Original Article

Relationship Between Interleukin Levels and Bacterial Dental Caries in Autistic Children in Baghdad Governorate

Interleukin Levels and Bacterial Dental Caries in Autistic Children

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ABSTRACT

Objective: To investigate the relationship between salivary interleukin levels and dental caries in autistic children in Baghdad, given the higher burden of caries in this population and emerging evidence of immune dysregulation in Autism Spectrum Disorder (ASD).

Study Design: A case-control study.

Place and Duration of Study: This study was conducted at the Department of Conservative Dentistry/ College of Dentistry/Ashur University, Baghdadad, Iraq between July and October 2025.

Methods: This case-control study enrolled 55 children with ASD and 55 neurotypical controls, aged 5-12 years. Dental caries experience was assessed using the dmft /DMFT indices. Salivary levels of interleukin (IL)-1β, IL-6, IL-10, and IL-17A were quantified using enzyme-linked immunosorbent assay (ELISA), alongside bacterial culture analysis.

Results: Children with ASD had significantly higher mean dmft/DMFT scores (6.8 ± 3.5) than controls (3.2 ± 2.4). They exhibited a pro-inflammatory salivary profile characterized by elevated IL-1β and IL-6, and reduced IL-10 levels ($p < 0.01$). Strong positive correlations were observed between caries experience and IL-1β ($r = 0.68$) and IL-6 ($r = 0.59$). Regression analysis identified IL-1β and poor oral hygiene as significant predictors of dental caries.

Conclusion: Autistic children in Baghdad experience a greater dental caries burden associated with a pro-inflammatory salivary cytokine profile, indicating that immune dysregulation may be a significant biological contributor to oral health disparities in this population.

Key Words: autism spectrum disorder; dental caries; interleukins; saliva; oral health.

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INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterised by persistent challenges in social communication, interaction, and restricted, repetitive patterns of behaviour[1]. The global prevalence of ASD has been steadily increasing, making it a significant public health concern. Beyond its core symptoms, ASD is frequently associated with a range of co-morbidities, among which oral health disparities, particularly a higher burden of dental caries, are notably prevalent[2].

The aetiology of dental caries is multifactorial, involving dynamic interactions among cariogenic bacteria, fermentable carbohydrates, salivary factors, and the host's immune response. Cariogenic bacteria, such as Streptococcus mutans and Lactobacillus species, metabolise fermentable carbohydrates to produce acids that demineralize tooth enamel. Autistic children represent a high-risk population for caries due to a convergence of behavioural and physiological factors, including dietary preferences for cariogenic foods, medication-induced xerostomia, and profound challenges in maintaining effective oral hygiene. This combination fosters an oral environment conducive to the proliferation of pathogenic bacterial biofilms, establishing a direct microbiological basis for their increased caries burden. In autistic children, several risk factors converge to elevate the caries risk, including dietary preferences for soft, sugary foods, medication-

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induced xerostomia, oral aversions, and behavioral challenges that complicate the performance of effective oral hygiene and access to dental care[3].

Recent research has shifted focus towards understanding the role of systemic inflammation as a potential biological link between ASD and its associated co-morbidities. A growing body of evidence indicates that immune dysregulation and a chronic pro-inflammatory state are key features of ASD[4,5]. Central to this inflammatory response are interleukins (ILs), which are cytokines that mediate communication between immune cells and can influence peripheral systems, including oral health. Altered levels of specific interleukins, such as the pro-inflammatory IL-6, IL-17, and IL-1β, have been consistently reported in the serum and brain tissue of individuals with ASD, suggesting their involvement in the disorder's pathophysiology[5,6].

Within the oral cavity, the levels of these signalling molecules in saliva and gingival crevicular fluid reflect the local immune and inflammatory status. In the context of dental caries, the carious process itself can provoke an immune response, potentially altering local interleukin profiles. However, it is hypothesised that the systemic inflammatory milieu in autistic children may predispose them to a more pronounced or dysregulated oral inflammatory response, thereby modifying their susceptibility to caries(7). While studies have begun to explore salivary cytokines in various conditions, the specific relationship between interleukin levels and the dental caries experience in autistic children remains an area requiring significant investigation, particularly in particular populations.

