

Relationship between gingivitis and autism in children: A matched case-control study

ABSTRACT

Autism is a neurodevelopmental disorder diagnosed in early childhood. Studies have shown that autistic children have poorer gingival health and hence chances of higher incidence of gingivitis may occur in autistic than non-autistic children. Gingivitis is characterized by gingival inflammation without loss of attachment due to continuous exposure to dental plaque. Studies on the gingival health of autistic children in Malaysia are lacking. Hence, this study aims to evaluate and compare the gingival health status and prevalence of these three bacteria among autistic and non-autistic children.

Material and Methods:

A total of 86 children were recruited and an oral assessment was performed on each child to assess the degree of calculus, plaque accumulation and gingival inflammation using the calculus, plaque and gingival indices respectively. Pooled dental plaque samples were collected and transferred to Stuart transport media.

Samples were diluted and 100 µl aliquots were cultured on Columbia agar with 5% sheep blood. Plates were incubated in an anaerobic environment at 37°C for 7 days. Colonies were Gram stained and examined for cellular morphology followed by indole and catalase test. Bacterial identification was done using the Rapid ID 32 A kit. Kolmogorov-Smirnov, Chi-square, Mann-Whitney U, Odds ratio and Spearman's correlation analyses were used to analyse the data.

Results

The prevalence of dental calculi was significantly elevated in the autistic group (p = 0.001). However, the prevalence of gingivitis and plaque were not significantly different between the two groups. The presence of plaque (p = 0.001) and calculi (p = 0.004) were significantly associated with the development of gingivitis. In this study, 11 different bacterial species were identified from 44 samples that were tested. *Actinomyces naeslundii* was found in 8(%) autistic samples while *Prevotella intermedia* and *Porphyromonas gingivalis* were not detected in any of the samples that were tested.

Conclusions

While the autistic children had significantly more calculus, the occurrence of gingivitis and plaque were similar between autistic and control groups. The prevalence of *Actinomyces naeslundii* was significantly higher in the autistic group. Measures to control dental plaque, calculi and *Actinomyces naeslundii* among the

autistic children should be improved to prevent gingivitis.

Keywords: [Autism, gingivitis, Prevotella intermedia, Porphyromonas gingivalis, gingival indices]

1. INTRODUCTION

Autism was first described by Leo Kanner in 1943, symptoms usually appear in early childhood and they differ in severity from mild to severe (Rekha CV et al., 2012). The prevalence of autism is rising and studies conducted in the United Kingdom, United States of America, Japan and South Korea report prevalence estimates of 157, 113, 181 and 264 per 10,000 individuals respectively (Sun X et al., 2013). In Malaysia, the prevalence is approximately 1.6 per 1000 among children aged 1.5 to 3 years old (Yeo KJ and Lu X., 2012).

Gingivitis is a reversible type of periodontal disease which is distinguished by inflammation of the gingival tissue without loss of periodontal attachment due to prolonged exposure of the gingiva to dental plaque. It can develop into periodontitis in susceptible individuals causing permanent destruction of periodontal tissue (Kistler JO et al., 2013).

Studies have shown that autistic children have poor oral hygiene and gingival health along with a higher incidence of periodontal disease than non-autistic children (Rekha CV et al., 2012; Vajawat M and Deepika P., 2012; Luppanapornlarp S et al., 2010; Murshid EZ., 2005; Jaber MA., 2011; Friedlander AH et al., 2006; DeMattei R et al., 2007). They have higher plaque and gingival index scores with increased need for periodontal treatments such as scaling and root planning (Vajawat M and Deepika P., 2012; Luppanapornlarp S et al., 2010). Generalized gingivitis seen in autistic children was attributed to several reasons such as irregular brushing habits, lack in manual dexterity, poor hand coordination, poor dental awareness, lack of dental education and adverse effects of certain medications used to manage some manifestations of autism (Murshid EZ., 2005; Jaber MA., 2011; Friedlander

AH et al., 2006). Study reported a higher occurrence of gingivitis in those with mixed and permanent dentition whereas another researcher reported significantly more gingivitis among elder autistic children. Gingivitis was attributed to hormonal factors and excessive plaque accumulation due to insufficient oral hygiene practices in both studies (Rekha CV et al., 2012; DeMattei R, et al., 2007).

