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ORIGINAL ARTICLE

Alteration of cytokines in saliva of children with caries and obesity

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Abstract
Caries and obesity are multifactorial diseases with inflammatory components, whose processes involve cells and molecules, such as cytokines. Therefore, the objective of this work was to compare the concentrations of IL-6, IL-8, IL-15, and IL-18 in the salivary samples of children with caries and obesity. The study was carried out with 80 children: 43 with a normal weight and 37 with obesity. The diagnosis of caries was carried out using the ICDAS system. Salivary samples were used to measure the cytokine levels via the ELISA technique. Our results show that children with obesity and dental cavities have high levels of IL-6 and IL-15. Similarly, obese children have elevated levels of these two cytokines, while children with cavitated carious lesions presented alterations in their concentrations of IL-6 and IL-8. In conclusion, our data suggest that IL-6 has a significant effect on both obesity and caries, although IL-8 is more related to caries, and IL-15 is more related to obesity.

Keywords Caries · Obesity · Cytokine · ICDAS · Children

Introduction.

The development of dental caries is a dynamic process that involves repeated cycles of demineralization and remineralization throughout the day, as a result of microbial metabolism on the dental surface, upon which, over time, a net mineral loss can result, allowing a cavity to be formed [1]. The global prevalence of caries in the year 2010 was calculated at 35.4% [2]. Mexico is one of the countries most affected by this pathology; previous studies have reported a caries prevalence of above 60% in children [3]. In contrast, the secretary of health of Mexico reports that about 75% of children suffer from caries [4].

On the other hand, obesity is defined as excessive body weight for height with an excessive accumulation of adipose tissue that is usually accompanied by mild, chronic, and systemic inflammation [5]. Estimates in the adult population show that by 2030, up to more than 50% of the population could suffer from obesity [6]. For the child population, there is slight evidence that these rates are leveling off in some populations of the United States and Europe. Nevertheless, these rates remain high at around 30% [7]. In Mexico, however, a prevalence greater than 45% has been reported [8].

A large number of papers have been published on the potential association, possibly bidirectional, between caries and obesity [9–11]. It has been widely documented that both caries and obesity are involved in an inflammatory process, in which both cells and molecules participate [12–15]. Macrophages are one of the principal types of cells of the innate immune system involved in the caries response. It has been reported that when the carious process progresses through the dentin, the number of macrophages increases in the dental pulp. This process reflects a higher vascular permeability and favors the elimination of bacteria through the activation of other immune cells, such as cytotoxic T lymphocytes and NK cells [14].

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Macrophages perform most of their functions through the secretion of cytokines, such as interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 15 (IL-15), and interleukin 18 (IL-18), among others.

IL-6 is a proinflammatory cytokine, which has been previously studied in the carious process, showing an increase in caries patients in most studies [16–20]. IL-8 is a cytokine with a chemotactic effect on neutrophils and has been extensively studied in caries; however, the results have been controversial [17, 19–22]. Some cytokines have been poorly studied in caries, such as IL-15 and IL-18, which participate in the activation of NK cells. So far, to our knowledge, IL-15 has not been studied in caries, and for IL-18, there is only one previous report [23]. Further, the concentrations of these cytokines in children with caries have not yet been studied using the International Caries Detection and Assessment System (ICDAS).

In contrast, cytokines represent a vast field of study in the pathophysiology of obesity. It has been widely demonstrated that IL-6 significantly participates in this pathology. However, the measurement of this molecule in the saliva samples of obese subjects has been less commonly studied [24–27]. IL-8 has not been studied in the saliva samples of obese subjects, while the serum results have been controversial [28–30]. IL-15 and IL-18 have also not been studied in the saliva of obese subjects (serum only) [31–35].

Although there is evidence of the involvement of these cytokines, both in caries and in obesity, patients suffering from both diseases have been rarely studied. Therefore, the objective of this research was to compare the concentrations of IL-6, IL-8, IL-15, and IL-18 in the salivary samples of children with caries and obesity.

Materials and methods

Study design

This is a cross-sectional and observational study, which included children from 3 to 8 years of age who attend the clinic of the Specialty in Pediatric Dentistry, of the Center for Comprehensive Medical Care, belonging to the Centro Universitario de Los Altos, of the Universidad de Guadalajara. The study population was obtained via consecutive non-probabilistic sampling, including 80 children of both genders. Children who were under any medical treatment, presenting periodontal disease, taking antibiotics or anti-inflammatory drugs, or whose parents did not authorize their participation in the study were excluded. The research protocol was approved by the local ethics committee, with the official number: CUA/CEI/004/2018.