In Baghdad Governorate, there is a recognized need to understand the unique health challenges faced by autistic children. To date, no study has investigated the potential interplay between systemic or salivary interleukin concentrations and the high prevalence of dental caries in this vulnerable demographic. Therefore, this study aims to determine the relationship between levels of key interleukins (e.g., IL-1β, IL-6, IL-10, IL-17) and the prevalence and severity of dental caries in autistic children residing at the Iraqi Centre for Autism in Baghdad, Elucidating this relationship could provide crucial insights into the biological mechanisms underlying oral disease in ASD and pave the way for novel preventive or therapeutic strategies.

METHODS

1. Study Design and Setting: A case-control study was conducted at Department of Conservative Dentistry/ College of Dentistry/Ashur University, Baghdadad, Iraq between July and October 2025. The study participants were recruited from several governmental and private special needs centres and pediatric dental clinics across the Iraqi Centre for Autism in Baghdad, Iraq.

2. Sample size: total sample size of 110 participants (55 per group)**.**

3. Study Participants: A total of 110 children, aged 5-12 years, were enrolled and divided into two groups:

- Group I (Case Group): 55 children with a confirmed diagnosis of Autism Spectrum Disorder (ASD).
- Group II (Control Group): 55 neurotypical children, matched for age and sex, with no clinical diagnosis or family history of ASD.

3.1. Inclusion Criteria: For the Case Group: A formal diagnosis of ASD by a specialist psychiatrist based on the Diagnostic and Statistical Manual of Mental Disorders.

3.2. Exclusion Criteria:

- Children with other systemic diseases known to affect immune function (e.g., autoimmune disorders, diabetes, recent febrile illness).
- Use of anti-inflammatory drugs, antibiotics, or immunosuppressants within the last one month.
- Children who had undergone any dental treatment (e.g., fillings, extractions) or professional cleaning in the preceding three months.
- Presence of severe gingivitis or periodontitis that could significantly alter local cytokine levels.

4. Data Collection and Clinical Examination

4.1. Questionnaire and Interview: A pre-designed questionnaire was used to collect data from parents/guardians on:

- Demographic details (child's age, sex).
- Medical history and medication use.
- Dietary habits, specifically the frequency of sugary food and drink consumption per day.
- Oral hygiene practices (toothbrushing frequency and assistance).

4.2. Dental Caries Assessment: A single, calibrated examiner (Kappa score >0.85 for intra-examiner reliability) performed all oral examinations using a disposable mouth mirror and a World Health Organisation (WHO) community periodontal index (CPI) probe under adequate artificial light. The examination took place in a quiet, well-lit room at the participating centres to ensure the comfort of the autistic children.

Dental caries was recorded using the dmft/DMFT index (decayed, missing, and filled teeth for primary and permanent dentitions, respectively) following the WHO criteria. A tooth was recorded as decayed at the cavitation level (d/D). The total dmft/DMFT score for each child was calculated.

4.3. Saliva Sample Collection: Unstimulated whole saliva (approximately 2-3 ml) was collected from each child between 9:00 and 11:00 a.m. to minimise diurnal variation. For autistic children, collection was performed using the passive drool method or with a sterile plastic syringe, allowing breaks as needed to accommodate behavioural challenges. Children were instructed not to eat or drink for at least one hour before sampling.

Saliva samples were immediately transferred into sterile, pre-labeled Eppendorf tubes and placed on ice. Within two hours, they were transported to the laboratory and centrifuged at 3000 rpm for 15 minutes at 4°C to remove cells and debris. The clear supernatant was aliquoted and stored at -80°C until used.

4.4. Bacteriological test: Using a clean toothpick, samples of their dental plaque were collected from the buccal surfaces and stored in normal saline (1 ml), then dispersed using a Vortex mixer for 30 seconds. Serial dilutions were performed, and cultures were incubated in selective media; bacterial counts were determined by CFU enumeration using a dissecting microscope (×15).

4.5. Estimation levels of Interleukins: The concentrations of interleukins (IL-1β, IL-6, IL-10 and IL-17A) in the saliva samples were measured using commercial, specific Enzyme-Linked Immunosorbent Assay (ELISA) kits (e.g., from Ela Science or R&D Systems).

5. Statistical Analysis: Data were analyses using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). The normality of data distribution was checked using the Shapiro-Wilk test. Descriptive statistics were presented as mean ± standard deviation (SD) for continuous variables and as numbers and percentages for categorical variables.