Studies on the gingival health of autistic children in Malaysia are lacking. Hence, this study aims to evaluate and compare the gingival health status and prevalence of *Actinomyces naeslundii*, *Prevotella intermedia* and *Porphyromonas gingivalis* among autistic and non-autistic children. Knowledge about the presence of these bacteria could help to better identify children at risk for periodontal disease and create awareness on the oral health of autistic children.

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55 2. MATERIAL AND METHODS

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57 A case-control study was conducted for six months (1st July to 31st Dec 2013) at three different centers of one of the autism society in Malaysia in Klang Valley. The children who were diagnosed by a psychiatrist as autistic were considered as cases. Children who were residing in three orphanage homes located at the same areas as the respective centres of the autism society, but not suffering from autism were considered as controls. Stratified Random Sampling matched with age groups was used to select the study sample.

63 The sample size was calculated for matched case-control study by using the following parameters: Confidence interval = 95%, power=80%, ratio of case against control was 1:1, proportion of dental plaques and calculi in control group = 5%, the difference between the proportion of dental plaques and calculi among autistic and non-autistic children =30%, the expected odds ratio or design effect was 8.14. due to feasibility constraints in statistical analysis, the researchers decided to replace any drop-outs or lost to follow up cases with a randomly selected new one according to the respective groups.

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Forty-three autistic children were selected from three centres of an autism society in Malaysia located at the Klang Valley. The cases belonged to (4–16) years of age group and suffering from mild to moderate autism with no comorbidities. Matched with age-groups 43 healthy children without autism (control) were randomly selected from 3 different children homes in the Klang Valley. A study info sheet was distributed and informed written consent was obtained from every participants before proceeding with this research.

Considering a proportion of 10% oral bacterial assessment with a precision of 9% and a confidence coefficient of 95%, 43 subjects were required for this study. A convenience sample of 43 autistic individuals aged 4 to 16 years old were obtained from three branches of one of the autism society.

The inclusion criteria were mild to moderate cases of autism between the ages of 4 to 16 years old for the test group and non-autistic individuals of similar age group as controls. The exclusion criteria were severe cases of autism with co-morbidities and uncooperative subjects

Ethical consideration:

The present study was independently reviewed and approved by the Research Ethics Committee of the internal university and board members of one of the autism society in Malaysia where study had been conducted. The children's parents provided informed written consent after receiving information about the objectives and procedures of this study. The control group consisted of 43 non-autistic individuals of the same age group solicited from children homes. Informed written consents were provided by highest management authorities of these establishments as well as parents of the participants.

Dental assessment and sample collection:

The dental assessment for all subjects was performed by the registered dentist using a sterile dental mirror and probe. The children were assessed for the presence and degree of calculus, plaque and gingivitis using the calculus, plaque and gingival indices in Table 1 (Vajawat M and Deepika P., 2012; Hamissi J et al., 2009; Summers A., 2009). These

findings along with subject demographics (age, gender and ethnicity) were recorded in a dental form.

Table 1: Description of indices used (Hamissi et al., 2009; Summers A., 2009).

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Description	
Calculus Index	
Score	
0	No calculus present.
1	Supragingival calculus covering not more than one third of the exposed tooth surface.
2	Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.
3	Supragingival calculus covering more than two thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both.
Plaque Index	
Score	
0	No plaque in the gingival area.
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be recognized only by running a probe across the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket and on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.
3	Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.
Gingival Index	
Score	
0	No inflammation. Gingival tissue is normal.
1	Mild inflammation. There is a slight change in colour and slight oedema of the gingival tissue but there is no bleeding on probing.
2	Moderate inflammation. There is some redness, oedema and glazing of the gingival tissue which bleeds on probing.
3	Severe inflammation. There is marked redness, oedema and ulceration of the gingival tissue which tends to bleed spontaneously.

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Pooled plaque samples were collected using a sterile probe and cotton swab from the supragingival margins and gingival crevices of the buccal surface of the anterior maxillary molar and lingual of mandibular molar teeth and were immediately placed into Stuart

106 transport medium (Improswab™, Guangzhou Improve Medical Instruments Co. Ltd.,
107 Guangzhou, China).

