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Oxidative Stress Levels and the Oral Health Status of Children with Down Syndrome at The Helena Health Center in Erbil City, Kurdistan Region, Iraq

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Abstract

Background and objectives: Down syndrome is the most frequently occurring hereditary reason for cognitive disability, arising from the duplication of either the whole or a section of chromosome 21. The study's goal was to evaluate the association between salivary oxidative stress indicators, including total antioxidant capacity, nitric oxide, and salic acid, and oral health parameters such as dental caries and periodontal disease in individuals with Down syndrome.

Method: This case-control study conducted at Helena Center, in Erbil City, Kurdistan Region, Iraq between June 1st 2023 and June 1st, 2024.The study included 37 persons with Down syndrome and 37 healthy-matched control volunteers aged 7 to 12 years. The study compared Oral health status (DMFT, Plague Index, and Gingival Index) among individuals with Down syndrome and healthy volunteers aged 7-12 years, using Plaque Index and Gingival Index. The unstimulated salivary samples were used for oxidative stress assessment.

Results: In children with Down syndrome, the value for (Decayed, Missing, Filled Teeth) for permanent teeth (0.75), for (Decayed, Filled Teeth) for deciduous teeth (0.94), plaque index (0.68), gingival index (0.88), Total antioxidant capacity (4.17), Nitric oxide (98.8), Sialic acid (1). whereas the values in the control group were DMFT (1.07), dft (1.27), plaque index (0.69), gingival index (0.56), Total antioxidant capacity (6.37), Nitric oxide (75.8), Sialic acid (0.72). No significant differences were seen in the two groups' regarding (Decayed, Missing, Filled Teeth) for permanent teeth, (Decayed, Filled Teeth) for deciduous teeth, plaque index and Nitric oxide. Down syndrome had significantly higher gingival index, while Total antioxidant capacity was significantly low and Sialic acid was significantly higher.

Conclusions: this study highlights differences in salivary biomarkers in children with Down syndrome, including lower total antioxidative capacity and higher sialic acid levels, which may impact oxidative stress and plaque formation.

Keywords: Down syndrome, Nitric oxide, Oral health status, Sialic acid, Total antioxidant capacity

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Introduction

Down syndrome, alternatively referred to as trisomy 21, manifests in approximately 1 in every 1000 to 1 in every 100 live births.[1] Down syndrome is commonly accompanied by a range of systemic symptoms, including as heart abnormalities, weakened immune systems resulting in repeated infections, and hypothyroidism. The oral cavity exhibits specific anomalies associated with Down syndrome, including dental agenesis, microdontia, macroglossia, and a constricted maxilla.[2,3] The maintenance of dental health is a crucial component of general well-being and has a substantial effect on a person's standard of living. Oral illnesses refer to a range of disorders comprising dental trauma, dental caries, periodontal disease, tooth loss, and oral cancer, Noma, and congenital abnormalities such cleft lip and palate.[4,5] Oxidative stress is the result of discrepancy between generation and aggregation of reactive oxygen species (ROS) in cells and tissues, and the biosystem's capacity to get rid of these reactive materials due to chemical reactions.[6] In addition, there is a worldwide documented decrease in the occurrence of dental cavities in persons with Down syndrome as compared to those without the disorder. This reduced prevalence may stem from factors such as delayed tooth eruption, alterations in saliva composition, and differences in anatomic structure of teeth, including less pronounced pits and fissures. Saliva serves the crucial function of protecting against dental caries.[7,8] Changes in the biochemistry of salivary secretion rate and composition within individuals with Down syndrome can impact the colonization of oral bacteria.[9] Studies suggest that dental caries development and progression may be partially influenced by imbalances between free radicals and saliva antioxidants.[10, 15] Down syndrome patients experience significant oxidative stress, with overexpressed genes linked to OS and neuronal death, potentially due to imbalanced free radical metabolism.[7,8] This study was undertaken to examine the total antioxidant capacity (TAC), nitric oxide (NO), and sialic acid (SA) in the saliva of children with Down syndrome, and to investigate the association between these parameters and their oral health condition.