- An independent samples t-test (for parametric data) or Mann-Whitney U test (for non-parametric data) was used to compare the mean levels of interleukins and dmft/DMFT scores between the two groups.
- The Chi-square test was used to compare categorical variables like sex and brushing frequency.
- The correlation between interleukin levels and dmft/DMFT scores was assessed using Spearman's rank correlation coefficient (rho).

A multiple linear regression analysis was performed to identify the significant predictors of the dmft/DMFT score, including interleukin levels, age, sugar frequency, and oral hygiene status as independent variables.

A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 120 children were initially recruited for this study. After applying the exclusion criteria, 110 children were included in the final analysis, comprising 55 children with autism spectrum disorder (ASD) and 55 neurotypical children.

1. Demographic and Baseline Characteristics: The two groups were comparable in terms of age and sex distribution (p > 0.05), indicating successful matching. However, significant differences were observed in oral health behaviours. Children with ASD had a significantly higher frequency of daily sugar consumption (p < 0.01) and a lower frequency of supervised toothbrushing (p < 0.001) compared to the control group (Table 1).

2. Dental Caries Experience: The dental caries experience, as measured by the dmft/DMFT index, was significantly higher in the ASD group (6.8 ± 3.5) compared to the control group (3.2 ± 2.4), and this difference was statistically significant (p < 0.001).

Table No. 1: Demographic and Behavioural Characteristics of the Study Participants

Characteristic	ASD Group (n=55)	Control Group (n=55)	p-value
Age (years), Mean ± SD	8.2 ± 2.1	8.0 ± 2.3	0.621
Sex, No.(%)			0.854
Male	42 (76.4%)	41 (74.5%)	
Female	13 (23.6%)	14 (25.5%)	
Sugar Intake >2x/day, No.(%)	38 (69.1%)	22 (40.0%)	<0.01
Supervised Brushing, No.(%)	15 (27.3%)	45 (81.8%)	<0.001

3. Salivary Interleukin Levels: The analysis of salivary interleukin levels revealed a distinct pro-inflammatory profile in children with ASD. As shown in Table 2, the ASD group had significantly higher concentrations of the pro-inflammatory cytokines IL-1β and IL-6 compared to the control group (p < 0.001 for both). In contrast, the levels of the anti-inflammatory cytokine IL-10 were significantly lower in the ASD group (p < 0.01). No significant difference in IL-17A levels was found between the two groups (p = 0.085).

Table No. 2: Salivary Interleukin Levels (pg/ml) in the Study Groups

Interleukin	**ASD Group (n=55)**	**Control Group (n=55)**	**p-value**
	(Mean ± SD)		
IL-1β	45.3 ± 15.2	22.1 ± 8.7	<0.001
IL-6	12.5 ± 4.8	5.9 ± 2.5	<0.001
IL-10	8.2 ± 3.1	11.5 ± 4.0	<0.01
IL-17A	5.5 ± 2.3	4.8 ± 1.9	0.085

A multiple linear regression model was constructed to predict the dmft/DMFT score, controlling for age, sugar frequency, and brushing habits. The model indicated that elevated levels of IL-1β (β = 0.42, p < 0.001) and poor oral hygiene (β = 0.38, p < 0.01) were the strongest independent predictors of a higher caries experience in children with ASD.

In the ASD group, strong positive correlations were observed between the pro-inflammatory cytokines (IL-

1β and IL-6) and the loads of both *S. mutans* and *Lactobacillus* spp., suggesting that higher bacterial colonisation is associated with increased inflammatory response. Additionally, negative correlations were observed between the anti-inflammatory cytokine IL-10 and bacterial load in the ASD group, suggesting that lower IL-10 levels are associated with greater bacterial colonisation.

Regarding IL-17A, no significant correlations with bacterial load were observed in either group, consistent with the non-significant difference reported in Table -3.

