

Article

Difference Between Pro- and Anti-Inflammatory Interleukins in Saliva of Children with Early Childhood Caries

Juan Carlos Hernández Cabanillas ¹, Nuria Patiño-Marín ², María Guadalupe Martel Gallegos ³, Marco Felipe Salas Orozco ², Laura Matilde Ubaldo Reyes ⁴, Martha Imelda Maldonado Cervantes ³ and Jesús Ramón Castillo Hernández ^{3,*}

¹ Department of Pediatric Dentistry, Faculty of Dentistry, Universidad Autónoma de Baja California, Blvd. Universitario, Tijuana 22457, Mexico

² Clinical Research Laboratory, Faculty of Stomatology, Autonomous University of San Luis Potosí, San Luis Potosí City 78290, Mexico

³ Multidisciplinary Academic Unit of the Middle Zone, Autonomous University of San Luis Potosí, San Luis Potosí City 79615, Mexico

⁴ Department of Anatomy, Faculty of Medicine, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico; lm.ubaldo@facmed.unam.mx

* Correspondence: jesus.castillo@uaslp.mx

Abstract

Background: Early childhood caries (ECC) is a common chronic disease in young children, influenced by multiple factors, including the activity of bacteria and other microorganisms, diet, and immune response. Pro-inflammatory cytokines like interleukin-8 (IL-8) and anti-inflammatory cytokines like interleukin-10 (IL-10) play crucial roles in the inflammatory process of caries. However, their relationship with ECC severity remains unclear. This study aimed to compare salivary IL-8 and IL-10 levels in children with and without ECC and analyze their association with caries severity using the International Caries Detection and Assessment System (ICDAS). Children with and without central obesity were included to evaluate the potential influence of nutritional status on cytokine expression.

Methods: A cross-sectional study was conducted from March 2022 to December 2023 in San Luis Potosí, México, including 76 children aged 3 to 5 years (40 with ECC and 36 caries-free). Anthropometric measurements were taken to classify children as centrally obese or non-centrally obese. Unstimulated saliva samples were collected, and IL-8 and IL-10 levels were measured using ELISA. Statistical analysis included the Mann–Whitney U test, Spearman’s rank correlation coefficient, and binary logistic regression analysis, considering $p < 0.05$ as statistically significant. **Results:** IL-8 levels were higher in the ECC group (85 ± 119 pg/mL) than in the control group (45 ± 74 pg/mL), but this difference was not significant ($p = 0.3613$). IL-10 levels were lower in the ECC group (3 ± 2 pg/mL) than in the control group (11 ± 44 pg/mL; $p = 0.6481$). The difference between IL-8 and IL-10 levels was greater in the ECC group (27 ± 41 pg/mL) than in the control group (17 ± 33 pg/mL; $p = 0.1709$). No significant correlation was found between cytokine levels and ICDAS scores ($p > 0.05$), and binary logistic regression did not show an association between IL-8, IL-10, WHtR, and cavitated caries lesions. **Conclusions:** Although IL-8 tended to be elevated and IL-10 reduced in children with ECC, the differences were not statistically significant. The observed trend suggests a possible local immunological imbalance in children with caries, which may contribute to disease progression independently of bacterial activity or behavioral influences.



Received: 7 July 2025

Revised: 9 August 2025

Accepted: 9 September 2025

Published: 17 October 2025

Citation: Hernández Cabanillas, J.C.; Patiño-Marín, N.; Martel Gallegos, M.G.; Salas Orozco, M.F.; Ubaldo Reyes, L.M.; Maldonado Cervantes, M.I.; Castillo Hernández, J.R. Difference Between Pro- and Anti-Inflammatory Interleukins in Saliva of Children with Early Childhood Caries. *Oral* **2025**, *5*, 82. <https://doi.org/10.3390/oral5040082>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Keywords: early childhood caries; saliva; interleukins; IL-8; IL-10; inflammation; pro-inflammatory cytokines; anti-inflammatory cytokines; immune response; pediatric dentistry

1. Introduction

Early childhood caries (ECC) is one of the most prevalent chronic diseases among the pediatric population, both in Mexico [1] and worldwide [2]. It is characterized by the progressive demineralization of the dental hard tissues, caused by the action of cariogenic microbes in the presence of fermentable carbohydrates and poor oral hygiene. Its etiology is multifactorial, involving biological, behavioral, and environmental factors that contribute to its development and severity [2].

Beyond its microbial etiology, ECC involves complex host immune responses that may influence disease progression [3–5]. Salivary cytokines are increasingly recognized as valuable biomarkers for assessing immune activity in the oral cavity [6,7]. In the context of ECC, the balance between pro-inflammatory and anti-inflammatory mediators may influence disease progression and severity [3,8]. Among the wide array of cytokines present in saliva, interleukin-8 (IL-8) and interleukin-10 (IL-10) have been identified as key regulators of the immune response [7,9], yet their specific roles in ECC remain unclear.

IL-8 is a pro-inflammatory chemokine involved in neutrophil recruitment and activation [7], and its salivary levels have been reported to increase in children with ECC [10–12]. However, findings across studies are inconsistent, with some reporting no association [9,13,14] or even decreased IL-8 levels in caries-affected individuals [15]. IL-10, in contrast, is an anti-inflammatory cytokine that suppresses the expression of pro-inflammatory mediators [16]. Its role in ECC has been even less explored, with limited and conflicting evidence regarding its salivary expression in caries [9,13,17–20].

This study aims to compare salivary levels of IL-8 and IL-10 based on their complementary immunological functions—pro- and anti-inflammatory, respectively—to reflect the immunoinflammatory balance in the oral environment in children with and without ECC. The understanding of the host immune response in ECC could help identify immune profiles associated with caries risk. Moreover, these biomarkers could have diagnostic or prognostic value, facilitating early and personalized intervention strategies in vulnerable pediatric populations.