Patients and methods

The case-control study was conducted at the Helena Center, in Erbil City, Kurdistan Region, Iraq between June 1st 2023 and June 1st, 2024. Following approval of the research protocol by the Ethical Committee of the Kurdistan Higher Council of Medical Specialties. Post-explanation of the study's goals, the parents of the participants provided informed consent. Exclusion criteria included children with medical issues, those on long-term drugs, and persons unable to cooperate sufficiently for saliva collection. A detailed record of case histories and clinical presentations was obtained through a questionnaire that included information such as age, gender, social economic status, systemic diseases, and medications for each child. Consequently, (Group I) Down syndrome group was covered 37 children, (Group II) the control group consisted of 37 normally developing, healthy children, all aged between 7 and 12 years, from poor socioeconomic backgrounds. Sample Size for Unmatched Case-Control Study: Two-sided confidence level (1-alpha) (95), Power (%chance of detecting) (80), Ratio of Controls to Cases (1), Hypothetical proportion of controls with exposure (78.3), Hypothetical proportion of cases with exposure (44.25), Least extreme Odds Ratio to be detected: (0.22), Therefore, the minimum Sample Size: 74 (Case:37, Control 37).[16] The intraoral examination of children with Down syndrome was conducted with the assistance of a parent to facilitate cooperation from the children.[17] The assessment was conducted under standardized settings

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utilizing disposable mouth mirrors, calibrated periodontal probes, and personal protective equipment including masks and gloves. Dental caries status was determined using the Decayed, Missing, and Filled Teeth (DMFT) index for permanent teeth and dft for primary teeth. Dental caries was evaluated and reported using the World Health Organization's (WHO) standards. [18] The examination adhered to a systematic methodology, commencing with the last upper right molar and advancing in a sequential way to the final lower right molar. Only substantial, clinically observable carious surfaces were documented, consistent with the WHO's description of dental decay as a "cavity with a softened dentin floor." Additionally, the Plaque Index (PI) by Silness and Loe and the Gingival Index (GI) by Loe and Silnesswere utilized to assess the periodontal condition of the research subjects.[19,20] The first variable examined during the clinical assessment was the Plaque Index (PI), recorded as the presence or absence of visible dental biofilm. Both PI and Gingivitis Index (GI) were assessed by examining four surfaces (buccal, lingual/palatal, mesial, and distal) of six index teeth (teeth 18, 23, 26, 38, 43, and 46). Each site was probed with a periodontal probe, with a 10-second observation period for gingival bleeding. Dental plaque was noted when visible or when there was soft material accumulation in the gingival pocket or along the tooth and gingival margins, aligning with scores of 2 and 3 on the PI scale. Gingivitis was confirmed by bleeding at any site, with scores of 2 and 3 on the GI scale. Both indices ranged from 0 (excellent) to 3 (poor), with the Gingival Index assessing inflammation and the Plaque Index measuring plaque accumulation. Composite scores for each participant were then derived by combining both indices.[11] To commence saliva collection, children washed their mouths with 15ml of diluted water to eliminate food particles and desquamated cells. Before the clinical examination, in the morning between 9:00 and 10:00 the patient provided about two milliliters of unstimulated whole saliva samples. The child assumed the "coachman" posture.[20] Sitting in a peaceful setting with their head slightly slanted downwards. Participants were directed to avoid from swallowing or suckling their tongue or lips while undergoing the collection procedure.[17,20] The child was instructed to empty the saliva into a sterile graded container with a wide enough aperture, as excess saliva was permitted to deposit on the bottom of the mouth. The salivary samples were promptly transported to the laboratory within 20 minutes, and stored in sterile Eppendorf tubes. Each sample underwent centrifugation for 10 minutes at 3000 rpm to remove cellular debris and was then stored at 8°C until analysis was conducted.[10,11,17,20, 22] Salivary Total Antioxidative Capacity was assessed using a commercial kit (Assay Kit Rel Diagnostics, Turkey). Measurements involved radical reduction of 2,2'-casino-bis (3-ethylbenzothiazoline-6-sulphonic acid). Absorbance was measured at 420 nm before and after adding Reagent 2, and TAC was determined using absorbance changes. The test was adjusted with Trolox.[11, 13, 20] The study uses the Griess reaction method to quantify the concentration of nitrates and nitrites in saliva, a reactive free radical gas. The enzyme nitrate reductase converts NO_3^- to NO_2^- in saliva, and NO_2 levels are measured using a colorimetric technique. The samples were distributed with sulfanilamide and naphthyl ethylene diamine solutions.[22] This study used the thiobarbituric acid assay to measure sialic acid levels in saliva samples. The sample was hydrolyzed in sulfuric acid, preserved with periodate reagent, and incubated with sodium arsenite. The intensity of the color was evaluated at 549 nm. The same protocol was used to