Clinical examinations

Once the consent was signed, each patient's weight and height were obtained to establish his or her body mass index (BMI). The weight was obtained with a digital scale, and the height was obtained with a stadiometer. The children were separated into groups of normal weight or excess weight (overweight/obesity), using the BMI and its corresponding percentiles as a reference via the Center for Disease Control and Prevention (CDC) online tool: https://www.cdc.gov/healthyweight/spanish/bmi/calculator.html.

Caries severity was assessed using the criteria of the International Caries Detection and Assessment System (ICDAS), on a scale from 0 to 6, where 0 = a sound tooth surface; 1 = a visual change in the enamel (when dry); 2 = a visual change in the enamel (when wet); 3 = localized enamel breakdown due to caries, with no visible dentin or underlying shadow; 4 = underlying dark shadow from the dentin with or without localized enamel breakdown; 5 = visible dentin cavity on less than half of the surface; 6 = visible dentin cavity on more than half of the surface. One examiner conducted the clinical examinations. This examiner had training and experience in the use of ICDAS with high consistency (kappa = 0.89). Before the examinations, a dental prophylaxis was administered by a pediatric dentist and then dried with an air syringe.

The frequency found for the different ICDAS codes of the study subjects is shown in Table 1. However, for the majority of statistical analyses, the sample was divided into two groups, according to the severity of caries: The group of subjects with cavitated caries lesions only (ICDAS codes 3–6) was compared with a group of children who had ICDAS codes 0–2 for all their included teeth. Each child was classified based on his or her most advanced stage of tooth decay—that is, if a child had one tooth surface with an initial lesion and another with a severe lesion, only the latter was recorded.

Unstimulated saliva was collected using the spitting method. Children were asked to avoid eating, drinking, brushing, and using dental floss for 90 min before sampling. Saliva samples were homogenized and clarified by centrifugation at 10,000*g* for 15 min at 4 °C. The aliquots of the clarified supernatants were stored at -80 °C for future use in cytokine measurements.

On the day of the experiment, all samples were evaluated by an ELISA MAX™ Deluxe Set Human IL-8 (Biolegend, USA), according to the manufacturer's instructions. The concentrations of IL-6, IL-15, and IL-18 were also determined using a commercially available ELISA kit (R&D Systems Inc, USA), following the manufacturer's instructions in the same way. Absorbance was measured at 450 and 570 nm using a microplate reader (Multiskan GO,

Table 1 General characteristics of the study subjects

Characteristic	Total $n=80$ (%)	Normal weight $n=43$ (%)	Overweight/obesity $n=37$ (%)
Caries			
ICDAS 0	4 (5.0)	3 (7.0)	1 (2.7)
ICDAS 1	2 (2.5)	1 (2.3)	1 (2.7)
ICDAS 2	1 (1.3)	1 (2.3)	0 (0)
ICDAS 3	5 (6.3)	2 (4.7)	3 (8.1)
ICDAS 4	3 (3.8)	2 (4.7)	1 (2.7)
ICDAS 5	14 (17.5)	8 (18.6)	6 (16.2)
ICDAS 6	51 (63.7)	26 (60.5)	25 (67.6)
Caries			
Non-cavitated carious lesions (ICDAS-II code 0–2)	7 (8.8)	5 (11.6)	2 (5.4)
Cavitated caries lesions (ICDAS-II code 3–6)	73 (91.3)	38 (88.4)	35 (94.6)
Gender			
Female	31 (38.8)	15 (34.9)	16 (43.2)
Male	49 (61.3)	28 (65.1)	21 (56.8)
Tooth wash frequency			
1 a day	27 (33.8)	14 (32.6)	13 (35.1)
2 or more per day	53 (66.2)	29 (67.4)	24 (64.9)
Sinuous process			
Absence	74 (92.5)	38 (88.4)	36 (97.3)
Presence	6 (7.5)	5 (11.6)	1 (2.7)
Pain			
Absence	62 (77.5)	31 (72.1)	31 (83.8)
Presence	18 (22.5)	12 (27.9)	6 (16.2)

Thermo Scientific, Finland). The results were presented as the picogram per milliliter (pg/mL).