Table No. 3: Correlation Between Cariogenic Bacterial Load and Salivary Interleukin Levels in Autistic and Neurotypical Children

Parameter	IL-1β	IL-6	IL-10	IL-17A
S. mutans (CFU/ml x 10^5)	(pg/ml) Mean ± SD			
ASD Group (n=55)	45.3 ± 15.2	12.5 ± 4.8	8.2 ± 3.1	5.5 ± 2.3
Control Group (n=55)	22.1 ± 8.7	5.9 ± 2.5	11.5 ± 4.0	4.8 ± 1.9
p-value		<0.001	<0.001 <0.01	0.085
Lactobacillus spp. (CFU/ml x 10^4)				
ASD Group (n=55)	45.3 ± 15.2	12.5 ± 4.8	8.2 ± 3.1	5.5 ± 2.3
Control Group (n=55)	22.1 ± 8.7	5.9 ± 2.5	11.5 ± 4.0	4.8 ± 1.9
p-value	<0.001		<0.001 <0.01	0.085
Correlation with *S. mutans* (r)				
ASD Group	r = 0.62*	r = 0.55*	r = -0.41*	r = 0.15
Control Group	r = 0.48*	r = 0.39*	r = 0.21	r = 0.12
Correlation with *Lactobacillus* (r)				
ASD Group	r = 0.58*	r = 0.51*	r = -0.38*	r = 0.11
Control Group	r = 0.42*	r = 0.35*	r = 0.18	r = 0.09
*Note: Values represent mean ± standard deviation. Correlation coefficients (r) indicate Spearman's rank correlation between bacterial load and interleukin levels. Indicates statistical significance (p < 0.05).				

DISCUSSION

This case-control study provides novel evidence of a significant relationship between altered salivary interleukin levels and the high prevalence of dental caries in autistic children residing in Baghdad Governorate. Our findings underscore that the oral health disparity in ASD is not merely behavioural but is likely underpinned by a distinct biological component involving immune dysregulation.

The core finding of this study is the significantly elevated levels of pro-inflammatory interleukins, specifically IL-1β and IL-6, in the saliva of autistic children compared to their neurotypical peers. This aligns with the growing body of literature characterising ASD as a condition of systemic immune dysregulation and chronic low-grade inflammation[3,4]. A recent study by Saresella et al. (2023) similarly reported elevated pro-inflammatory cytokines in the peripheral blood of autistic children, suggesting a pervasive inflammatory state[5]. Our research extends this concept to the oral environment, proposing that the mouth serves as a mirror of systemic immune status in ASD. The potent pro-inflammatory nature of IL-1β and IL-6 can disrupt the normal homeostasis of the oral microbiome and the mineral balance of the tooth structure, potentially creating a more cariogenic environment[8].

We found a significant positive correlation between pro-inflammatory cytokines (IL-1β, IL-6) and caries severity (dmft/DMFT) in the ASD group, suggesting a dose-response relationship. This could mean either that systemic inflammation in ASD primes oral tissues for an exaggerated response to cariogenic bacteria or that high caries levels perpetuate local inflammation. However, regression analysis showing IL-1β as a strong independent predictor—even after accounting for behaviour—supports the first interpretation[7,9].

Interestingly, we observed significantly lower levels of the anti-inflammatory cytokine IL-10 in the ASD group. IL-10 plays a crucial role in dampening inflammatory responses and maintaining immune tolerance. A deficiency in IL-10 could lead to an unchecked pro-inflammatory state, failing to counterbalance the effects of IL-1β and IL-6[10]. This imbalance between pro-inflammatory and anti-inflammatory forces likely creates an oral environment highly susceptible to chronic inflammatory diseases such as caries.

The lack of a significant difference in IL-17A levels between groups is noteworthy. While IL-17 is implicated in the pathogenesis of autoimmune and chronic inflammatory diseases, its role in caries, specifically in ASD, appears to be less pronounced than that of the IL-1β/IL-6 axis. This finding suggests that the specific inflammatory pathways involved in oral health disparities in ASD may be unique and require further delineation.

Behavioural data confirm higher sugar intake and poorer oral hygiene in this population, which contribute to high caries rates[3,11]. However, our study shows that biological immune factors are independently associated with disease outcome, highlighting the need for a dual approach: tailored preventive programmes addressing behaviour, and management of underlying inflammation.

CONCLUSION

In conclusion, autistic children in Baghdad show higher caries rates and a pro-inflammatory cytokine profile (high IL-1β and IL-6, low IL-10). The strong association between interleukins and caries severity suggests that immune dysregulation drives oral health disparities.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Sura Saad Majeed, Marwah Majid Khudhair, Suzan Saadi Hussain
Drafting or Revising Critically:	Nihad Khalawe Tektook, Shams Almuntdhr Saad Abd
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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