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determine free sialic acid levels, with protein concentration measured using Folin's phenol reagent and bovine serum albumin.[23] The acquired data were statistically analyzed using version 22 of the Statistical Package for the Social Sciences software. The normality of the data was evaluated using the Kolmogorov–Smirnov test. Chi-square and Fisher's exact tests were utilized for categorical data, whilst the independent sample t-test and Mann-Whitney test were employed for continuous variables. The correlation between various variables was assessed by means of the Pearson correlation test. A p-value of 0.05 or less was viewed as statistically significant.

Results

This study had 37 children with Down syndrome and 37 healthy youngsters serving as controls. No significant differences were detected between the two studies in terms of age and gender, Table (1).

Table (1): Demographic characteristics distribution by study groups

Variable	Study groups				*p-value
	Down syndrome		Controls		
	No.	%	No.	%	
Age					.16
<10 years	11	29.7	18	45.0	
≥10 years	26	70.3	22	55.0	
Gender					.56
Male	20	54.1	19	47.5	
Female	17	45.9	21	52.5	

*p ≤ 0.05 (significant)

There were no significant differences within the 2 groups in terms of DMFT dft or plaque index. The mean gingival index was significantly higher in children with Down syndrome compared to controls, Table (2).

Table (2): Distribution of oral health measures according to study groups.

Oral measures	Study groups		*p-value
	Down syndrome	Controls	
	Mean±SD	Mean±SD	
DMFT	.75±1	1.07±1.2	.24
dft	.94±1.07	1.27±1.28	.25
Plaque index	.68±.53	.69±.6	.9
Gingival index	.88±.67	.56±.49	.02

*p ≤ 0.05 (significant)

The mean salivary Total Antioxidative Capacity level was significantly lower in children with Down syndrome compared to controls. The mean salivary Sialic Acid level was significantly higher in children with Down syndrome compared to controls. There were no significant differences between the two research groups in terms of salivary Nitric Oxide levels, Table (3).

Table (3): Distribution of salivary antioxidants assessed based on study groups

Antioxidant measures	Study groups		*p-value
	Down syndrome	Controls	
	Mean±SD	Mean±SD	
TAC (µg/ml)	4.17±2.3	6.37±1.42	<.001
SA (mg/dl))	1±.33	.72±.27	<.001
NO (µm/L)	98.8±56.7	75.8±61.9	.09

*p ≤ 0.05 (significant)

No significant differences were observed between male and female DS children regarding DMFT, dft, plaque index, gingival index, TAC, SA and NO, Table (4).

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Table (4): Distribution of study measures in regard to gender of children with Down syndrome

Study measures	Gender		*p-value
	Male	Female	
	Mean±SD	Mean±SD	
DMFT	1.05±1.02	1.09±1.37	.8
dft	1.26±1.37	1.28±1.23	.49
Plaque index	.67±.63	.72±0.59	.8
Gingival index	.6±0.57	.53±0.43	.6
TAC (µg/ml)	6.08±1.09	6.6±1.6	.2
SA (mg/dl))	.67±.24	.77±0.29	.26
NO (µm/L)	71.5±55.2	79.7±68.5	.68

*p ≤ 0.05 (significant)