2. Materials and Methods

2.1. Study Design

A comparative cross-sectional study was conducted between March 2022 and December 2023 in San Luis Potosí, San Luis Potosí, Mexico. The study was carried out in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of the Universidad Autónoma de San Luis Potosí (UASLP), under approval number CEI-FE-079-022. Written, voluntary informed consent was obtained from the parents or legal guardians of all participating children prior to the start of the study.

2.2. Study Population

A total of 76 children aged 3 to 5 years were included and divided into two groups:

1. Early childhood caries (ECC) group ($n = 40$; ICDAS 1–6);
2. Caries-free control group ($n = 36$; ICDAS 0).

2.3. Sample Size Calculation

To determine the required sample size, salivary IL-8 levels in children with and without caries were considered. The following formula for comparing two means was used [21], assuming a 95% confidence level ($Z\alpha = 1.96$) and 95% statistical power ($Z\beta = 1.64$). According to the available data, the caries group had a mean IL-8 level of 1489.24 pg/mL with a standard deviation of 960.32, while the caries-free group had a mean of 619.19 pg/mL and a standard deviation of 311.79 [21]. Therefore, the minimum required sample size was 18 subjects per group.

2.4. Sampling Method

A non-probabilistic consecutive sampling method was used in public schools in the municipality of Rio Verde, San Luis Potosí, Mexico. Seventy-six preschoolers met the following inclusion criteria:

- Enrolled in invited public schools;
- Apparently healthy;
- Of either sex;
- Aged between 3 and 5 years.

Exclusion criteria included: lack of informed consent, presence of infectious diseases, chronic non-communicable or autoimmune diseases, or being under pharmacological treatment (including steroidal and non-steroidal anti-inflammatory drugs or antibiotics) within the past 30 days. Elimination criteria: Participants who do not allow clinical, anthropometric, and/or salivary sample collection and subjects whose salivary samples cannot be processed.

2.5. Clinical and Anthropometric Evaluation

Each participating child underwent a physical and dental evaluation, which included the following:

- Anthropometric measurements: Weight, height, and waist circumference were recorded. Waist-to-height ratio (WHtR) was calculated using a cut-off point of ≥ 0.50 to classify children as centrally obese or non-centrally obese [22].
- Habit questionnaire: Parents answered a questionnaire on dietary and oral hygiene habits, as well as other risk factors for caries development.
- Dental caries assessment: The International Caries Detection and Assessment System (ICDAS) was used to classify caries severity [23], with the following scale:
 - 0: Sound tooth surface;
 - 1: First visual change in enamel (dry surface);
 - 2: First visual change in enamel (wet surface);
 - 3: Localized enamel breakdown without visible dentin;
 - 4: Underlying dark shadow from dentin, with or without enamel breakdown;
 - 5: Distinct cavity with visible dentin involving $<50\%$ of the surface;
 - 6: Extensive cavity with visible dentin involving $>50\%$ of the surface.

Two calibrated examiners performed the clinical assessments with high interobserver agreement ($\kappa = 0.89$ and 0.85). Prior to the clinical evaluation, each child received a dental prophylaxis performed by a pediatric dentistry specialist, followed by air drying with a syringe to ensure precise visualization of dental surfaces. Based on this system, children were classified into two groups: ECC (ICDAS 1–6) and caries-free (ICDAS 0).

2.6. Saliva Sample Collection

Unstimulated saliva samples were collected using the spitting technique into microtubes in the morning (9:00–12:00 h). Children were instructed to refrain from eating, drinking, or brushing their teeth for at least 120 min before sample collection. Each sample (~0.5 mL) was labeled with a reference number and transported in a refrigerated container at -4°C to the laboratory at the Universidad Autónoma de San Luis Potosí, where they were stored at -80°C until further processing for cytokine analysis (IL-8 and IL-10).

2.7. Determination of IL-8 and IL-10 in Saliva by ELISA

Human IL-8 and IL-10 levels in saliva were quantified using sandwich enzyme-linked immunosorbent assay (ELISA) following the manufacturer's standard protocols (KHC0081 and KHC0101 Invitrogen Corporation CAT; Camarillo, CA, USA). A total of 50 μL of each saliva sample was added to microplate wells pre-coated with anti-IL-8 or anti-IL-10 capture antibodies for binding antigen. Subsequently, biotin-labeled detection antibodies specific to human IL-8 and IL-10 were added. After 4X washing, a streptavidin-HRP detection system was applied, followed by the addition of a chromogenic substrate that generated a colorimetric reaction. Optical density was measured at 450 nm using a microplate reader (Multiskan FC, Thermo Scientific, Waltham, MA, USA). The concentrations were calculated and expressed in pg/mL from standards curves of IL-8 and IL-10 for analyzed their association with the presence and severity of dental caries.

2.8. Ethical Considerations

This study was conducted in accordance with the ethical principles established in the Declaration of Helsinki and followed national and institutional regulations for research involving human participants in research protocols. Ethical approval was granted by the Research Ethics Committee of the Universidad Autónoma de San Luis Potosí (approval code: CEI-FE-079-022).

Prior to participation, written informed consent was obtained from the parents or legal guardians of all children included in the study. They were informed about the study's objectives, procedures, potential risks, and their right to withdraw at any time without consequences.

To ensure confidentiality, all data were anonymized and coded prior to analysis. No personal identifiers were collected or stored. Saliva samples were collected using non-invasive procedures and handled in accordance with established biosafety protocols for biological fluids. All research personnel were trained in ethical conduct and biosafety practices to minimize any risk to participants.

