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RELATION BETWEEN SALIVARY IMMUNOGLOBULINS LEVELS AND DENTAL CARIES IN EGYPTIAN CHILDREN WITH GAUCHER DISEASE

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ABSTRACT

Background: Gaucher disease (GD) is one of the most prevalent autosomal recessive lysosomal storage diseases. Dental caries is commonly encountered in the pediatric age. The current study aimed to evaluate the association between salivary bacteriological findings, immunoglobulins levels, and dental caries in children with GD. The current case-control study included 55 children with GD and 40 age and sex-matched siblings of the included patients served as the control group. Dental caries was diagnosed and graded according to WHO criteria. Collected saliva was examined for bacteriological findings and salivary immunoglobulins: sIgA and sIgG.

Results: Comparison between patients and controls revealed significantly lower frequency of dental caries in the patients' group (65.5 % versus 90.0 %, p=0.007) as well as significantly lower dental caries severity scores as compared to controls. In addition, patients had significantly higher sIgA [median (IQR): 1880.0 (1440.0-2250.0) versus 1000.0 (0.0-2172.5) mg/L, p= 0.002] and sIgG [median(IQR): 24.0 (13.0-28.0) versus 11.5 (4.5-23.5) mg/L, p= 0.002] levels when compared with healthy siblings. GD patients with dental caries had significantly lower sIgA levels as compared with patients without dental caries [median (IQR): 1655.0 (777.5- 1947.5) versus 2050.0 (1890.0-2660.0) mg/L, p= 0.001]. Multivariate logistic regression analysis recognized low sIgA levels as a predictor of dental caries in the studied patients [OR (95% CI): 1.002 (1.001-1.003), p=0.012].

Conclusions: Increased sIgA levels in children with GD probably contribute to their protection against dental caries.

KEYWORDS: Gaucher patients, dental caries, *streptococcus mutans*, Salivary IgA, Salivary IgG

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INTRODUCTION

Gaucher disease (GD) is recognized as one of the most prevalent autosomal recessive diseases of lysosomal storage[1,2]. GD may present as the non-neuronopathic form (type 1), type 2 or chronic neuronopathic form (type 3). Type 3 GD is prevalent in some Asian countries e.g. China and Korea and in Egypt [3,4]. Systemically, the syndrome is characterized by hypersplenism, hepatosplenomegaly, and bone disorders[5]. Common oral findings include gingival bleeding, and low salivary flow[6,7].

Dental caries is commonly encountered in children aged 5-17 years[8,9]. Associated risk factors include poor oral hygiene, family history of dental caries, diminished salivary flow, unhealthy diet, and diminished fluoride level in drinking water [10].

Salivary IgA (sIgA) represents almost 60% of the total salivary immunoglobulins amount. It serves to improve oral immunity through prevention of microbial adherence, neutralization of enzymes, toxins, and viruses, or synergistic effect with other factors such as lysozymes and lactoferrin [11].

Salivary IgG (sIgG) is almost entirely derived from the gingival fluid in response to periodontal irritation and inflammation. An increase in gingival fluid and sIgG has been seen in association with a variety of oral microbial agents including *streptococcus. Mutans* [12].

The present study aimed to evaluate the relation between salivary bacteriological findings and immunoglobulins levels and dental caries in children with GD.

METHODS:

The present case-control study was conducted at Specialized Children Hospital, Egypt. Data collection was initiated after ethical approval from the Research Ethics Committee of my University (Approval no: N-52-2015). Informed consent was obtained from the guardians of included children.

The study included 55 children with GD. Diagnosis was established by identification of decreased β-glucocerebrosidase enzyme activity in white blood cells by the standard laboratory methods. All patients were under enzyme replacement therapy. In addition, there were 40 age and sex-matched siblings of the included patients who served as the control group.

Dental examination

The oral cavity of the child was clinically examined while he was seated in a regular chair by a single operator using a disposable mirror, a dental probe, a portable light, and cotton pellets. Dental caries was identified based on WHO diagnostic criteria [13]. In children with permanent teeth, the severity of caries was assessed by DMFT index (D: decayed tooth, M: missing tooth, F: filled teeth. In children with primary teeth, we used the dmft index (d: decayed teeth, m: missed teeth, f: filled teeth. For those with mixed dentition, we used both DMFT and the deft index (d: decayed teeth indicated for filling, e: decayed teeth indicated for extraction, f: filled teeth) [14].