Discussion

The present study investigates salivary levels of Total Antioxidative Capacity (TAC), nitric oxide (NO), and sialic acid (SA) in children with Down syndrome (DS) to examine their impact on oral health, comparing these results with those of children without systemic conditions. Saliva was chosen as a diagnostic medium due to its non-invasive nature and relevance in disease diagnosis and prognosis, proving to be a valuable alternative to blood.[10-14] Research has demonstrated strong links between salivary components and dental health, suggesting saliva as a reliable indicator for dental conditions.[10, 11, 13, 14, 17, 20-22] The biomarkers TAC, SA, and NO were assessed in both DS and healthy children to detect oxidative stress, as these parameters indicate free radicals in saliva. In this study, no significant differences were found in DMFT (Decayed, Missing, and Filled Teeth) scores between the groups, consistent with previous findings showing that DMFT in DS children is often similar or slightly lower than that of typically developing children. [24-28] A recent meta-analysis by Nilchian et al. found that the mean DMFT in DS patients tends to be lower, though not statistically significant.[29] AlSarheed et al. also reported no significant difference in caries incidence between DS and non-DS children, suggesting DS may affect salivary gland function, altering electrolyte composition and potentially reducing caries risk.[27] Al Habashneh et al. similarly observed comparable caries levels in both groups, though adolescents with DS presented more dental abnormalities and poorer periodontal health than younger children of the same age and gender. [26, 29] The study found no significant differences in plaque index scores between DS and control groups, likely due to well-matched participants in terms of age, sex, socioeconomic status, and similar oral hygiene practices. However, the mean gingival index was notably higher in children with DS compared to controls, aligning with numerous previous studies reporting higher incidences of gingival diseases in DS populations. [20, 24, 26, 34-36] Contributing factors to this increased vulnerability include cognitive challenges, subgingival plaque composition, and distinct immune and inflammatory responses. [20, 30-36] Microbiological studies have also highlighted elevated levels of periodontopathic bacteria, such as *Porphyromonas gingivalis* and *Tannerella forsythia*, in individuals with DS, which—along with increased production of destructive enzymes like matrix metalloproteinases—facilitates periodontal breakdown.[34, 37] In this study, the mean salivary TAC levels were significantly lower in DS children compared to controls ($p<0.001$). Past research has noted a relationship between increased TAC levels and higher caries indices, although Subramaniam et al. found an inverse association between TAC levels and caries in DS children. [20, 38, 39] Additionally, sialic acid (SA), a key component of salivary glycoproteins that promotes bacterial

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aggregation and contributes to plaque formation, was significantly higher in DS children ($p<0.001$), consistent with findings by Subramaniam and Yarat et al. [20, 25] However, other studies by Siqueira and Nicolau and Anandan et al. found no significant difference in SA levels between DS and control groups.[23, 28] Nitric oxide (NO), a metabolite in saliva produced through dietary and metabolic processes, aids oral defense by inhibiting bacterial growth and enhancing immune responses. NO functions as an antibacterial agent, potentially suppressing caries-causing bacteria and enhancing macrophage-mediated cytotoxicity. [20, 40] NO in saliva may thus serve as a defense mechanism against cariogenic bacteria, partly through its conversion from nitrate to antimicrobial nitrogen oxides by nitrate-reducing bacteria.[43] Nonetheless, the present study found no significant difference in NO levels between DS and control groups ($p=0.09$), with research on NO's role in caries prevention yielding mixed results.[41, 42] Variations in saliva analysis across studies may stem from differences in collection methods, stimulant type, duration of stimulation, and saliva analysis techniques, as well as sample size and sampling approaches.[28] Saliva's physical and chemical properties can influence oral disease onset, and its ease of collection and antioxidant capacity assessment make it a potentially cost-effective tool for large-scale health evaluations.[44]

Conclusion

In conclusion, this study highlights significant differences in salivary biomarkers in children with Down syndrome (DS), particularly with lower Total Antioxidative Capacity (TAC) and higher sialic acid (SA) levels, which may influence oxidative stress and plaque formation. While no differences in DMFT and plaque indices were observed, DS children had higher gingival index scores, indicating a greater susceptibility to gingival issues. Future research should focus on antioxidant supplementation and other interventions to reduce oxidative damage. Longitudinal studies could refine clinical practices and improve preventive care strategies. Expanding studies to include adolescents and adults with DS may help identify age-related trends, while examining dietary and lifestyle factors could reveal modifiable influences. Integrating these studies with quality-of-life assessments would further deepen our understanding of the broader implications for this population, ultimately enhancing preventive care and quality of life for individuals with DS.

Disclosure

The authors assert that they have no conflicts of interest.

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