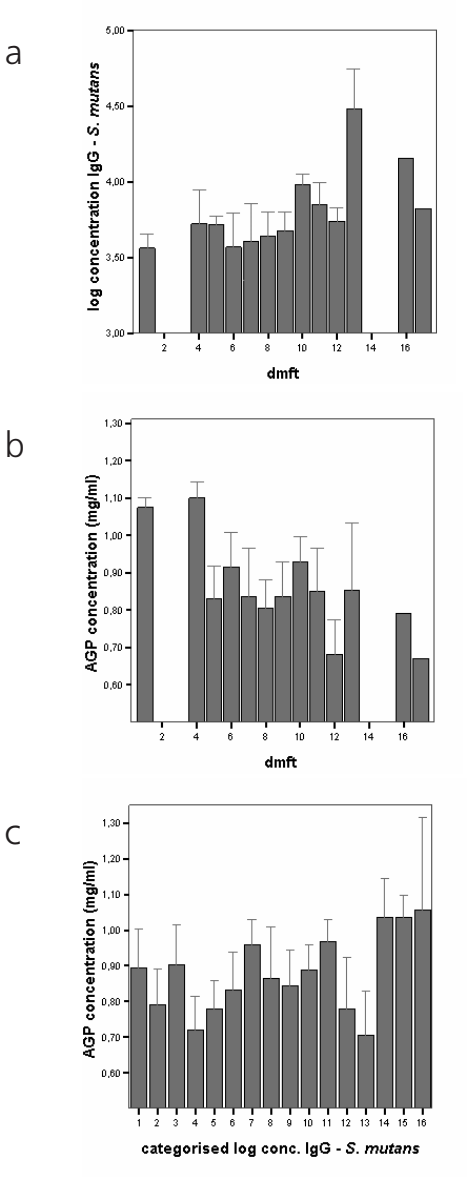

Figure 2 Mutual correlations between dmft, the concentration of AGP and the concentration of IgG against *S. mutans* in the plasma of the patients. The bars indicate the means with standard deviation.
a Correlation of dmft with log-transformed concentrations IgG to *S. mutans* (r=0.31, p=0.003).
b Correlation of dmft with AGP concentrations (r=–0.25, p=0.04).
c Correlation of log-transformed concentrations of IgG to *S. mutans* with AGP concentration (r= 0.26, p=0.04). For graphical presentation, the IgG concentration on the x-axis is categorized.

in all children, a high number of salivary lactobacilli and *S. mutans* could be detected [Kreulen et al., 1997a]. Although data are not available for Indonesia, a high caries incidence is usually associated with high salivary mutans streptococci counts. Moreover, the infrastructural problems in Bali did not allow us to perform bacterial cultures on a laboratory level.

The numbers of caries lesions found in the present study are similar to what has been reported before. For Indonesia, Koloway and Kailis [1992] reported a caries prevalence of more than 90% in pre-school children and a mean dmft of 8.0. The high percentage of untreated caries may be due to the shortage of dentists in Indonesia: 1 dentist/20,300 people [Sekiguchi and Machida, 1999].

A wide distribution in concentration of IgG to *S. mutans* was found. The log-transformed concentrations correlated significantly with dmft, indicating that the infection by *S. mutans* affects the immune system. Similar results were reported earlier [Challacombe and Lehner, 1976; Huis in 't Veld et al., 1979; Parkash et al., 1994]. The concentration of the acute-phase protein AGP was more widely distributed than expected for a healthy population [Koj et al., 1993]. The results indicate a relationship between dmft, AGP and antibodies specific for *S. mutans*. Moreover, dmft and IgG specific for *S. mutans* were positively correlated, while dmft and AGP were negatively correlated. Similar results were found in a study on infection, length growth and acute phase proteins, such as AGP. It was reported that AGP is negatively correlated with retardation in growth [Hautvast et al., 2000]. Our findings of a negative correlation coefficient in only one parameter can be explained by assuming two counteracting mechanisms. Some data are available showing that different phases of a caries process are associated with a different cytokine response, which has possibly to do with the difference between infections caused by *S. mutans* and lactobacilli. In both cases, a different cytokine response has been found [Plitnick et al., 1998; Hahn et al., 2000]. In the present study, we could not find any definite proof for such a mechanism, possibly because we were not able to investigate the children before and after restorative treatment. Moreover, the group was possibly too small to prove this two-phase system.

In this study, we showed that dental caries has systemic effects on the production of IgG, but also on induction of acute-phase proteins in the liver, such as AGP. These results are supported by the findings of others. Hahn and co-workers suggested that the presence of antibodies against cariogenic bacteria might be protective against invasion of these bacteria during the caries process [Hahn and Falkler, 1992]. Furthermore, it was reported that when AGP levels increase, infections might take a longer time to heal [Wasunna et al., 1995]. Therefore, it is suggested that severe dental caries might influence the general health of the affected child.

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