Saliva collection and examination

Collection of unstimulated (resting) salivary samples was achieved using Dawes's method[15,16]. Teeth brushing, using mouthwash, eating, or drinking weren't allowed one hour before collection of samples between 10:00 and 11:00 a.m. Children were asked to pool salivary fluid on mouth floor and spit it out into a collection cup. The collected saliva was refrigerated at −10°C until evaluation of sIgA and sIgG and bacteriological assessment [17].

Salivary bacterial evaluation

A 50 μl sample of the saliva was cultured directly on Saselect (BioRad, USA) mitis salivarius agar for isolation of *streptococcus mutans, staphylococcus aureus* and *staphylococcus epidemidis* and MRS medium agar (Oxoid, United Kingdom) for isolation of *lactobacilli*. *Candida* was isolated on Sabaroud's

dextrose agar. All plates were incubated at 37˚C for 24–48 h aerobically except for MRS, which was anaerobically incubated (nitrogen 85%, hydrogen 10%, carbon dioxide 5%) at 37˚C for 48–72 h in an anaerobic jar using Oxoid anaerogen compact gas packs (Oxoid, UK) and mitis salivarius was incubated at 37˚C in a candle jar for 48–72 h [18]. The remainder of each sample was centrifuged and divided into several aliquots stored at –70˚C until required. The amounts of sIgA and sIgG were estimated in saliva using human sIgG and human sIgA ELISA kits (Komabiotech, Korea) as directed by the manufacturer in duplicates, and absorbance measurements were obtained by a SpectraMax® microplate reader.

Statistical analysis

All statistical calculations were operated using SPSS 21.0 (IBM Inc, USA). Data were presented as number and percent or median with interquartile range (IQR). Comparative statistics was processed using Fisher's exact, chi-square or Mann-Whitney U tests as appropriate. Univariate and multivariate logistic regression analysis were utilized to determine predictors of dental caries. Receiver Operator Characteristic (ROC) curve analysis was executed to assess the diagnostic value of salivary IgA. P value < 0.05 was identified as statistically significant.

RESULTS

The present study included 55 patients with GD and 40 age and sex-matched siblings who served as controls. Comparison between patients and controls revealed significantly lower frequency of dental caries in the patients' group (65.5% versus 90.0%, p=0.007) and significantly lower dental caries severity scores as compared to controls. Moreover, patients expressed significantly lower salivary *streptococcus mutans* count [median (IQR): 300.0 (20.0-1200.0) versus 1100.0 (600.0-2000.0) cell/ml, p<0.001] when compared with controls. In addition, it was found that patients had significantly higher sIgA [median (IQR): 1880.0 (1440.0-2250.0) versus 1000.0 (0.0-2172.5) mg/L, p=0.002] and sIgG [median (IQR): 24.0 (13.0-28.0) versus 11.5 (4.5-23.5) mg/L, p=0.002] when compared with controls (Table-1, Fig.1).

TABLE (1) Clinical and laboratory findings in the studied groups

	Patients N=55	**Controls N=40**	**p value**
Age (years) median (IQR)	9.0 5.0-14.0)	10.5 (6.5-14.8)	0.43
Age categories (years) n (%)			
< 6	14 (25.0)	6 (15.0)	0.18
6-12	20 (36.4)	22 (55.0)	
> 12	21 (38.2)	12 (30.0)	
Male/female n	28/27	22/18	0.84
Dental caries n (%)	36 (65.5)	36 (90.0)	0.007
dfm	1.0 (0.0-4.0)	5.0 (0.0-8.0)	<0.001
deft	2.5 (0.3-5.8)	4.0 (2.0-6.0)	<0.001
DFMT	2.0 (0.0-2.5)	3.5 (2.0-7.0)	<0.001
***Streptococcus mutans* count** (cell/ml) median (IQR)	300.0 (20.0-1200.0)	1100.0 (600.0-2000.0)	<0.001
Other bacterial isolates n (%)			
Staph. epidermidis	17 (30.9)	6 (15.0)	0.092
Staph. aureus	36 (65.5)	26 (65.0)	0.96
Lactobacilli	38 (69.1)	28 (70.0)	0.92
Candida albicans	16 (29.1)	18 (45.0)	0.13
Salivary Immunoglobulins (mg/L) median (IQR)			
IgA	1880.0 (1440.0-2250.0)	1000.0 (0.0-2172.5)	0.002
IgG	240.0 (130.0-280.0)	115.0 (45.0-235.0)	0.002

