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# **Original research**

# Oral health experiences of Turkish children with acute rheumatic fever or rheumatic heart disease

#### Purpose

Children with acute rheumatic fever (ARF) or using depot-penicillin because of rheumatic heart disease (RHD) are prone to the risk of infective endocarditis (IE) and poor oral hygiene. This cross-sectional study aimed to investigate oral health experiences of a group of healthy children and a group of children with ARF or who were using depot-penicillin because of RHD (study group).

#### **Materials and Methods**

Medical and dental data of 86 children aged between 5-12 years were investigated in this study. Medical histories, decayed, missing, and filled teeth, plaque index, gingival index, toothbrushing frequencies, and the study and healthy groups' socioeconomic levels were recruited and examined.

#### Results

The 'dmft' of the study and healthy groups were found to be  $5.51\pm3.81$  and  $2.37\pm2.31$ , respectively, while the 'DMFT' of the study and healthy groups were  $1.71\pm2.28$  and  $1.06\pm1.59$ , respectively. There was no significant difference between the gingival indexes of the study group  $0.89\pm0.39$  and the healthy group  $0.62\pm1.03$  (p=0.112). Nevertheless, the groups significantly differed regarding the plaque index, which were  $0.87\pm0.40$  and  $0.45\pm0.41$ , respectively (p<0.001). The tooth brushing frequencies in the study and healthy groups being twice a day or more were 23.3% and 46.5%, respectively.

#### Conclusion

The children with ARF or using depot-penicillin because of RHD had more permanent and primary tooth caries and poorer oral health than the healthy group in this study.

**Keywords:** Acute rheumatic fever, dental caries, cardiac disease, rheumatic heart disease, oral health

# Introduction

The acute rheumatic fever (ARF) is an inflammatory disorder mainly involving the heart and joints, but rarely the central nervous system, skin, and subcutaneous tissues as a late-stage finding, due to Group A beta-hemolytic streptococcal (GABHS) infections (*Streptococcus Pyogenes (SP)*) during childhood (1). This disease manifests the peak ratio in school-age children aged 5-15 years (2). While it has nearly evanesced in the affluent countries (North America and Western Europe), it is still a significant problem in developing countries (2,3). Although there has been no factual statistical data about this disease in Turkey, it has been suggested to be seen with a frequency similar to the Middle Eastern and Mediterranean countries (25-100 per 100.000) (4,5). The status of both ARF and rheumatic heart disease (RHD) have changed due to improved living conditions, good nutrition, advanced medical nursing, penicillin, oral and medical hygiene (2,3).

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ARF diagnosis is made with either two major criteria or one major and two minor criteria (1). The 2015 revision of the Jones Criteria for ARF indicates only two groups regarding this illness: moderate-high and low-risk groups (6). The critical symptom of ARF (60%) with long-term sequelae is carditis (1,2). While RHD may be seen after known ARF, the disease might be formerly asymptomatic, or patients may not recall ARF history (7). The minor complications of ARF are arthralgia, fever, first-degree heart block, and the high ratio of acute phase reactants (1).

If ARF patients do not have intramuscular injections of benzathine penicillin G (BPG) every 21 days, they will likely have more cardiac problems, heart failure risk, palsy, and early demise (3,7). The prophylaxis may avoid an initial ARF attack following a sore throat (Table 1) (3). The American Heart Association has suggested that children with a complaint of ARF or RHD should receive secondary penicillin prophylaxis (SPP) as either oral diurnal penicillin (penicillin V) or BPG every 21 or 28 days (3). Patients might have infective endocarditis (IE) in the presence of three major components: a cardiac lesion and endocardial harm, significant mucosal lesions responsive to bacteremia and the microbial inoculant capacity, and the bacterial factor's virulence. IE might be diagnosed and treated early; however, the mortality rate varies between 10-15%. IE, which has complicated relationships with invasive dental therapies and poor oral health, occurs in ARF patients' mitral or aortic valves since they are most frequently disrupted (7). Thorney et al. (8) claimed that dental caries is associated with sugar, and ARF is fed by sugar. Thus, if the patient has untreated caries, ARF's prognosis might worsen. Untreated dental caries has adverse impacts on children's well-being and public health status, and may create pain, abscess, and fever, together with chewing, growing, and sleeping problems.

