Egyptian Journal of Medical Microbiology Volume 34 / No.3 / July 2025 337-344 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Bacterial Distribution in Autistic and Non-Autistic Children with Dental Caries, in Thi-Qar Province Iraq

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Key words:
Autism, dental caries, Streptococcus mutans, bacterial distribution, oral microbiota

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ABSTRACT

Background: *Dental caries is a widespread oral disease, with autistic children potentially being at higher risk due to challenges in maintaining oral hygiene and specific dietary habits. Particularly in Iraq, the role of oral microbiota in autistic individuals remains largely unexplored. This study aimed to isolate bacterial pathogens of dental caries in three groups of children, with special emphasis on Streptococcus mutans and its role in dental caries diseases.* ***Methodology****: This cross-sectional study was conducted in Thi-Qar province, Iraq, including 170 children (70 autistics with dental caries, 70 non-autistic with dental caries, and 30 healthy controls). Dental plaque samples were collected and cultured on selective media. Bacterial isolates were identified using biochemical tests, API 20 Strep, and the VITEK 2 Compact system. Statistical analysis was performed using SPSS v.26.* ***Results****: Gram-positive bacteria were the most prevalent (77%), with Streptococcus spp. being the dominant genus (55.34%). Streptococcus mutans was significantly more common in autistic children compared to non-autistic children ($p < 0.05$). Urban residence was associated with a higher prevalence of dental caries in autistic children, while non-autistic children from rural areas exhibited more bacterial diversity.* ***Conclusion****: Autistic children exhibited a distinct bacterial distribution in dental caries, with S. mutans being the predominant pathogen. This highlights the need for targeted oral health interventions to reduce dental caries risk in this population.*

INTRODUCTION

Autism Spectrum Disorder (ASD) is a complicated neurodevelopmental disease with a frequency of roughly one in 54 children in the United States, according to the Centres for Disease Control and Prevention (CDC)[1]. Individuals with ASD frequently display sensory sensitivities and issues with oral hygiene habits, which can lead to the development of dental caries[2]. ASD is characterized by difficulties in social interaction, communication, and repetitive activities[3]. These individuals may have challenges with oral hygiene practices, eating habits, and sensory sensitivity, which could predispose them to greater incidence of dental caries[4] .

Dental caries, also known as tooth decay, is a complex illness impacted by food, oral hygiene, and microbiological factors. Dental caries is a significant dental health concern affecting children globally [5]. While dental caries is a concern for all children, evidence suggests that some populations, such as those with (ASD), may be at a higher risk [4]. Dental caries, a common oral illness that affects people all over the world, is caused by a complex interaction of microbial, nutritional, and host variables. Dental plaque, a microbial biofilm that adheres to tooth surfaces, plays an important role in its growth. Many bacterial species thrive in this matrix, with *Streptococcus mutans* emerging as a significant actor in caries pathogenesis [6].

Dental plaque (dental biofilm), a thin and sticky layer of microorganisms (with more than 1010 bacterial cells per milligram) embedded in a matrix composed mainly of insoluble polysaccharides and colonize the teeth surface, is a common etiological factor of dental caries and other oral diseases such as gingivitis and periodontal disease[7]. *Streptococcus* is a genus of Gram-positive bacteria in the Firmicutes phylum. The mouth cavity is known for having one of the highest levels of bacterial biodiversity in the human body[8]. The principal colonizers of microorganisms that can attach to the teeth surface and generate dental plaque are mostly Gram-positive cocci, Gram-positive rods, filaments, and a few Gram-negative cocci [9]. Species of Gram-positive cocci that were among the first to colonize include various *Streptococcus* species such as *S. mutans*, *S.oralis*, *S.sanguis*, and *S.sobrinus*, as well as other gram-positive rods such as *Lactobacillus spp*. [10, 11]. *S. mutans*, a Gram-positive bacterium, is thought to be the principal cause of dental caries[12, 13]. It's a significant contributor to the development of dental caries because of its capacity to ferment dietary carbohydrates (e.g., sucrose and glucose) and form acidic byproducts that erode tooth enamel[14].Several factors contribute to its

pathogenicity, including its capacity to cling to tooth surfaces, create biofilms, and produce acids that demineralize tooth enamel [15]. The role of bacterial communities in autistic individuals has not been extensively studied, particularly in Iraq[16]. Understanding the bacterial composition in dental caries among autistic and non-autistic children can help in developing preventive strategies.