Statistical analysis

A statistical analysis for the ordinal and nominal variables is presented according to the pure frequency and percentage. The scale variables are presented as the mean and standard error or median and interquartile range. For the inferential analysis, the corresponding normality tests were performed before undertaking the statistical test. A comparison of the nominal and ordinal variables was performed using a chi-squared test. At the same time, a comparison between the scale variables was carried out by a Student's *t* test, Mann–Whitney or Kruskal–Wallis tests, with a post hoc analysis. $P<0.05$ was considered significant.

Results

Eighty children between 3 and 8 years of age were enrolled, with an average age of 5.8. Of these participants, 43 had normal weight and 37 had excess weight (18 overweight and 19 obesity). The different degrees of caries, according to ICDAS, together with other characteristics, are shown in Table 1. The majority of the study subjects were classified under codes 5 and 6 of the ICDAS, for those with both normal weight and overweight/obesity. Secondly, the group of subjects with cavitated caries lesions only (ICDAS-II codes 3–6) was compared with the group of children who were classified under ICDAS-II codes 0–2 (non-cavitated carious lesions). Interestingly, the percentage of cavitated carious lesions was higher in the group of overweight/obese children (94.6%) but without being statistically significant.

There was a greater participation of the female gender. Around two-thirds of female participants reported brushing their teeth two or more times a day, and only some of them mentioned suffering pain or a sinuous process. No statistical difference was found between the two groups studied according to body composition or according to carious lesions for the variables shown in Table 1. Besides, it was observed that children with cavitated lesions did not have had a higher BMI compared to the infants with carious lesions without cavitation (17.7 ± 0.3 vs. 16.9 ± 0.2; respectively; $P=0.075$).

On the other hand, as seen in Fig. 1 and Table 2, the salivary levels of IL-6 and IL-15 were found to be increased in children with overweight/obesity compared to those in the normal-weight group (Fig. 1a, g). Furthermore, IL-6 was also found to be increased in children with cavitated carious lesions (Fig. 1b). Conversely, IL-8 decreased in the same study group (Fig. 1e). Interestingly, the salivary levels of IL-6 were higher among the female than the male participants (56.8 ± 17.4 vs. 23.8 ± 8.7, respectively; $P=0.003$). Notably, the frequency presented by both genders was similar.

Subsequently, a cytokine analysis was performed on body composition and caries. In this analysis, the overweight/obesity group with cavitated carious lesions was not considered because only two children formed this study group (Table 1). Therefore, as seen in Fig. 1c, i, only salivary levels of IL-6 and IL-15 were found to have a statistical difference in the group with overweight/obesity and cavitated carious lesions compared to the group with a normal weight and cavitated carious lesions or un-cavitated carious lesions. The other two cytokines did not show any statistical differences between the groups studied.