Oral health is a crucial part of public health, with a two-sided correlation (9). The oral cavity is the most prevalent origin for bacteremia due to periodontal pockets, dental pulp, and periradicular bone. Tooth brushing, flossing, chewing, and breathing are the daily activities for spreading bacteremia (7). Administration of drugs with sucrose is a risk factor for caries and periodontal diseases in children (10). A dental therapy, such as performing an extraction, periodontal surgery, scaling, and root planning, are risk factors for bactere-

Table 1 Methods for prevention from streptococcal infections in acute rheumatic fever (3)

mia (7). Ultimately, a team involving a pediatric cardiologist and a pediatric dentist should collaborate for their patients' oral health (11). If the cardiac patient is in the high-risk group for IE, a pediatric cardiologist may recommend dental therapy for the patient (Table 2) (7).

If an invasive dental procedure is indicated, patients under IE risk should have proper oral health. In the literature, children with congenital heart disease (CHD) have been more susceptible to caries lesions when compared to healthy control groups (10,12). To our best knowledge, only a few studies have assessed the impacts of interference with oral health in children with ARF (8,13-15). Moreover, there is no such previously conducted study in Turkey.

Hence, this cross-sectional study aimed to investigate oral health experiences in healthy children and a group of children with ARF or using depot-penicillin because of RHD. The null hypothesis tested in the present study is that there would be no difference between the children with ARF or using depot-penicillin for RHD and healthy children.

#### **Materials and Methods**

#### Ethical approval

The ethical approval of this study was obtained from the Kahramanmaras Sutcu Imam University Faculty of Medicine

Table 2. The high risk of the patients with CHD (7).
- Prosthetic cardiac valve or prosthetic material used for cardiac valve repair
- Prior IE
- Unrepaired cyanotic CHD, inclusive of palliative shunts and conduits

- In patients who have cyanotic CHD with residual defect or palliative residual defect

- In patients who have full-repaired CHD without residual defects up to 6 months after surgery

- In patients who have undergone transplantation and whose valve insufficiency continues

Medicine	Application criteria	Dose/Method of administration	Primary protection time	Secondary protection time
Benzathine penicillin G	>27 kg ≤27 kg	1.200.000 unit, IM 600.000 unit, IM	Single-dose Single-dose	Every 21 days*
Penicillin V	Primary protection Secondary protection	3x250 mg/day, PO 2x250 mg/day, PO	10 days	Everyday
Sulfadiazine or sulfisoxazole	>27 kg ≤27 kg	1x1 gr/day, PO 1x0.5 gr/day, PO	-	Everyday
Erythromycin**	Primary protection Secondary protection	20-40 mg/kg/day, 2-4 dose, PO 2x250 mg/day, PO	10 days	Everyday
1st generation cephalosporin***	Primary protection	Variable by drug	10 days	-

\* In regions where rheumatic fever is not endemic, this period can be extended to 28 days. \*\* Given to those with penicillin allergies\*\*\* 15% of people with penicillin allergy also develop an allergic reaction to cephalosporins. IM: Intramuscular, PO: Peroral.

Clinical Investigations Ethics Board (2019/18-294). Written informed consent was obtained from the parents of the children included in the study.

#### Study design and participants

The present study was a cross-sectional one, conducted with all children admitted to the Kahramanmaras Sutcu Imam University Faculty of Medicine of Pediatric Cardiology Department between August 2019 and March 2020 and diagnosed with cardiac disease. A group of children with ARF or using depot-penicillin because of RHD (study group) was compared to a randomized group of healthy children (control group; no medical history and systemic disease).

A total of 86 children aged between 5-12 years were included in the study. The participants were divided into two groups: study group (n=43) and healthy group (n=43). Fourteen males and 29 females with a mean age of  $10.3\pm1.81$  years were in the study group, whereas the healthy group comprised 22 males and 21 females with a mean age of 9.65  $\pm$ 2.64 years.

#### Inclusion criteria

Children with ARF or using depot-penicillin because of RHD and referred to the Pediatric Dentistry Department during the study period were included.

#### Exclusion criteria

Children with a medical history of cardiac or other disorder (e.g., cancer, chronic renal disease, chronic liver disease, autoimmune disease, congenital heart disease, aplastic or different types of anemia, immune disorder, cardiomyopathy, congenital malformation, neoplasm, metabolic syndrome, nervous system disorder, chronic lower respiratory disease, neonatal cerebral disorder) were excluded from the study (8,16). Inputs of the study were realized by a single pediatric cardiologist (U.U.G).