2.9. Statistical Analysis

Shapiro–Wilk and Brown–Forsythe tests were applied to assess the distribution of the variables. Quantitative variables were expressed as mean $\pm$ standard deviation and range (minimum–maximum), while categorical variables were presented as frequencies and percentages. Differences between groups were identified using the Chi-square test for categorical variables and the Mann–Whitney U test for non-parametric quantitative variables.

Additionally, Spearman correlation analysis was used to evaluate relationships between quantitative variables. A binary logistic regression analysis was performed, with the presence of cavitated carious lesions as the dependent variable (1 = presence; 0 = absence). Independent variables included IL-8, IL-10 levels, and waist-to-height ratio (WHtR).

To assess multicollinearity among the independent variables, the variance inflation factor (VIF) was applied. A specification test was also conducted to verify the assumption that the logit of the response variable is a linear combination of the independent variables.

After determining the main effects, interactions were tested; however, none were statistically significant. Model fit was evaluated using a goodness-of-fit test, and results are presented as odds ratios (OR) with 95% confidence intervals (CI). A p -value of <0.05 was considered statistically significant. Data analysis was performed using JMP software, version 15 (SAS Institute, Cary, NC, USA) [24].

3. Results

A total of 76 children participated in the study, including 36 without early childhood caries (control group) and 40 with early childhood caries (ECC group). Table 1 presents the demographic characteristics, ICDAS scores, and oral hygiene habits of the study groups. No statistically significant differences were observed between the groups in terms of age and sex ($p > 0.05$; Table 1). However, a comparison of the groups with respect to oral diagnosis revealed statistically significant differences in caries prevalence ($p = 0.0001$), with the highest frequencies corresponding to ICDAS scores 5 and 6 in the ECC group (Table 1).

Table 1. Characteristics, ICDAS codes and oral hygiene habits of the study groups.

Groups	Control Group ($n = 36$)	With Dental Caries ($n = 40$)	
Variables	Frequency (%)		p Value
Age distribution (years)			
3	5 (14)	5 (12)	0.2407
4	17 (47)	12 (30)	
5	14 (39)	23 (58)	
Gender			
Female	17 (47)	19 (48)	0.9807
Male	19 (53)	21 (52)	
Decayed teeth	0 (100)	40 (100)	0.0001 *
Missing teeth	0 (100)	0 (100)	
Filled teeth	0 (100)	0 (100)	
Caries (ICDAS codes)			
0	36 (100)	0	0.0001 *
1	0	1 (2)	
2	0	4 (10)	
3	0	6 (15)	
4	0	9 (23)	
5	0	10 (25)	
6	0	10 (25)	
ICDAS code groups			
0	36 (100)	0	0.0001 *
1–2	0	5 (12)	
3–4	0	15 (38)	
5–6	0	20 (50)	
Visible plaque presence	30 (83)	31 (78)	0.5221
Fluoride toothpaste use	29 (80)	35	

$n = 76$. Control Group: No early childhood caries; With dental caries group: Early childhood caries. Chi-square tests and Mann–Whitney U test. * Statistically significant difference at $p \leq 0.05$ value.

The comparison of salivary IL-8, IL-10, and the difference between both interleukins in children with caries and those in the control group is presented in Table 2. No statistically

significant differences were observed between groups for any of the variables analyzed ($p > 0.05$). However, a non-significant trend toward highest mean values of both IL-8 (85 pg/mL) and the IL-8/IL-10 difference (27 pg/mL) was noted in the ECC group, whereas IL-10 levels were lowest mean values (3 pg/mL) compared to the control group.

Table 2. Comparison of IL-8, IL-10 and difference between IL-8 and IL-10 in the saliva of children with caries and the control group.

Groups	Control Group ($n = 36$)	With Dental Caries ($n = 40$)	
Variables (pg/mL)	Mean (Rango) $\pm$ Standard Deviation		p Value
IL-8	45 (0–293) $\pm$ 74	85 (0–530) $\pm$ 119	0.3613
IL-10	11 (0.7–268) $\pm$ 44	3 (0.7–9) $\pm$ 1	0.6481
Difference between IL-8 and IL-10	17 (0–154) $\pm$ 33	27 (0–190) $\pm$ 41	0.1709

$n = 76$. Control Group: No early childhood caries; With dental caries group: Early childhood caries. Mann–Whitney U test.

Table 3 presents the association between IL-8, IL-10, and the difference between these interleukins across ICDAS scores in the study groups. No statistically significant differences were observed for any of the variables analyzed. However, descriptive data showed that the highest mean values for the IL-8/IL-10 difference were recorded in ICDAS grades 5–6 (35 pg/mL) and in cavitated carious lesions (29 pg/mL). No correlations were identified between caries frequency and IL-8 or IL-10 levels ($p > 0.05$). Similarly, binary logistic regression analysis revealed no significant association between interleukin levels, their difference, or the waist-to-height ratio and the presence of cavitated carious lesions.

Table 3. Association between IL-8, IL-10, and the interleukin difference with the ICDAS index in the study groups.

Variable	IL-8 (pg/mL)		IL-10 (pg/mL)		Difference Between IL-8 and IL-10	
ICDAS	Mean $\pm$ Standard Deviation	p Value	Mean $\pm$ Standard Deviation	p Value		p Value
0 ($n = 36$)	45 $\pm$ 74	0.6843	11 $\pm$ 44	0.4444	17 $\pm$ 33	0.3876
1–2 ($n = 5$)	39 $\pm$ 46		3 $\pm$ 1		13 $\pm$ 2	
3–4 ($n = 15$)	76 $\pm$ 142		3 $\pm$ 2		20 $\pm$ 31	
5–6 ($n = 20$)	103 $\pm$ 114		3 $\pm$ 2		35 $\pm$ 51	
Caries free or not cavitated carious lesions ($n = 41$)	44 $\pm$ 71	0.4928	10 $\pm$ 41	0.2530	17 $\pm$ 33	0.2530
Cavitated carious lesions ($n = 35$)	92 $\pm$ 125		3 $\pm$ 2		29 $\pm$ 44	

$n = 76$. ICDAS 0 = Caries free, ICDAS 1–2 = Not cavitated carious lesions, ICDAS 3–6 = Cavitated carious lesions. Mann–Whitney U test.