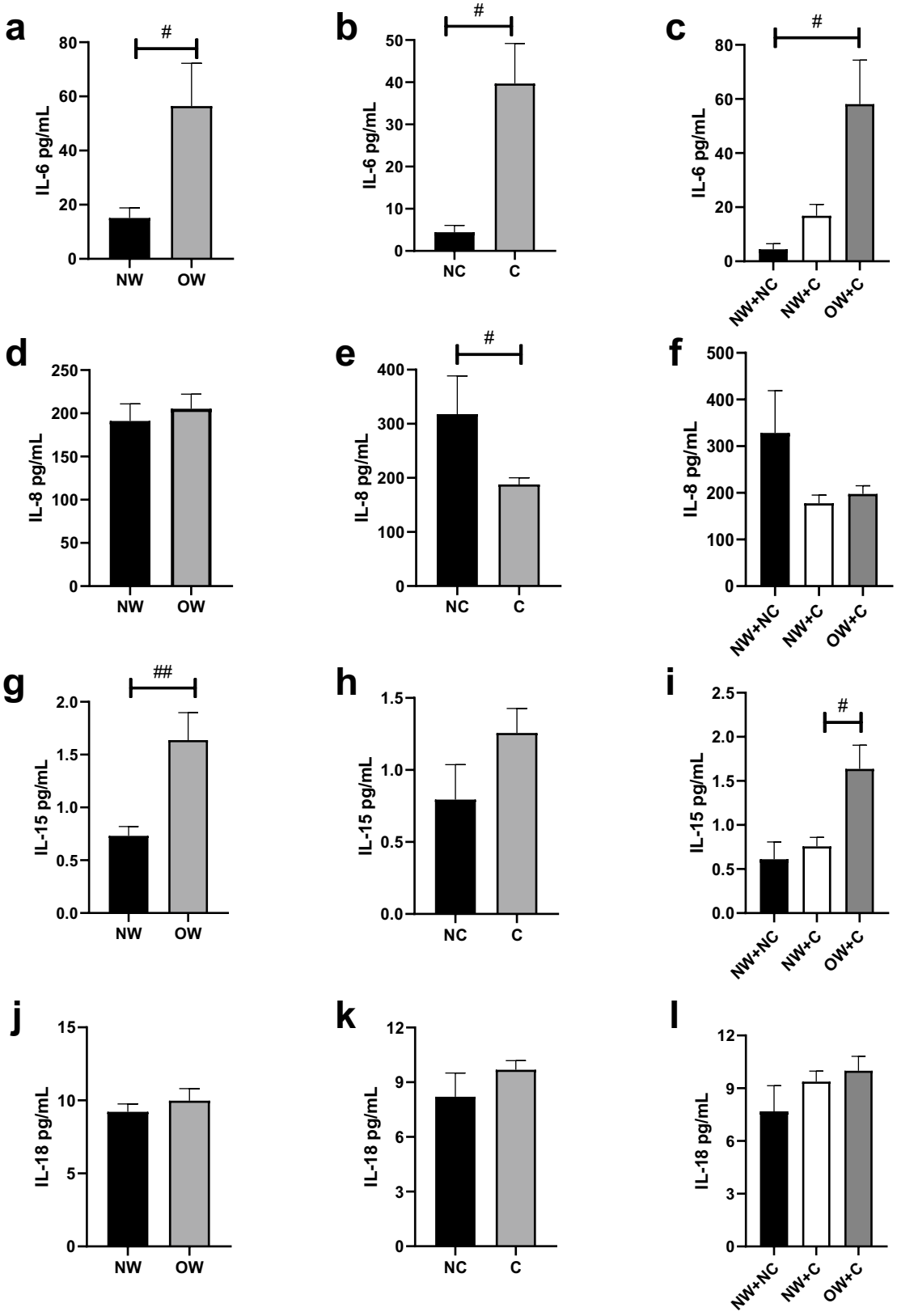

Fig. 1 Comparison of IL-6, IL-8, IL-15, and IL-18 in the saliva of children with caries and obesity. **a**, **d**, **g** and **j** show the comparisons of cytokines concerning overweight/obesity. **b**, **e**, **h** and **k** show the comparisons of cytokines for caries. **c**, **f**, **i** and **l** show the comparisons of cytokines to the two pathologies. *NW* normal weight; *OW* overweight/obesity; *NC* carious lesions not cavitated; *C* cavitated carious lesions. Data are presented as the mean and standard error. # $P < 0.05$, ## $P < 0.01$

Table 2 Concentrations, in children's saliva, according to the presence of caries and obesity

Cytokines (pg/mL)	NW	OW	*P*	NC	C	*P*	NW + NC	NW + C	OW + C	*P*
IL-6 median (IQR)	5.36 (3.31–21.99)	12.47 (4.63–32.54)	0.025	3.32 (1.78–7.54)	12.1 (4.08–26.53)	0.042	2.04 (1.78–6.23)	7.65 (3.93–23.81)	12.85 (5.77–32.54)	0.028
IL-8 median (IQR)	197.90 (133.75–235.00)	216.35 (117.20–274.40)	0.608	237.20 (198.95–304.20)	189.90 (118.30–260.00)	0.044	200.00 (198.95–267.00)	183.90 (125.35–235.00)	210.30 (117.20–270.90)	0.152
IL-15 median (IQR)	0.55 (0.36–0.83)	1.14 (0.65–1.56)	0.003	0.45 (0.24–1.17)	0.86 (0.51–1.39)	0.365	0.27 (0.24–0.45)	0.59 (0.40–0.94)	1.10 (0.65–1.52)	0.013
IL-18 median (IQR)	8.05 (6.91–9.36)	9.21 (6.33–11.66)	0.640	7.21 (5.55–9.23)	8.65 (6.87–11.26)	0.390	6.76 (5.55–7.21)	8.52 (7.00–10.30)	8.65 (6.33–11.66)	0.475

NW normal weight, *OW* overweight/obesity, *NC* carious lesions, not cavitated, *C* cavitated carious lesions, *IQR* interquartile range

Discussion

In the present study, we compared the levels of IL-6, IL-8, IL-15, and IL-18 in the salivary samples of children with caries and obesity. Our results show that salivary levels of IL-6 and IL-15 increased in children with caries and obesity compared to the results for children who were only overweight/obese. IL-8 decreased in children with dental cavities, while IL-6 increased.