The aim of this study

Isolate and identify bacterial causes of dental caries in three groups of children (autistic, non-autistic, and healthy children) by traditional methods (Gram stain, culture, biochemical tests, Api 20 strep, and Vitk2 compact system), with a particular focus on *Streptococcus mutans* and its contribution to caries pathogenesis.

METHODOLOGY

Study Design and Population

This study design is a cross-sectional study that included 170 children (70 autistics with dental caries, 70 non-autistic with dental caries, and 30 without dental caries), aged 3–11 years. Sample Collection: Dental caries swabs were collected from Thi-Qar Rehabilitation Centre for Autism and local dental clinics located Thi-Qar province, Iraq.

Ethical approval:

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki. The committee of researchers at the Thi-Qar Health Directorate (No. 2023/166 on 8/8/2023) has viewed and approved this study. The person's informed consent was obtained

Isolation and Identification of Bacteria

This was done by: Culture Media (Blood agar, Mitis Salivarius Bacitracin Agar), Biochemical Tests (Catalase, oxidase, and sugar fermentation tests), Advanced Identifications (API 20 Strep and VITEK 2 Compact system) All the bacteria were subjected to morphotyping, and the suspected colonies were identified by biochemical tests. Api20 and vitek2 compact.

Statistical Analysis

Data were analysed using SPSS v.26

RESULTS

This study identified 468 bacterial isolates in the dental caries using the bacteriological culture, 362 isolates (77%) of these isolates were Gram-positive bacteria, while 106 isolates (23%) were Gram-negative bacteria, as shown in figure (1).

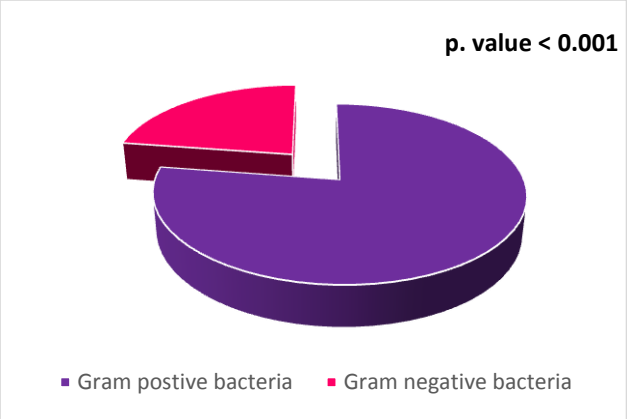

Fig. 1: Distribution of bacterial species according to Gram stain.

As shown in table 1; the presence of *Streptococcus* spp. (55.34%) was the most common genus, with *S. mutans*, followed by *Staphylococcus* spp. at 60 isolates (12.82%), *Lactobacillus* spp. 40 isolates (8.55%), *Gemella morbillorum* 30 isolates (6.41%), and *Enterococcus faecalis* 27 isolates (0.64%).

Three species of *Staphylococcus* spp. were isolated; *S. epidermidis* was 25 isolates (41.66%), *S.aureus* 22 isolates (36.67%), and *S.homins* 13 isolates (21.67%).As for the bacteria *Lactobacillus acidophilus*, which belongs to the genus Lactobacillus spp. 31 isolates (77.5%), superior to *Lactobacillus casei* bacteria of the same genus, 9 isolates (22.5 %).The Gram-negative bacteria were also seen in the dental caries isolates. The species and percentage of these bacteria were as follows *Neisseria* Spp. 24 isolates (11.75%), *K. pneumoniae*,3.85 %, *E. coli* 3.42%, *P. aeruginosa* 2.35%, *Proteus* spp. 1.50%, and *Enterobacter* spp. 0.64% as shown in table (1).

Table 1: Number and percentage of Gram positive and Gram-negative bacteria that isolated from dental caries of the study groups.