4. Discussion

ECC is a multifactorial disease that affects children between the ages of 3 and 5 and is characterized by the demineralization of dental tissues due to bacterial activity in the oral cavity [25]. The host's immune response, including the production of pro- and anti-inflammatory cytokines, plays a crucial role in the progression of this condition [3–5]. This cross-sectional study analyzed salivary levels of interleukin-8 (IL-8) and interleukin-10 (IL-10) in children with and without ECC to examine differences in pro- and anti-inflammatory interleukin levels.

The finding of statistically significant differences in caries distribution ($p = 0.0001$), particularly in ICDAS codes 5 and 6 within the ECC group, reflects a high prevalence of advanced cavitated carious lesions in this population. This suggests that, in the analyzed sample, caries progression was not detected or treated in its early stages, allowing it to evolve into more destructive clinical forms.

This severity pattern may be explained by several individual and contextual factors. Caries progression to advanced stages is often associated with poor oral hygiene [26], frequent intake of fermentable sugars [27], limited access to preventive dental services [28], and inadequate supervision of toothbrushing in young children [29]. Moreover, many parents or caregivers do not recognize the importance of primary dentition, leading to delays in seeking dental care and allowing lesions to progress [30]. From an immunological perspective, the high frequency of cavitated lesions may also be related to an imbalance in the local inflammatory response, as discussed in other sections of this study [31].

In our study, children with ECC exhibited higher mean salivary IL-8 levels (85 pg/mL) compared to controls (45 pg/mL), although the difference was not statistically significant ($p = 0.3613$). This trend is consistent with some previous studies, but contrasts with others that reported statistically significant elevations in IL-8 among children with caries. These discrepancies highlight the complexity of interpreting IL-8 as a biomarker and underscore the need to consider methodological and biological variability across studies.

For example, Biria et al. [10] and Sharma et al. [12] found significantly elevated IL-8 levels in caries-affected children, suggesting a strong inflammatory response. Sharma et al. also reported correlations with IL-6 and TNF- α , indicating a broader immune activation [12]. In contrast, studies such as those by Borsting et al., Rinderknecht et al., and Seyedmajidi et al. observed elevated or even reduced IL-8 levels without statistical significance [9,13,14]. These conflicting results may stem from differences in sample size, age range, saliva collection methods (stimulated vs. unstimulated), lesion severity, and cytokine quantification techniques.

Moreover, the inflammatory stage of the lesion may influence IL-8 expression. Acute lesions may trigger strong neutrophil recruitment, while chronic or advanced lesions could lead to immune exhaustion or dysregulation, potentially explaining the paradoxical reductions in IL-8 observed in some studies. This hypothesis is supported by findings from Seyedmajidi et al. [14] and Ramírez-De los Santos et al. [15], who reported lower IL-8 levels in ECC groups compared to controls.

A meta-analysis by Alarcón-Sánchez et al. reviewed 15 studies on salivary cytokines in caries, including seven on IL-8. Although five studies reported increased IL-8 levels, the overall effect was not statistically significant [32], reinforcing the variability observed in individual studies and aligning with our findings.

Importantly, our data suggest a non-significant trend toward increased IL-8 and decreased IL-10 levels with greater lesion severity, as indicated by ICDAS scores. Although not statistically significant, IL-8 levels were highest in ICDAS grades 5–6, suggesting a potential association between lesion progression and pro-inflammatory mediator activation [3]. This supports the notion that IL-8 may reflect ongoing tissue destruction and immune response, even in the absence of statistical significance.

In the same context, IL-10 is an anti-inflammatory cytokine that plays a key role in regulating immune responses and preventing excessive tissue damage [3,5]. In our study, children with ECC showed lowest mean salivary IL-10 levels (3 pg/mL) compared to controls (11 pg/mL), although the difference was not statistically significant. This trend aligns with most of the available literature, which generally reports decreased or unchanged IL-10 levels in individuals with caries.

For instance, Indrawati et al. and Mohammed IJ et al. both observed reduced IL-10 levels in children and young adults with caries, respectively [19,20]. Similarly, Pakdedemirli et al. found lower IL-10 levels in adults at high caries risk [33]. Although these differences were not always statistically significant, they suggest a consistent pattern of diminished anti-inflammatory activity in caries-affected individuals. In contrast, Gur et al. reported significantly elevated IL-10 levels in ECC children [17], highlighting the variability in findings across studies.

This inconsistency may be attributed to methodological differences, such as the type of saliva collected (stimulated vs. unstimulated), age and health status of participants, and cytokine detection techniques.

Another factor that may contribute to variability in salivary cytokine levels is central obesity, which was assessed in our study using the waist-to-height ratio (WHtR). Obesity is known to induce a systemic low-grade inflammatory state that may influence local immune responses in the oral cavity [34]. Although our logistic regression analysis found no significant association between WHtR and salivary levels of IL-8 or IL-10, previous research suggests that childhood obesity may alter baseline inflammatory status. A recent scoping review identified IL-6, IL-1 β and IL-12p70, among several salivary biomarkers affected by pediatric obesity [35], though findings remain inconsistent [15]. Given the overlap between systemic and oral inflammation, obesity may act as a confounding factor in cytokine-based biomarker studies. Therefore, while our data suggest that the impact of obesity on IL-8 and IL-10 levels in ECC may be limited or context-dependent, this variable should be carefully considered in future research to better understand its modulatory role in oral immune responses.