#### Data collection

The study was conducted on data related to gender, age, socioeconomic status, toothbrushing habits, cardiac status detection, and dental health.

#### Clinical oral investigation

The caries experiences of the patients were noted according to the World Health Organization (WHO) criteria for primary (dmft) and permanent teeth (DMFT) indexes (i.e., the sum of decayed, missing (due to caries), and filled teeth). The Gingival and Plaque Indexes were measured at the Kahramanmaras Sutcu Imam University of Faculty of Dentistry of Pediatric Dentistry Department. A pediatric dentist (A.S.O) examined the dried teeth with a probe and mount mirror in a dental unit.

Gingival Index was measured for six surfaces using a probe pressed into the gingival margin parallel to the buccal surfaces (55/16, 52/12, 64/24, 75/36, 72/32, and 84/44). The scoring was recorded as follows: 0 – no inflammation and no bleeding; 1- bleeding on at least one surface or mild in-

flammation, such as a slight color change; 2- moderate inflammation, such as moderate glazing, redness, edema, and hypertrophy; 3- progressive inflammation, such as advanced redness, edema, and spontaneous glazing (17).

Plaque Index was measured for six surfaces using a probe pressed into the gingival margin parallel to the buccal surfaces (55/16, 52/12, 64/24, 75/36, 72/32, and 84/44). The scoring was recorded as follows: 0- no plaque; 1- a thin film of plaque along the free gingival margin; 2- moderate accumulation with a plaque in the sulcus; 3- a large amount of plaque in the sulcus or pocket along the free gingival margin (17).

The panoramic radiographs and clinical photographs of children were obtained when possible and necessary. The children in the study group were offered prophylaxis involving dental examination and treatment (extracting and periodontal scaling) by their pediatric cardiologist. The European Society of Cardiology's recommendations were considered while deciding on the need for IE prophylaxis (18).

#### Statistical analysis

The Jamovi (Version 1.0.4) Computer Software was used for all statistical analyses. Descriptive statistics and Chi-square tests were utilized for participants' demographic characteristics. The normality of data distribution was checked using the Shapiro–Wilk test. Since the assumptions for normal distribution were met, one-way analysis of variance (ANO-VA) was used for the dmft/DMFT scores, plaque index, and gingival index. The pairwise comparisons were done using Tukey's post hoc test (p=0.05).

#### Results

#### Demographic data

A total of 86 children, 43 with ARF or using depot-penicillin because of RHD [14 males (32.6%), 29 females (67.4%)], and 43 healthy children [22 males (51.2%), 21 females (48.8%)] participated in the study. The demographic characteristics of the participants were presented in Table 3. The children's ages were between 5-12 years; the mean age of children in the study group was  $10.3\pm1.81$  years, whereas it was  $9.65\pm2.64$  years for children in the healthy control group. The

Table 3. Demographic information of the study participants.					
Variables	Study Group n= 43	Healthy Group n= 43	p value		
Age (years ±SD)	10.3±1.81	9.65±2.64	0.072		
Sex					
Female	29 (67.4%)	21 (48.8%)	0.080		
Male	14 (32.6%)	22 (51.2%)			
Toothbrushing frequency					
≥2 times a day	10 (23.3%)	20 (46.5%)	0.024*		
<2 times a day	33 (76.7%)	23 (53.5%)			
Socioeconomic status					
Low	13 (30.2%)	16 (37.2%)			
Medium	25 (58.1%)	22 (51.2%)	0.778		
High	5 (11.6%)	5 (11.6%)			

study and healthy groups' socioeconomic status were low in 13 (30.2%) and 16 (37.2%) children, moderate in 25 (58.1%) and 22 (51.2%) children, and high in 5 (11.6%) and 5 (11.6%) children, respectively. There was no statistically significant difference between the two groups regarding age, gender, and socioeconomic status (p=0.072, p=0.80, p=0.778, respectively). The study and healthy groups' toothbrushing frequencies being two or more a day were 23.3% and 46.5%, respectively. A significant difference was present between the two groups regarding the toothbrushing frequency (p=0.024).

#### Medical diagnosis

Thirty-four patients (79.1%) had mild mitral insufficiency, and two patients (4.7%) had severe mitral insufficiency in our study. Twenty-three patients (53.5%) had mild aortic regurgitation and, only one patient (2.3%) had severe aortic regurgitation. The numbers of patients with first-degree, second-degree, and third-degree carditis were 37 (86%), 4 (9.3%), and 2 (4.7%), respectively (Table 4).