	Characteristics	No.	%	Autisticn		Non-autistic children		Control		Total		P.value
				No.	%	No.	%	No.	%	No.	%	
	Streptococcus Spp											
Gram positive Bacteria	*Streptococcus mutans*	259	55.34	31	44.29	32	45.71	0	0.00	63	24.33	0.833
	Streptococcus sobrinus			15	21.43	20	28.57	6	20.00	41	15.83	0.352
	Streptococcus salivarius			19	27.14	19	27.14	0	0.00	38	14.67	1.00
	Streptococcus parasanguinis			8	11.43	14	20.00	0	0.00	22	8.49	0.106
	Streptococcus sanguinis			5	7.14	9	12.86	2	6.67	16	6.18	0.264
	Streptococcus mitis			16	22.86	9	12.86	0	0.00	25	9.65	0.096
	Streptococcus oralis			16	22.86	6	8.57	0	0.00	22	8.49	0.013
	Streptococcus pyogenes			3	4.29	0	0.00	2	6.67	5	1.93	0.366
	Enterococcus faecalis			11	15.71	15	21.43	1	3.33	27	10.43	0.002
	Total			124	26.50	124	26.50	11	2.35	259	100	
	Staphylococcus Spp											
	Staphylococcus aureus	60	12.82	10	14.29	11	15.71	1	3.33	22	36.67	0.012
	Staphylococcus homins			9	12.86	4	5.71	0	0.00	13	21.67	0.108
	Staphylococcus epidermidis			13	18.57	6	8.57	6	20.00	25	41.66	0.099
	Total			32	6.84	21	4.49	7	1.50	60	100	
	Lactobacillus Spp											
	Lactobacillus acidophilus	40	8.55	15	21.43	16	22.86	0	0.00	31	77.5	0.763
	Lactobacillus casei			5	7.17	4	5.71	0	0.0	9	22.5	0.782
	Total			20	4.27	20	4.27	0	0.00	40	100	
	Gemella morbillorum											
	Gemella morbillorum	30	6.41	5	7.14	15	21.43	10	33.33	30	100	< 0.001
	Total			5	1.07	15	3.21	10	2.14	30	100	
Gram negative Bacteria	Gram negative Bacteria											
	Enterobacter Spp	3	0.64	1	1.43	2	2.86	0	0.00	3	100	0.317
	Proteus Spp	7	1.50	1	1.43	6	8.57	0	0.00	7	100	0.011
	Klebsiella pneumoniae	18	3.85	7	10.00	8	11.43	3	10.00	18	100	0.968
	Pseudomonas aeruginosa	11	2.35	1	1.43	10	14.29	0	0.00	11	100	0.001
	Escherichia coli	16	3.42	4	5.71	10	14.29	2	6.67	16	100	0.121
	Neisseria Spp	55	11.75	14	2.99	36	7.69	5	1.07	55	100	0.109
	Total	*468*		28	5.98	72	15.38	10	2.14	110	23.50	
	Overall Total			209	44.66	222	47.44	37	7.91	468	100	< 0.001

The results of the present study showed the distribution of *Streptococcus* species compared to other bacterial species in all studies groups.In autistic children, *Streptococcus* species accounted for 54.07%(124) of the bacterial population, while other bacterial species constituted 45.93%(85). Among non-autistic children with dental caries, *Streptococcus* species accounted for 49.10%(98) of the bacterial population, while other bacterial species constituted 50.90%. While in the control group without dental caries, *Streptococcus* species were detected only in 27.03%(11) of the bacterial isolates, while other bacterial species constituted 72.97%(26), (figure 2).