From an immunological perspective, reduced IL-10 levels may reflect an insufficient anti-inflammatory response, allowing persistent inflammation and tissue damage. This imbalance between pro- and anti-inflammatory mediators supports the view of ECC as a chronic inflammatory condition rather than a purely infectious disease.

To further explore this imbalance, we calculated the difference between IL-8 and IL-10 as an indicator of the inflammatory “range.” The ECC group showed a wider mean difference (27 pg/mL) compared to controls (17 pg/mL), suggesting a dominant pro-inflammatory profile in children with ECC. Although not statistically significant, this trend is consistent with studies reporting elevated IL-8 [9–13,32] and reduced or unchanged IL-10 levels [9,13,18–20,33], reinforcing the hypothesis of immune dysregulation in ECC.

This analysis is clinically relevant, as it may reflect a local inflammatory imbalance contributing to disease development. The onset of new carious lesions typically results from an imbalance between pathological factors—such as acidogenic bacteria, reduced salivary flow, and sugar-rich diets—and protective factors including fluoride, calcium, phosphate, and local immune activity. When this balance is disrupted, the risk of lesion formation increases, along with treatment costs, clinical burden, and the negative impact on children’s short- and long-term quality of life [36].

Understanding cytokine imbalance in children with caries represents a significant step toward elucidating the immunological component of this multifactorial disease. Traditional diagnostic methods—such as clinical examination, radiographs, and ICDAS scoring—identify the presence and severity of carious lesions. However, salivary cytokine profiling could serve as a complementary tool to detect subclinical inflammatory states associated with caries development and progression [7]. Such biomarkers may help identify high-risk children before visible lesions appear and support the evaluation of preventive intervention efficacy [37]. This opens the door to therapeutic strategies focused on modulating immune responses and restoring immunological balance. Examples include promoting diets rich in vitamins A, C, and D; zinc, iron, and omega-3 fatty acids—which help regulate

inflammation and support both systemic and oral immunity [38]; encouraging breastfeeding, which provides natural antibodies that protect the oral mucosa during infancy [39]; avoiding unnecessary antibiotic use, which can disrupt the oral and gut microbiome and perpetuate chronic inflammation [40]; and ensuring adequate sleep [41], regular physical activity [42], and stress management [43]. This immunobiological approach may enhance ECC diagnosis and follow-up, while reducing its clinical, economic, and emotional burden and improving overall child health [44].

The finding of elevated IL-8, an increased IL-8/IL-10 difference, and reduced IL-10 in children classified with ICDAS codes 5–6 suggests greater inflammatory response activation in advanced stages of caries. When ECC prevalence remains high despite widespread preventive strategies—including oral hygiene, fluoride use, parental education, and early dental care—this raises the possibility of an overlooked immunological component predisposing certain children to more aggressive disease progression [45].

This immunobiological perspective opens new therapeutic avenues beyond mechanical or chemical interventions. Clinical strategies aimed at restoring immune balance in children may include the use of probiotics [46], regulation of the oral microbiome [47], and reduction in systemic pro-inflammatory factors such as obesity [48]. Integrating the immunological component into ECC diagnosis and prevention represents an innovative step toward a more predictive, personalized, and biologically grounded model of dental care.

Strengths and Limitations

This pioneering study simultaneously assessed salivary pro-inflammatory (IL-8) and anti-inflammatory (IL-10) cytokines in relation to caries status in children aged 3–5 years, using ICDAS for precise lesion classification and a non-invasive saliva collection method [23]. However, despite meeting the calculated sample size, high interindividual cytokine variability, biological factors such as genetics, diet and microbiota may have influenced results, and these variables were not analyzed independently [9,49–51]. Although our study did not include microbiome analysis, the imbalance between elevated IL-8 and reduced IL-10 observed in our study may indicate shifts in the oral microbiota, as ECC is often linked to a more acidogenic profile with increased *Streptococcus mutans* and reduced commensals, contributing to sustained inflammation. These findings support the hypothesis that immune imbalance and microbial dysbiosis may act synergistically in ECC [52,53]. Additionally, the cross-sectional design prevents determining causality between cytokine levels and caries progression, underscoring the need for larger, longitudinal studies using advanced detection methods to clarify these relationships [54].

5. Conclusions

This study explored the immunoinflammatory profile in children with ECC by analyzing salivary levels of IL-8 and IL-10. Although no statistically significant differences were found, a non-significant trend was observed toward the highest mean values of the IL-8, and the lowest mean values of the IL-10 in children with ECC compared to controls. These findings do not confirm a difference but may suggest a potential imbalance between pro- and anti-inflammatory responses in the oral environment, in addition to bacterial factors, reflecting a complex interplay between immunological, microbial, behavioral, and nutritional determinants. The IL-8/IL-10 ratio could serve as a potential biomarker for inflammation in caries progression. In our sample, several children presented advanced cavitated lesions (ICDAS 5–6) and poor oral hygiene, indicating that they would clearly benefit from targeted preventive and restorative dental interventions. Further studies with larger sample sizes and standardized methodologies are needed to validate the clinical relevance of these biomarkers.