#### Oral health experiences: prevalence and severity

The children's data relevant to caries, gingival index, and plaque index were presented in Table 5. While the 'dmft' index is used for primary teeth of children aged 5-12 years, the 'DMTF' index refers to permanent teeth of children aged 12-15 years. The 'dmft' indexes of the study and healthy groups were found to be 5.51±3.81 and 2.37±2.31, respectively, and these values significantly differed between the groups (p<0.001). The patients in the study group had more teeth with caries than the healthy group; the difference was statistically significant (p<0.001). Forty-one children in the study group and 35 children in the healthy group had permanent teeth. The 'DMFT' indexes of the groups were 1.71±2.28 and 1.06±1.59, respectively. However, these values did not significantly differ between the groups (p=0.150). There were more permanent teeth with caries in the study group, and the difference was statistically significant (p=0.029).

It was found that there was no significant difference between the gingival indexes of the study group  $(0.89\pm0.39)$  and the healthy group  $(0.62\pm1.03)$  (p=0.112). On the other hand, the groups significantly differed regarding the plaque index, which were  $(0.87\pm0.40)$  and  $(0.45\pm0.41)$ , respectively (p<0.001).

Table 4. The medical diagnoses of the patients in the study group.						
Mitral Insufficiency (MI)						
Absent	3 (7.0%)					
Mild	34 (79.1%)					
Moderate	4 (9.3%)					
Severe	2 (4.7%)					
Aortic Regurgitation (AR)						
Absent	19 (44.2%)					
Mild	23 (53.5%)					
Moderate	0 (0.0%)					
Severe	1 (2.3%)					
Degree of Carditis						
First	37 (86.0%)					
Second	4 (9.3%)					
Third	2 (4.7%)					

**Table 5.** The oral health experiences of children with acute rheumatic fever or using depot-penicillin because of rheumatic heart disease and the healthy group.

Caries index	Group	Ν	Mean ± SD	p-value
D	Study group	43	5.05±3.61	<0.001*
	Healthy group	43	1.14±1.92	
м	Study group	43	0.33±0.78	0.028*
	Healthy group	43	0.05±0.21	
F	Study group	43	0.14±0.52	<0.001*
	Healthy group	43	1.19±1.40	
Dmft	Study group	43	5.51±3.81	<0.001*
	Healthy group	43	2.37±2.31	
D	Study group	41	1.32±1.71	0.029*
	Healthy group	35	0.57±1.19	
м	Study group	41	0.22±0.82	0.154
_	Healthy group	35	0.03±0.17	
F	Study group	41	0.17±0.77	0.158
	Healthy group	35	0.46±0.95	
DMFT	Study group	41	1.71±2.28	0.150
	Healthy group	35	1.06±1.59	
Gingival	Study group	43	0.89±0.39	0.112
index	Healthy group	43	0.62±1.03	-
Plaque	Study group	43	0.87±0.40	<0.001*
index	Healthy group	43	0.45±0.41	_

### Discussion

ARF and RHD are estimated to affect almost 20 million children in developing countries and constitute the leading cause of cardiovascular death during the first five years of life (2). The incidence of ARF has decreased with improvement in living conditions and common antibiotics for treating streptococcal transmission (7).

To our best knowledge, this study is the first dental study conducted on Turkish children with ARF or using depot-penicillin because of RHD. This study had some restrictions, such as that the examined study group was small, no power analysis was performed due to unclear prevalence of ARF in the Turkish population, and some patients with ARF but no cardiac involvement were excluded. This study aimed to emphasize the importance of oral health in these diseases. The study group diagnosed by an experienced pediatric dentist disclosed some significant results for their previous dental therapies. The oral health examinations relied on clinical and radiographic diagnoses. The children were first examined when they were admitted to the Pediatric Cardiology Department of Medical Faculty to receive intramuscular injections of BPG.

The literature embodies several studies comparing children with ARF and healthy children regarding their caries experiences (8,13-15). Thornley *et al.* (8) claimed that there was a positive correlation between caries and ARF. Intake of sugar leads to dental caries; therefore, the relationship between sugar intake and ARF might be significant. *Streptococcus Mutans (MS)* 

is a bacterium associated with dental caries; therefore, there may be a correlation between this bacterium and ARF (19). *SP*, which is related to ARF, ferments sugar (especially glucose and fructose), and the sugar is proposed to be a fundamental source of ARF owing to the growing number of *SP* in sugar (20). Nevertheless, in their study, Thornley *et al.* (8) used the hospital records for the participants' medical histories. In our study, the children were diagnosed by a pediatric dentist when they presented to the Department of Pediatric Cardiology.