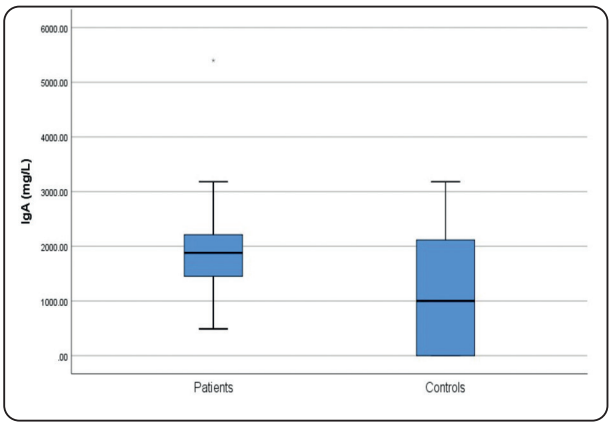

Fig. (1) Salivary IgA levels in patients and controls

TABLE (2) Comparison between patients with dental caries and patients without regarding the clinical and laboratory data

	Dental Caries n=36	**No Dental Caries N=19**	**p value**
Age (years) median (IQR)	10.0 (6.0-14.8)	7.0 (5.0-14.0)	0.46
Age categories (years) n (%)			
< 6	8 (22.2)	6 (31.6)	0.51
6-12	15 (41.7)	5 (26.3)	
> 12	3 (8.3)	8 (42.1)	
Male/female n	15/21	13/9	0.089
Gaucher disease type n (%)			
1	14 (38.9)	9 (47.4)	0.58
3	22 (61.1)	10 (52.6)	
***Streptococcus Mutans* count** (cell/ml) median (IQR)	400.0 (70.0-1150.0)	200.0 (20.0-1200.0)	0.35
Other bacterial isolates n (%)			
Staph. Epidermidis	13 (36.1)	4 (21.1)	0.36
Staph. Aureus	23 (63.9)	13 (68.4)	0.78
Lactobacilli	29 (80.6)	9 (47.4)	0.016
Candida albicans	12 (33.3)	4 (21.1)	0.53
Salivary Immunoglobulins (mg/L) median (IQR)			
IgA	1655.0 (777.5-1947.5)	2050.0 (1890.0-2660.0)	0.001
IgG	230.0 (92.5-280.0)	250.0 (200.0-300.0)	0.2

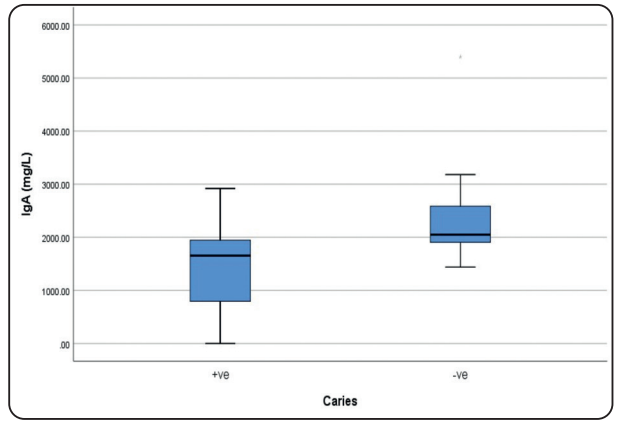

Fig. (2) Salivary IgA levels in patients with and without dental caries

Comparison between GD patients with and without dental caries showed significantly higher frequency of patients with salivary *lactobacilli* isolates (80.6% versus 47.4%, p=0.016). and significantly lower sIgA levels in patients with dental caries [median (IQR): 1655.0 (777.5- 1947.5) versus 2050.0 (1890.0- 2660.0) mg/L, p=0.001]. (Table-2, Fig.2). Multivariate logistic regression analysis recognized low salivary IgA levels as predictor of dental caries in the studied patients [OR (95% CI): 1.002 (1.001-1.003), p=0.012] (Table-3). ROC curve analysis showed that salivary IgA levels could reliably distinguish between patients with dental caries and patients without (AUC:0.78, sensitivity: 78.9 %, specificity: 66.7 %) (Fig.3).