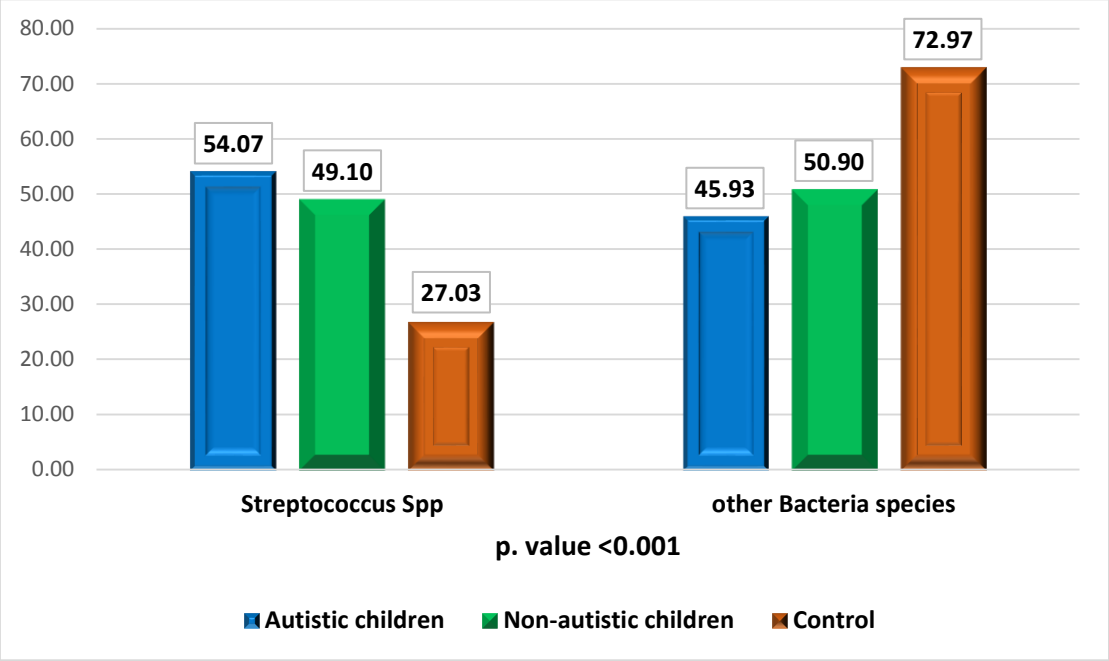

Fig. 2: Distribution of *Streptococcus Spp.* compared with other bacteria species between study group.

Urban residence was associated with higher caries prevalence in autistic children, while non-autistic children from rural areas showed increased bacterial diversity as shown in figure (3). Among autistic children, the majority reside in urban areas, constituting 85.71% of the group, while only 14.29% reside in rural areas.

In non-autistic children, there was a higher proportion residing in rural areas (58.57%)compared to urban areas (41.43%). The distribution according to residence, showed high significant differences at $p<0.001$.

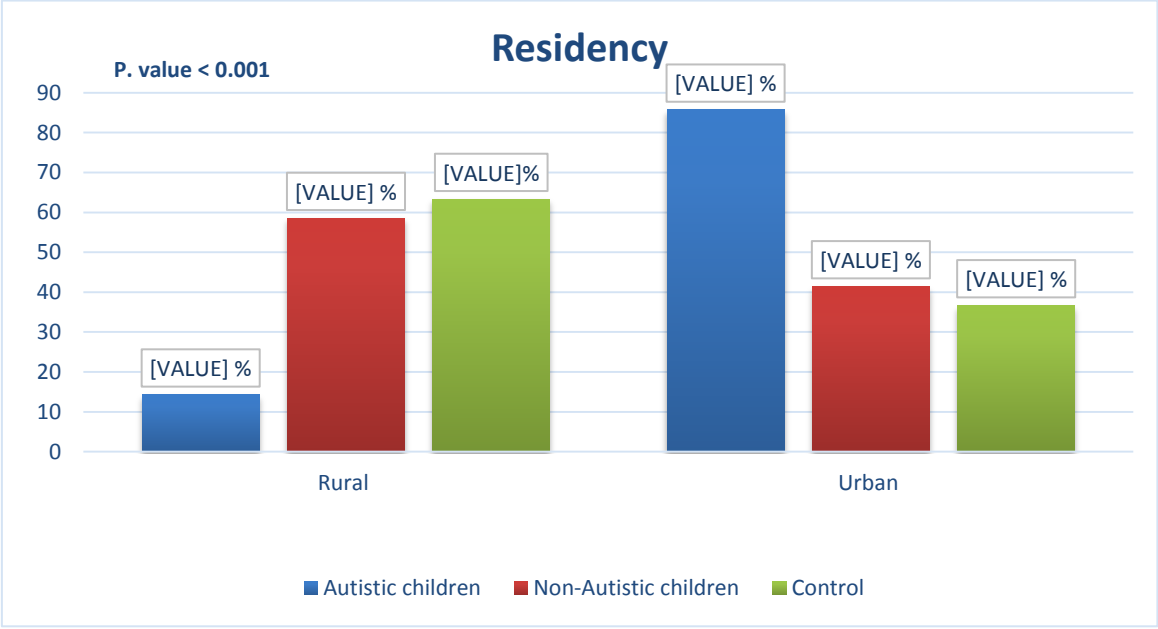

Fig. 3: Distribution of the study groups according to the residence

DISCUSSION

The composition of bacteria isolated from tooth decay and dental plaque can provide valuable insights into the microbial etiology of oral diseases. Gram-positive and Gram-negative bacteria are two major categories of microorganisms commonly found in the oral cavity, each with distinct characteristics and potential roles in the development of dental caries.