The dmft/DMFT outcomes in our study suggest that the null hypothesis should not be accepted. The dmft index of primary teeth in the study group was found to be significantly higher than that of the healthy group (p<0.001), similar to various studies conducted with cardiac patients in the literature (10,12). One study concluded that the patient group with ARF had significantly more caries than the control group (14). Such a result in this study may suggest that using depot-penicillin because of RHD accelerates caries in primary teeth (21). Dental treatments may cause significant problems, such as monetary, psychological, somatic, and educational problems, in small-age children. A team consisting of a pediatric dentist and a pediatric cardiologist might inform patients about the importance of optimal oral health. A general dentist might consider that his/her knowledge may be insufficient for children with special needs and may direct such patients to a pediatric dentist.

This study group's missing primary teeth were significantly more than that of the healthy group (p=0,028). When the pulp tissue is involved in caries lesions, the pediatric dentist uses radical treatment such as extraction instead of root endodontic treatment or vital pulp therapy (22). The extraction might be performed under antibiotic prophylaxis (18).

The study group's filled primary teeth were significantly less than that of the healthy group (p<0.001). It is known that if dental caries of a patient with ARF or who is receiving depot-penicillin for RHD is not treated, the patient will have an increased risk of dental sepsis. The sepsis outcome may be lethal or critical complications may develop in surgically undermined children (7). Optimal oral health restrains bacteremia and IE. When the patient's dental therapy was tooth extraction or a periodontal operation, it was performed by the pediatric dentist (A.S.O) under antibiotic prophylaxis. The guideline claims that dental procedures should be performed under antibiotic prophylaxis when oral mucosal perforation involving the tooth's periapical area and manipulation of gingival tissue are present (18). As formerly reported, caries in primary teeth increases the possibility of developing caries in permanent teeth, and plague and gingivitis are risk factors for periodontitis in the future (23,24).

The DMFT index of permanent teeth was found to be 1.71  $\pm$  2.28 in the study group, and there was no significant difference between the groups (p=0.150). Similar to our study, Stecksen-Blicks *et al.* (10) reported no significant differences between patient and control groups regarding permanent teeth and suggested that such a result might have been related to the participants' age. Our findings were also parallel with what was previously reported for cardiac patients in the literature (10,25). Moreover, the study group's permanent tooth caries was significantly more than that of the healthy group in our study (p=0.029), similar to Franco *et al.*'s study (26). On the other hand, the number of filled permanent teeth

in the study group was less than that of the healthy group, but it did not significantly differentiate them (p=0.158). In a study, the children with CHD were more worried than their healthy peers due to their former therapy experience and hospitalization; thus, such children might delay visiting their dentists due to their anxiety (27). The missing permanent teeth in the study group were higher in number than that of the healthy group, and there was no statistical difference between the groups (p=0.154). This result may be related to the the patients' age. In this age group, children are more aware of the importance of tooth brushing.

In our study, the plaque index of the study group was significantly higher than that of the healthy group (p<0.001), which shows a similarity with the study of Ali *et al.* (28). Poor oral health among Turkish children might be related to the typical attitude towards dental treatments and less awareness of dental treatments' importance.

It has been strongly recommended that patients brush their teeth twice a day, use dental floss, and visit their dentists regularly, thus protecting their oral health and quality of life (7). In our study, 23.3 % of the children in the study group brushed their teeth more than twice a day. There was a significant difference between the groups in this study regarding tooth brushing (p=0.024). Berger (29) claimed that pediatric cardiac patients with more caries lesions might have had improper tooth brushing habits.

There was no statistical difference between the groups in our study regarding socioeconomic status (p=0.778). Previous studies investigating the relationship between socioeconomic status and dental caries lesions have suggested precise results (29,30). Like our study, Balmer *et al.* (22) claimed no association between socioeconomic status and dental caries lesions in children with CHD. The result in the present study may be expounded upon the free-of-charge dental therapy in Turkey.