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109 Cultural analysis:

110 All samples were processed within 12 hours of collection. They were diluted by 25 fold and
111 100 µl aliquots were cultured on Columbia agar with 5% sheep blood (Isolac®, Isolab Sdn
112 Bhd, Selangor, Malaysia). The plates were incubated in an anaerobic environment in
113 anaerobic jars at 37°C for 7 days (Lie M., 2001; Tanner A et al., 2011; Tsuruda K et al.,
114 1995). Morphologies of the resulting bacterial colonies were observed and morphologically
115 different colonies were marked on each plate. Smears were prepared by heat fixing on glass
116 slides, by using Gram stain (bioMérieux® SA, Marcy-l'Etoile, France Color Gram 2) followed
117 by microscopic examination to observe the Gram reactions and cellular morphologies.

118 Colonies were tested for the presence of catalase, an enzyme which decomposes hydrogen
119 peroxide to water and oxygen, and indole, a product from the decomposition of tryptophan
120 by the bacteria (Taylor WI and Achanzar D., 1972; Miller JM and Wright JW., 1982). A
121 positive catalase test is characterized by the presence of bubbles on the glass slide when
122 the bacteria was added to the ID color Catalase (ID-ASE, bioMérieux® SA, Marcy-l'Etoile,
123 France) reagent while a positive indole test is characterized by a blue coloration on the filter
124 paper after the bacteria was added to the ID indole TDA (ID-TDA, bioMérieux® SA, Marcy-
125 l'Etoile, France) reagent.

126 All observations were recorded and based on these findings, colonies that were presumptive
127 to be of the bacteria of interests were sub-cultured and incubated in an anaerobic condition
128 at 37°C for 2 days. Bacterial identification was performed using API Rapid ID 32 A Kit
129 (bioMérieux® SA, Marcy-l'Etoile, France), an identification system for anaerobes in 4 hours
130 using 29 miniaturized enzymatic tests and a database.

131 Statistical analysis:

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 17.0 (IBM SPSS Inc., Chicago, Illinois, USA). Chi-square was done to study the association between a factor and the outcome while odds ratio and Spearman's correlation coefficient were computed to find the direction and strength of association. Level of significance was set at $p < 0.05$.

3. RESULTS

In this study, majority of the participants were male 62 (72%). Among the autistic children, Majority of the participants 68 (79.1%) belonged to the age group of (4-12) years. The distribution of ethnicity revealed that majority of them were Malay 58 (67.4%). Oral characteristics of participants in Table 2 revealed that majority had gingivitis 63 (73.3%) and plaques 78 (90.7%). However, majority of participants were without dental calculi 45 (52.3%). There were no significant associations between gingivitis and gender or ethnicity or age of the participants.

Table 2: Oral characteristics of the participants.

Demographic and oral characteristics	Autistic (N₁ = 43) n₁ (%)	Non-autistic (N₂ = 43) n₂ (%)	Total (N = 86) n (%)
Gingival Index			
0 (None)	14 (32.5)	9 (20.9)	23 (26.7)
1 (Mild)	15 (34.9)	19 (44.2)	34 (39.6)
2 (Moderate)	11 (25.6)	13 (30.2)	24 (27.9)
3 (Severe)	3 (7.0)	2 (4.7)	5 (5.8)
Plaque Index			
0 (None)	3 (7.0)	5 (11.6)	8 (9.3)
1 (Mild)	10 (23.3)	8 (18.6)	18 (20.9)
2 (Moderate)	21 (48.8)	15 (34.9)	36 (41.9)
3 (Severe)	9 (20.9)	15 (34.9)	24 (27.9)
Calculus Index			
0 (None)	14 (32.5)	31 (72.1)	45 (52.3)
1 (Mild)	18 (41.9)	8 (18.6)	26 (30.2)
2 (Moderate)	6 (14.0)	3 (7.0)	9 (10.5)
3 (Severe)	5 (11.6)	1 (2.3)	6 (7.0)

Table 3 and 4 revealed that the autistic group had a lower incidence of gingivitis (46%), but a higher incidence of plaque (51.3%) than the non-autistic group (54% and 48.7% respectively). However, difference in the prevalence of gingivitis and plaque between the two groups was found to be statistically insignificant ($p=0.001$). The calculus index was positively and significantly correlated with autism (Spearman's $\rho=+0.389$)