Author Contributions: Conceptualization, J.C.H.C. and N.P.-M.; methodology, J.C.H.C. and M.G.M.G.; software, J.R.C.H.; validation, N.P.-M., M.F.S.O. and J.R.C.H.; formal analysis, M.F.S.O.; investigation, J.C.H.C. and L.M.U.R.; resources, M.I.M.C.; data curation, M.F.S.O.; writing—original draft preparation, J.C.H.C. and M.F.S.O.; writing—review and editing, N.P.-M., M.G.M.G., and J.R.C.H.; visualization, L.M.U.R.; supervision, N.P.-M.; project administration, J.C.H.C.; funding acquisition, M.I.M.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Stomatology at the Autonomous University of San Luis Potosí, México (protocol code CEIFE-022-2023, approved on 1 March 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. For children under the age of legal consent, written informed consent was provided by their parents or legal guardians.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical restrictions related to patient confidentiality.

Acknowledgments: We wish to thank the Autonomous University of San Luis Potosí and the schools in the municipality of Rio Verde, San Luis Potosí, Mexico.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ECC	Early Childhood Caries
ICDAS	International Caries Detection and Assessment System
IL-8	Interleukin 8
IL-10	Interleukin 10

References

1. Márquez-Pérez, K.; Zúñiga-López, C.M.; Torres-Rosas, R.; Argueta-Figueroa, L. Prevalencia reportada de caries dental en niños y adolescentes mexicanos. *Rev. Med. Inst. Mex. Seguro Soc.* **2023**, *61*, 653–660. [[CrossRef](#)] [[PubMed](#)]
2. Zou, J.; Du, Q.; Ge, L.; Wang, J.; Wang, X.; Li, Y.; Song, G.; Zhao, W.; Chen, X.; Jiang, B.; et al. Expert consensus on early childhood caries management. *Int. J. Oral Sci.* **2022**, *14*, 35. [[CrossRef](#)]
3. Farges, J.-C.; Alliot-Licht, B.; Renard, E.; Ducret, M.; Gaudin, A.; Smith, A.J.; Cooper, P.R. Dental Pulp Defence and Repair Mechanisms in Dental Caries. *Mediat. Inflamm.* **2015**, *2015*, 230251. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
4. Veerayutthwilai, O.; Byers, M.R.; Pham, T.T.; Darveau, R.P.; Dale, B.A. Differential regulation of immune responses by odontoblasts. *Oral Microbiol. Immunol.* **2007**, *22*, 5–13. [[CrossRef](#)] [[PubMed](#)]
5. Horst, O.V.; Horst, J.A.; Samudrala, R.; Dale, B.A. Caries induced cytokine network in the odontoblast layer of human teeth. *BMC Immunol.* **2011**, *12*, 9. [[CrossRef](#)]
6. Elsalhy, M.; Azizieh, F.; Raghupathy, R. Cytokines as diagnostic markers of pulpal inflammation. *Int. Endod. J.* **2013**, *46*, 573–580. [[CrossRef](#)] [[PubMed](#)]
7. Gonçalves, L.L.; Kim, E.; Bortolatto, J.F.; Buzalaf, M.R.; Alreshaid, L.; Prakki, A. Insights on the role of cytokines in carious lesions. *Braz. Dent. Sci.* **2023**, *26*, e366. [[CrossRef](#)]
8. Liu, C.; Chu, D.; Kalantar-Zadeh, K.; George, J.; Young, H.A.; Liu, G. Cytokines: From Clinical Significance to Quantification. *Adv. Sci.* **2021**, *8*, e2004433. [[CrossRef](#)] [[PubMed](#)] [[PubMed Central](#)]
9. Rinderknecht, C.; Filippi, C.; Ritz, N.; Fritschi, N.; Simmen, U.; Filippi, A.; Diesch-Furlanetto, T. Associations between salivary cytokines and oral health, age, and sex in healthy children. *Sci. Rep.* **2022**, *12*, 15991. [[CrossRef](#)]
10. Biria, M.; Sattari, M.; Iranparvar, P.; Eftekhari, L. Relationship between the salivary concentrations of proteinase-3 and interleukin-8 and severe early childhood caries. *Dent. Med. Probl.* **2023**, *60*, 577–582. [[CrossRef](#)] [[PubMed](#)]
11. Gornowicz, A.; Bielawska, A.; Bielawski, K.; Grabowska, S.Z.; Wójcicka, A.; Zalewska, M.; Maciorkowska, E. Pro-inflammatory cytokines in saliva of adolescents with dental caries disease. *Ann. Agric. Environ. Med.* **2012**, *19*, 711–716.