TABLE (3) Predictors of dental caries in the studied patients

	Univariate analysis			**Multivariate analysis**		
	OR	**95% CI**	**p**	**OR**	**95% CI**	**p**
Age	0.96	0.86-1.07	0.48	-	-	-
Sex	3.03	0.94-9.8	0.064	2.738	0.687-10.908	0.015
Lactobacilli	4.603	1.357-15.616	0.014	4.037	0.958-17.02	0.057
IgA	1.002	1.000-1.003	0.005	1.002	1.001-1.003	0.012
IgG	1.0	0.999-1.002	0.75	-	-	-

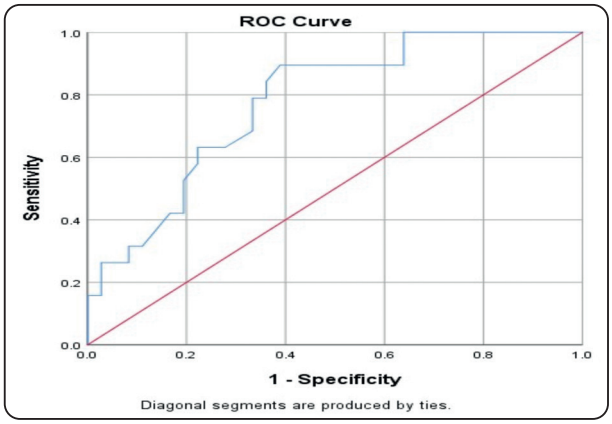

Fig. (3) ROC curve for salivary IgA and dental caries

DISCUSSION

Oral health in children with GD is a rarely investigated issue. In this study, we identified significantly lower prevalence of dental caries in children with GD in comparison to their age and sex-matched siblings. In harmony with these findings, the study of Fischman et al. [6], on 87 patients with GD including children and adults, concluded that patients had significantly fewer carious lesions than otherwise healthy carriers. They acknowledged that patients had better dental parameters despite the associated known comorbidities of anemia, tendency to bleeding, and poor healing. These findings were arguably due to the high awareness of the condition among patients and their families and consequently better care of their child's oral health.

Considering the fact that hyperimmunoglobulinemia is a well-documented abnormality in children with GD [19,22] the present study tried to evaluate the relation between salivary immunoglobulin levels and the development of dental caries in this population.

In our study, patients had significantly higher sIgA in comparison to controls,while it was found that patients with dental caries had significantly lower sIgA levels when compared with patients without. The protective role of sIgA against dental caries was previously reported in other conditions. In the study of Lee et al. [23], on children with Down syndrome (DS), the authors found significantly lower dental caries prevalence and severity in patients as compared to controls. Moreover, they noted that patients had significantly higher salivary *S. mutans* (serotype g and c) specific IgA concentrations. Another study also found that DS children had significantly higher total sIgA when compared with controls. However, they found no relation between caries experience and IgA levels in both groups [24].

In another longitudinal study that monitored the relation between caries development, colonization with caries-associated microflora, and immunity, it was found that lower baseline level of sIgA was associated with higher caries risk[25]. In contrast to our findings, Lo Giudice et al.[26], found no statistically significant differences between caries-free and caries-active children regarding sIgA levels.

Notably, the study of Parisotto et al. [25], identified a significant association between *S.Mutans and lactobacilli* colonization in the studied children. In our study, patients with caries demonstrated higher frequency of salivary *lactobacilli* isolates. Similar conclusions were reported by Angarita-Díaz et al.[27], and Ahmad et al [28].

Finally, this study showed no significant association between sIgG levels and dental caries in harmony with the findings of Mousavizadeh et al. [29], who noted that sIgA but not sIgG levels were related to development of dental caries.

CONCLUSION

The present study suggests that increased sIgA levels in children with GD probably contribute to their protection against dental caries. These conclusions are limited by the small sample size of the study. Further studies are recommended to detect other immunological mediators related to lower rates of dental caries in this population.

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