Moreover, patients with systemic disease and their parents should be more aware of preventive dental treatments and required measures to prevent dental caries because it is acknowledged in the literature that oral health and general health are interrelated (9). Some daily practices, such as tooth brushing, dental flossing, and chewing, tend to decrease oral bacteremia risks (7).

In almost half of the patients with this disease, the valvular endocardium may be impaired due to cardiac inflammation (31). The patients' medical histories were taken to investigate their cardiac conditions and suitability for dental operations (Table 4). A further limitation of this study was that the study group's caries could not be fully correlated with the patients' medical histories. All patients with ARF or using depot-penicillin because of RHD started regularly visit our dental clinic after relevant information pertinent to this study's results was provided.

Poor oral hygiene due to caries lesions is a significant problem for IE, and a pediatric dentist and cardiologist should perform proper dental therapy. This study's team recommended that patients visit the dentist regularly, take good care of their oral health (tooth brushing, dental flossing, dental fluoride treatment), and feed with less sugar. If caries is diagnosed initially, dental therapy will be easier, cheaper, and quicker. The parents of children with ARF or using depot-penicillin for RHD should be warned about the infective endocarditis risk.

# Conclusion

The children with ARF or using depot-penicillin because of RHD had worse oral health experience than the healthy group in this study.

Türkçe Özet: Akut romatizmal ateş veya romatizmal kalp hastaliği olan türk çocuklarinin ağiz sağliği deneyimleri. Amaç: Akut romatizmal ateş (ARA) veya romatizmal kalp hastalığına (RKH) bağlı depo penicillin kullanan çocuklar enfektif endokardit (EE) riskine ve kötü ağız hijyenine yatkındır. Bu kesitsel çalışmanın amacı bir sağlıklı çocuk grubu (kontrol grubu) ile ARA veya RKH nedeniyle depo penicillin kullanan çocukların (çalışma grubu) oral bulgularını karşılaştırmaktır. Gereç ve yöntem: Bu calısmada yaşları 5-12 arasında toplam 86 cocuğun tıbbi ve diş verileri incelenmiştir. Çalışma ve kontrol gruplarının tıbbi öyküleri, çürük, çekim ve dolgulu diş, plak indeksi, gingival indeks, diş fırçalama sıklıkları, sosyoekonomik durumları kaydedildi ve incelendi. Bulgular: Çalışma ve kontrol gruplarında 'dmft' sırasıyla 5,51  $\pm$  3,81 ve 2,37  $\pm$  2,31 ve bu gruplarda 'DMFT' sırasıyla  $1,71 \pm 2,28$  ve  $1,06 \pm 1,59$  olarak bulunmuştur. Çalışma ve kontrol gruplarının gingival indeksleri sırasıyla  $0,89 \pm 0,39$ ve 0,62 ± 1,03 olup, istatistiksel olarak aralarında anlamlı fark yoktur (p=0,112). Bununla birlikte, grupların plak indekleri sırasıyla  $0,87 \pm 0,40$ ve 0,45  $\pm$  0,41 dir ve aralarında anlamlı fark vardır (p<0,001). Çalışma ve kontrol gruplarının günde iki kere veya daha fazla diş fırçalama sıklığı %23,3 ve %46,5'dir. Sonuç: ARA veya RKH nedeniyle depo penicillin kullanan çocukların sağlıklı gruba göre süt dişlerinde ve daimi dişlerinde çürükleri daha fazladır ve ağız hijyenleri daha kötüdür. Anahtar Kelimeler: Akut romatizmal ateş; diş çürüğü; kalp hastalığı; romatizmal kalp hastalığı; ağız sağlığı.

**Ethics Committee Approval:** The ethical approval of this study was obtained from the Kahramanmaras Sutcu Imam University Faculty of Medicine Clinical Investigations Ethics Board (2019/18-294).

**Informed Consent:** The informed consents were obtained from the parents of the children included in the study.

Peer-review: Externally peer-reviewed.

**Author contributions:** ASO participated in designing the study. ASO participated in generating the data for the study. ASO, UUG, SI participated in gathering the data for the study. ASO, UUG, SI participated in the analysis of the data. ASO wrote the majority of the original draft of the paper. ASO participated in writing the paper. ASO has had access to all of the raw data of the study. ASO, UUG, SI have reviewed the pertinent raw data on which the results and conclusions of this study are based. ASO, UUG, SI have approved the final version of this paper. ASO, UUG, SI guarantee that all individuals who meet the Journal's authorship criteria are included as authors of this paper.

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