12. Sharma, V.; Gupta, N.; Srivastava, N.; Rana, V.; Chandna, P.; Yadav, S.; Sharma, A. Diagnostic potential of inflammatory biomarkers in early childhood caries-A case control study. *Clin. Chim. Acta* **2017**, *471*, 158–163. [\[CrossRef\]](#)
13. Børsting, T.; Venkatraman, V.; Fagerhaug, T.N.; Skeie, M.S.; Stafne, S.N.; Feuerherm, A.J.; Sen, A. Systematic assessment of salivary inflammatory markers and dental caries in children: An exploratory study. *Acta Odontol. Scand.* **2022**, *80*, 338–345. [\[CrossRef\]](#)
14. Seyedmajidi, M.; Khodadadi, E.; Maliji, G.; Zaghian, M.; Bijani, A. Neutrophil count and level of interleukin-1 β and interleukin-8 in the saliva of three to five year olds with and without dental caries. *J. Dent.* **2015**, *12*, 662.
15. Ramirez-De los Santos, S.; López-Pulido, E.I.; Medrano-Gonzalez, I.d.C.; Becerra-Ruiz, J.S.; Alonso-Sanchez, C.C.; Vázquez-Jiménez, S.I.; Guerrero-Velázquez, C.; Guzmán-Flores, J.M. Alteration of cytokines in saliva of children with caries and obesity. *Odontology* **2021**, *109*, 11–17. [\[CrossRef\]](#)
16. Vignali, D.; Collison, L.; Workman, C. How regulatory T cells work. *Nat. Rev. Immunol.* **2008**, *8*, 523–532. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Gur, E.; Keles, S.; Cevik, O. Concentrations of interleukin-32, interleukin-10, interleukin-6, and TNF- α are higher in saliva of children with early childhood caries. *Pediatr. Dent. J.* **2023**, *33*, 116–124. [\[CrossRef\]](#)
18. Cogulu, D.; Onay, H.; Ozdemir, Y.; Aslan, G.I.; Ozkinay, F.; Kutukculer, N.; Eronat, C. Associations of interleukin (IL)-1 β , IL-1 receptor antagonist, and IL-10 with dental caries. *J. Oral Sci.* **2015**, *57*, 31–36. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Indrawati, R.; Luthfi, M.; Oki, A.S.; Sosiawan, A.; Rachmadi, P.; Rifai, M. Analysis of interleukin-10 anti-inflammatory cytokines in salivary lymphocyte surface: A cross sectional study. *J. Int. Oral Health* **2020**, *12*, 439–442.
20. Mohammed, I.J.; Sarhat, E.R.; Hamied, M.A.S.; Sarhat, T.R. Assessment of salivary interleukin (IL)-6, IL-10, oxidative stress, antioxidant status, pH, and flow rate in dental caries experience patients in Tikrit Province. *Sys. Rev. Pharm.* **2021**, *12*, 55–59.
21. Charan, J.; Biswas, T. How to calculate sample size for different study designs in medical research? *Indian J. Psychol. Med.* **2013**, *35*, 121–126. [\[CrossRef\]](#)
22. Feng, Q.; Bešević, J.; Conroy, M.; Omiyale, W.; Woodward, M.; Lacey, B.; Allen, N. Waist-to-height ratio and body fat percentage as risk factors for ischemic cardiovascular disease: A prospective cohort study from UK Biobank. *Am. J. Clin. Nutr.* **2024**, *119*, 1386–1396. [\[CrossRef\]](#)
23. Rai, A.; Sundas, S.; Dhakal, N.; Khapung, A. Assessment of dental caries based on ICDAS and WHO criteria: A comparative study. *Int. J. Paediatr. Dent.* **2024**, *34*, 77–84. [\[CrossRef\]](#)
24. Bagley, S.C.; White, H.; Golomb, B.A. Logistic regression in the medical literature: Standards for use and reporting, with particular attention to one medical domain. *J. Clin. Epidemiol.* **2001**, *54*, 979–985. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Kawashita, Y.; Kitamura, M.; Saito, T. Early childhood caries. *Int. J. Dent.* **2011**, *2011*, 725320. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Wong, H.M. Childhood caries management. *Int. J. Environ. Res. Public Health* **2022**, *19*, 8527. [\[CrossRef\]](#)
27. Pang, L.; Zhi, Q.; Jian, W.; Liu, Z.; Lin, H. The oral microbiome impacts the link between sugar consumption and caries: A preliminary study. *Nutrients* **2022**, *14*, 3693. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Ismail, A.; Sohn, W.; Lim, S.; Willem, J. Predictors of dental caries progression in primary teeth. *J. Dent. Res.* **2009**, *88*, 270–275. [\[CrossRef\]](#)
29. Curnow, M.; Pine, C.; Burnside, G.; Nicholson, J.; Chesters, R.; Huntington, E. A randomised controlled trial of the efficacy of supervised toothbrushing in high-caries-risk children. *Caries Res.* **2002**, *36*, 294–300. [\[CrossRef\]](#)
30. Andrew, L.; Wallace, R.; Wickens, N.; Patel, J. Early childhood caries, primary caregiver oral health knowledge and behaviours and associated sociological factors in Australia: A systematic scoping review. *BMC Oral Health* **2021**, *21*, 521. [\[CrossRef\]](#)
31. Zhang, R.; Hou, B. Immune responses of pulpodentinal complex during caries progression. *Zhonghua Kou Qiang Yi Xue Za Zhi Zhonghua Kouqiang Yixue Za Zhi Chin. J. Stomatol.* **2024**, *59*, 966–970.
32. Alarcón-Sánchez, M.A.; Becerra-Ruiz, J.S.; Avetisyan, A.; Heboyan, A. Activity and levels of TNF- α , IL-6 and IL-8 in saliva of children and young adults with dental caries: A systematic review and meta-analysis. *BMC Oral Health* **2024**, *24*, 816. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Pakdemirli, A.; Çalibaşı Kocal, G.; Kılınç, G.; Daşkın, E.; Kemaloğlu, H.; Başbınar, Y.; Ellidokuz, H. Salivary Pro-Inflammatory Cytokines and Salivary Bacterial Challenge Effect on Dental Caries: A Clinico-Molecular Cross-Sectional Study. *J. Basic Clin. Health Sci.* **2020**, *1*, 77–81.
34. Deng, Q.; Wong, H.M.; Peng, S. Alterations in salivary profile in individuals with dental caries and/or obesity: A systematic review and meta-analysis. *J. Dent.* **2024**, *151*, 105451. [\[CrossRef\]](#)
35. Sabella, F.M.; Katzenelson, R.T.; de Carvalho, F.G.; Duque, C.; Darrieux, M.; Marson, F.A.L.; Parisotto, T.M. Exploring Salivary Biomarkers in Pediatric Obesity: A Scoping Review. *Int. J. Mol. Sci.* **2025**, *26*, 5789. [\[CrossRef\]](#)
36. Nishide, R.; Mizutani, M.; Tanimura, S.; Kudo, N.; Nishii, T.; Hatashita, H. Homecare protective and risk factors for early childhood caries in Japan. *Environ. Health Prev. Med.* **2018**, *23*, 57. [\[PubMed\]](#)
37. Dongiovanni, P.; Meroni, M.; Casati, S.; Goldoni, R.; Thomaz, D.V.; Kehr, N.S.; Galimberti, D.; Del Fabbro, M.; Tartaglia, G.M. Salivary biomarkers: Novel noninvasive tools to diagnose chronic inflammation. *Int. J. Oral Sci.* **2023**, *15*, 27. [\[CrossRef\]](#)
38. Malin, A.J.; Wang, Z.; Khan, D.; McKune, S.L. The potential systemic role of diet in dental caries development and arrest: A narrative review. *Nutrients* **2024**, *16*, 1463. [\[CrossRef\]](#)