The results of the current study, showed the dominance of Gram-positive bacteria with higher percentages in tooth decay and dental plaque by (77%) compared to Gram-negative bacteria at (23%) in the study groups. These results indicate that Gram-positive bacteria are the main factor contributing to dental caries. It was noted that total *Streptococcus* spp. had the highest percentage (55.34%), compare to other bacterial species in all study groups. Isolating *Streptococcus* species. from dental caries provides valuable insights into the microbial ecology of oral biofilms associated with caries development. It was observed that most of the streptococcal bacteria isolated were 54.07% in autistic children, and 49.1% in non-autistic children, compared with other bacterial species.

The results of the present study agree with the results of study conducted by Qiao *et al*[17] that showed alterations of oral microbiota distinguish children with autism spectrum disorders from healthy children and showed that *Streptococcus* spp. in plaques showed significantly higher abundance in ASD patients[17.] *S. mutans* had the highest percentage compared to other species of streptococci isolated from dental caries, recording the highest percentage (27.16%). The prevalence of *S. mutans*, a common cariogenic bacterium, is similar between autistic children (44.29%) and non-autistic children (45.71%). However, it's notably absent in the control group, suggesting its association with dental caries. The study conducted by Tulumbacı *et al*. was similar to the current results, There was no statistically significant difference in the *S. mutans* between the autistic and non- autistic groups[18].

As the results showed, 0.0% of the control group had isolates of *S. mutans*. own finding is consistent with the results of Al-Hamadani's study in Diwaniyah city, where *S. mutans* was also not isolated from the control group[١٩]. Also Teba and Osama[20] in Samarra city show that *S.mutans* constitutes about 33% of the rest of the bacterial species that cause dental caries[20] . Another study was carried out to detect the distribution of *S. mutans* with dental caries in Iraqi's patient samples. Out of 100 patient's samples only (22) with age (3-17) years old were found to be affected with *S. mutans*[21.]

S. mutans, in particular, is considered a primary etiological agent of dental caries due to its ability to produce acids from dietary sugars and its capacity to adhere to tooth surfaces and form biofilms. These acidogenic and aciduric properties make *S. mutans* especially adept at initiating and exacerbating enamel demineralization[22].

In addition to *S. mutans*, various other *Streptococcus* species have been isolated from dental caries, including *S. sobrinus* which shows a slight difference in prevalence between autistic children (21.43%) and non-autistic children (28.57%). Interestingly, its prevalence in the control group (20.00%) suggests a potential role in dental caries development regardless of autism status.

There's no significant difference in the prevalence of *S. salivarius* between autistic children (27.14%) and non-autistic children (27.14%). However, it's absent in the control group, indicating its potential association with dental caries. *S. parasanguinis*, *S. sanguinis*, *S. mitis*, and *S. oralis*; These *Streptococcus* species show varying prevalence rates across groups, but none exhibit statistically significant differences between autistic and non-autistic children with dental caries. However, *S. oralis* is significantly more prevalent in autistic children compared to non-autistic children.

S. pyogenes shows minimal prevalence across groups, with no significant difference between autistic and non-autistic children with dental caries. Its presence in the control group suggests a potential role in oral health beyond dental caries. The distribution of *Streptococcus* species. varies among autistic and non-autistic children. While some species show similar prevalence rates across groups, others exhibit differences that may indicate their role in dental caries development. Notably, *S. oralis* stands out as more prevalent in autistic children, this suggests a potential association between *S. oralis* and autism.

These percentages are consistent with Al-hamadani AH's study, where there was a percentage the *S. mutans* comprised the high percentage (40.47%) in comparison with the other *streptococcus* species. (*S.sangius, S. mitis, S.oralis, S.salivarius, S.pyogenes, S.sobrinus*)[19, 23]. This explains that other bacteria, such as various species of *Streptococcus*, have also been implicated in the development of dental caries, either directly through acid production or indirectly by modifying the oral environment to favour the growth of acidogenic bacteria[24].