The observation that IL-15 increased in the obesity and caries group is highly relevant because, to our knowledge, this cytokine has not been previously associated with caries. However, these results should be taken with caution, as this same cytokine increased in children who also had obesity. It is, therefore, plausible that this effect is due to obesity and not due to tooth decay. The increase in IL-6 levels in the overweight/obese children with cavitated carious lesions should also be taken with caution. Although this molecule increased both in children with obesity and in children with caries, we must consider the significant difference in the number of participants between both study groups. Therefore, it is also possible that the elevated levels of IL-6 are mainly due to obesity and not due to the presence of caries.

IL-15 was also increased in the obesity group, a result that is consistent with another report [31]. However, another study reported a decrease in this cytokine in subjects with a high BMI [32]. It should be noted, however, that in these two previous works, IL-15 was measured in serum samples, while we measured it in saliva. Therefore, this result supports the idea that the saliva of obese subjects differs from those with a normal weight [36, 37]. The salivary levels of IL-6 were also found to be increased in children with obesity, a result that is corroborated by previous research [24–27]. Some studies have shown that IL-8 and IL-18 are associated with obesity, but the results have been controversial. These studies have only been performed on serum samples but not in salivary samples [28–30, 33–35]. However, these results show that children with obesity present an inflammatory state, which is reflected even in their saliva.

The results obtained in the caries groups show that the salivary levels of IL-6 increased in the group with cavitated lesions. Other research groups have reported similar results in saliva samples [16–20]. These results demonstrate that proinflammatory cytokines, such as IL-6, have an essential role in caries pathophysiology. IL-15 and IL-18 did not show a significant difference in the presence of caries. However, to our knowledge, this is the first work on the relationship between IL-15 and caries in saliva samples. A study conducted via real-time PCR previously reported that IL-18 is increased in severe caries pulp [23].

On the other hand, we found low levels of IL-8 in children with cavitated carious lesions, whereas the

associations between salivary levels of IL-8 and previously published caries have been controversial. Some previously published research papers have shown that patients with caries have high salivary levels of IL-8; however, some of these studies examined adults, while in others, the diagnosis of caries was done through DMFT (Decayed, Missing, Filled, Teeth) [17, 19–22]. This last aspect is of particular importance because we used the ICDAS system to diagnose caries. So far, there are few investigations that use this system to perform salivary or serum cytokine measurements.

Another point to consider is the different factors involved in the development of caries and obesity. Some of these factors are cultural, sociodemographic, and biological. Cytokines are found in this last factor. However, there are also other very important factors, such as diet. Previous studies have reported that high sugar consumption increases the risk of developing both caries and obesity [38, 39] Therefore, one of our future perspectives is to conduct research where the interaction between the various factors that predispose to the development of obesity and caries is observed.

Some of the limitations of this study are the low number of caries-free children, as well as the presence of children with non-cavitated carious lesions. However, in a clinical environment, it is difficult to find real diagnoses (such as overweight children without cavities), especially in pediatric dental practices. Another point to consider is that we measured cytokine levels in saliva, which does not faithfully reflect the cellular origin of these molecules. Most saliva proteins come from the major and minor glands, as well as the gingival crevicular fluid [40]. Nevertheless, one of the criteria for non-inclusion of the children was the presence of gingivitis or other periodontal diseases. It would also be desirable to perform other tests, such as RT-PCR or western-blot, to identify the origins of the cytokines studied. Besides, we must take into account that salivary sampling is a non-invasive procedure that is ideal for use in children, unlike other sampling techniques, such as taking gingival crevicular fluid.

In conclusion, these results demonstrate that under the conditions of both obesity and caries, the immune system participates in the inflammatory process, in which a vast network of cytokines are involved. Additionally, the alterations in their concentrations are reflected in the salivary samples. Nevertheless, more thorough studies are needed to achieve a better understanding of the interactions between these two diseases and to reduce their prevalence and comorbidities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The research protocol was approved by the local ethics committee, with the official number: CUA/CEI/004/2018; the study was performed following the Declaration of Helsinki.

Informed consent Informed consent was obtained from all parents or guardians of the participants included in this study.

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