39. Córdova-Carrillo, K.; De la Peña-Lobato, C.; Cuevas-González, M.V.; Cuevas-González, J.C.; Espinosa-Cristóbal, L.F.; Tovar-Carrillo, K.L.; Saucedo-Acuña, R.A.; Zambrano-Galván, G.; Reyes-López, S.Y. Importance of Human Breast Milk in the Early Colonization of *Streptococcus mutans*. *Medicina* **2024**, *60*, 1308. [\[CrossRef\]](#)
40. Pandey, N.; Bodduluri, S.; Mathis, S.; Bodduluri, H.; Kumar, A. Role of Oral Microbiota in Preserving Health and Disease Management. *J. Clin. Immunol. Microbiol.* **2024**, *5*, 1–17. [\[CrossRef\]](#)
41. Tsai, H.; Tsai, T.; Wang, Y.; Chen, H.; Lee, C.; Tsai, S. Sleep and its association with dental caries or myopia in first graders. *Nurs Open*. **2024**, *11*, e2063. [\[PubMed\]](#)
42. Zhang, Q.; Bai, X.; Jin, H.; Dong, N. Combined effect of dietary calcium consumption and physical activity on dental caries in children and adolescents: A study of the NHANES database. *BMC Oral Health* **2024**, *24*, 281. [\[CrossRef\]](#)
43. Nakano, R.; Ohshima, T.; Mukai, Y.; Tsurumoto, A.; Maeda, N. Association between dental caries prevalence and stress levels in Japanese children. *Cureus* **2022**, *14*, e31074. [\[CrossRef\]](#)
44. Zaror, C.; Matamala-Santander, A.; Ferrer, M.; Rivera-Mendoza, F.; Espinoza-Espinoza, G.; Martínez-Zapata, M.J. Impact of early childhood caries on oral health-related quality of life: A systematic review and meta-analysis. *Int. J. Dent. Hyg.* **2022**, *20*, 120–135.
45. Davidovich, E.; Sarne, H.; Shmueli, A.; Polak, D. Is there an association between salivary immune and microbial profile with dental health in systematically healthy children? *Clin. Oral Investig.* **2024**, *28*, 564. [\[CrossRef\]](#)
46. Luo, S.C.; Wei, S.M.; Luo, X.T.; Yang, Q.Q.; Wong, K.H.; Cheung, P.C.; Zhang, B.B. How probiotics, prebiotics, synbiotics, and postbiotics prevent dental caries: An oral microbiota perspective. *NPJ Biofilms Microbiomes* **2024**, *10*, 14.
47. Grier, A.; Myers, J.; O'Connor, T.; Quivey, R.; Gill, S.; Kopycka-Kedzierawski, D. Oral microbiota composition predicts early childhood caries onset. *J. Dent. Res.* **2021**, *100*, 599–607. [\[CrossRef\]](#)
48. Hung, H.V.; Ngoc, V.T.N.; Vu Thi, H.; Chu, D.T. Early childhood caries in obese children: The status and associated factors in the suburban areas in Hanoi, Vietnam. *Int. J. Environ. Res. Public Health* **2021**, *18*, 8844. [\[PubMed\]](#)
49. Li, Y.; Oosting, M.; Deelen, P.; Ricaño-Ponce, I.; Smeekens, S.; Jaeger, M.; Matzaraki, V.; Swertz, M.A.; Xavier, R.J.; Franke, L.; et al. Inter-individual variability and genetic influences on cytokine responses to bacteria and fungi. *Nat. Med.* **2016**, *22*, 952–960. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Aziz, N.; Detels, R.; Quint, J.J.; Gjertson, D.; Ryner, T.; Butch, A.W. Biological variation of immunological blood biomarkers in healthy individuals and quality goals for biomarker tests. *BMC Immunol.* **2019**, *20*, 33. [\[CrossRef\]](#)
51. Alwarawrah, Y.; Kiernan, K.; MacIver, N.J. Changes in nutritional status impact immune cell metabolism and function. *Front. Immunol.* **2018**, *9*, 1055. [\[CrossRef\]](#)
52. Fakhruddin, K.S.; Samaranayake, L.P.; Egusa, H.; Ngo, H.C.; Pesee, S. Profuse diversity and acidogenicity of the candida-biome of deep carious lesions of Severe Early Childhood Caries (S-ECC). *J. Oral Microbiol.* **2021**, *13*, 1964277. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Astuti, E.S.Y.; Sukrama, I.D.M.; Mahendra, A.N. Innate immunity signatures of early childhood caries (ECC) and severe early childhood caries (S-ECC). *Biomed. Pharmacol. J.* **2019**, *12*, 1129–1134. [\[CrossRef\]](#)
54. Wang, X.; Cheng, Z. Cross-sectional studies: Strengths, weaknesses, and recommendations. *Chest* **2020**, *158*, S65–S71. [\[CrossRef\]](#) [\[PubMed\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.