Other Gram-positive bacteria commonly isolated from dental caries include species of *Staphylococcus* at 12.82%, *Lactobacillus8*.55%, and certain strains of *Gemella* at 6.41% and *Enterococcus* at 0.64%. The results of the present study agreed with the results of studies conducted in the city of Diwaniyah by Wafaa *et al* and Alkhafaf *et al*.[٢٧-٢٥] These bacteria may also contribute to caries progression through their metabolic activities and interactions within the plaque biofilm. The *Staphylococcus* spp., including *Staphylococcus epidermidis*, gave the highest percentage (41.66%)

when isolated from caries areas, compared to *Staphylococcus aureus* (36.67%), while *S. homins* had the lowest (21.67%).The results of the present study agree with the previous study[٢٥].

The appearance of *Staphylococcus* bacteria, especially *Staphylococcus aureus*, in the vicinity of the mouth may be due to it being one of the important pathogens with the ability to cause opportunistic infections due to its natural presence on the bodies of carriers, on the skin, and on the upper part of the mouth. Nose, digestive, and reproductive tracts[28],Also because it possesses many surface antigens and enzymes that help it penetrate the body's tissues[29].

As for the bacteria *L.acidophilus*, which belongs to the genus Lactobacillus spp. Its percentage reached 77.5%, superior to *L. casei* bacteria which of the same genus, which amounted to 22.5% were consistent with other results [30, 31] in the isolation ratio. While *S. mutans* is important in the initiation of caries, *Lactobacillus* species, contribute to the advancement of infection. They thrive in the acidic environment generated by *S. mutans*, contributing to additional enamel demineralization [32].

Gram-negative bacteria are less prevalent in dental plaque compared to Gram-positive bacteria but are still present and may play roles in oral disease pathogenesis .Which agreed with others studies [26]. Enterobacteriaceae family (Gram-negative) is due to their scarcity in the vicinity of the mouth, and this is confirmed by others studies [33, 34] that most Gram-negative bacteria come from respiratory system infections or from the digestive duct and appears in the mouth. While the result of Gram-negative bacteria is not typically considered primary agents of dental caries, their interactions with Gram-positive species and their potential to modulate the oral environment may indirectly affect caries development and progression. For example, certain Gram-negative bacteria can produce proteolytic enzymes and endotoxins that contribute to tissue damage and inflammation, which may influence the host response to cariogenic bacteria.

The influence of geographic location on bacterial distribution revealed that urban residence was associated with a higher prevalence of dental caries in autistic children, whereas non-autistic children from rural areas exhibited greater bacterial diversity. Disparities in dental caries prevalence between rural and urban areas are apparent in many regions, including Iraq. Urban areas typically have better access to dental services, education, and resources, which may result in lower prevalence rates compared to rural areas where access to dental care is limited. The children with non-autistic, they are more in rural areas. This is consistent with a study done by Al-Dabbagh et al [35]. They reported a higher prevalence in rural areas (70.4%) compared to urban areas (58.2%).

In rural areas, limited access to fluoridated water, poor oral hygiene practices, inadequate nutrition, and a lack of awareness about oral health contribute to higher rates of dental caries among children. A study done by Al-Huda *et al* [36] explored factors contributing to dental caries in rural Iraqi children. They identified poor oral hygiene practices, low fluoride exposure, and high sugar consumption as significant risk factors. Overall, the findings underscore the importance of targeted oral health interventions for autistic children, considering their distinct bacterial profiles and increased susceptibility to dental caries.

Recommendations

Future research should explore the underlying mechanisms driving these differences, including host immune responses, genetic predispositions, and behavioral factors, to develop more effective prevention and treatment strategies.

CONCLUSIONS

Autistic children in Thi-Qar provinces exhibited a distinct bacterial profile in dental caries, with S. mutans being more prevalent. Improved oral health awareness and targeted interventions are needed to reduce the risk of dental caries in this population.

Author contributions

All authors had seen and approved the submission of the manuscript with full responsibility, and this research had not been published or under consideration by any other journal.

Conflict of Interest:

The authors declare that they have no conflict of interest.

Financial disclosure

The authors deny receiving any financial support or grant from any organization

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