Table 3: Comparison between Gingivitis status/Dental plaques/Dental Calculi and autism

	Autistic (N ₁ = 43) n ₁ (%)	Non- autistic (N ₂ = 43) n ₂ (%)	Total (N = 86) n (%)	χ^2 value	OR	95% CI	P- value
Gingivitis status							
Present	29 (46.0)	34 (54.0)	63 (73.3)	1.484	0.548	0.207 – 1.451	0.223
Absent	14 (60.9)	9 (39.1)	23 (26.7)				
Plaque status							
Present	40 (51.3)	38 (48.7)	78 (90.7)	0.551	1.754	0.392 – 7.852	0.713
Absent	3 (37.5)	5 (62.5)	8 (9.3)				
Calculus status							
Present	29 (70.7)	12 (29.3)	41 (47.7)	13.471	5.351	2.127 – 13.460	0.001*
Absent	14 (31.1)	31 (68.9)	45 (52.3)				

* Here, p -value <0.05 was considered as significant

Table 4: Spearman's correlation analyses on dental indices with regards to autism status.

	Spearman's ρ	p-value
Gingival Index	-0.078	0.474
Plaque Index	-0.076	0.482
Calculus Index	+0.389	0.001*

* Here, p -value <0.05 was considered as significant

Table 5 & 6 revealed that there was a significant association between the presence of plaque and gingivitis ($p = 0.001$). The OR and 95% CI for the OR could not be computed because one cell had a frequency of 0 (Table 6). The plaque index was positively and significantly correlated with the prevalence of gingivitis (Spearman's $\rho = +0.601$).

Table 5: Comparison between Dental plaques or dental calculi and gingivitis status

Gingivitis status				χ^2 value	OR	95% CI	P- value
Present (N ₁ = 63) n ₁ (%)	Absent (N ₂ =23) n ₂ (%)	Total (N = 86) n (%)					
Plaque status							
Present	63 (80.8)	15 (19.2)	78 (90.7)	24.161	–	–	0.001*
Absent	0 (0)	8 (100)	8 (9.3)				
Calculus status							
Present	36 (87.8)	5 (12.2)	41 (47.7)	8.466	4.80	1.583 – 14.556	0.004*
Absent	27 (60.0)	18 (40.0)	45 (52.3)				

* Here, p -value < 0.05 was considered as significant

Table 6: Spearman's correlation analyses on dental indices with regards to gingivitis status.

	Spearman's ρ p- value	p-value
Plaque Index	0.601	0.001*
Calculus Index	0.342	0.002*

* Here, p -value < 0.05 was considered as significant

There was a significant association between the presence of calculus and gingivitis ($p = 0.004$). Individuals with calculus were 4.8 times more likely to develop gingivitis than

178 individuals without calculus (OR = 4.80). The calculus index was positively correlated with
 179 the presence of gingivitis by 34.2% (Spearman's $\rho = 0.342$).

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182 4. DISCUSSION

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184 The median plaque index and gingival index scores show that in general, the children
 185 experienced moderate accumulation of plaque and mild gingivitis. The autistic children had a
 186 higher incidence of calculus (calcified plaque deposits) than the control group. However, the
 187 prevalence of plaque and gingivitis were comparable between the two groups. The latter was
 188 due to good oral hygiene care given to the children by their parents and caregivers at the
 189 respective centres. A Swedish study involving 20 autistic and 20 non-autistic children
 190 reported similar findings in which there was no significant difference in the occurrence of
 191 gingivitis between the two groups (Fahlvik-Planefeldt C and Herrstrom P., 2001).

192 The presence of plaque and calculus were positively correlated with gingivitis, indicating that
 193 they pose as risk factors for the development of gingivitis. Similar findings had been reported
 194 in other studies (Loesche W and Syed S., 1978; El-Qaderi S and Quteish Ta'ani D., 2006;
 195 Kostadinović LB et al., 2011). In two separate studies in which one involved 1362 Jordanian
 196 children with mean plaque and gingival scores of 1.46 and 1.56 respectively while another
 197 involved 86 Serbian children with median plaque and gingival scores of 1.757 and 1.894
 198 respectively, researchers reported that dental plaque accumulation was associated with
 199 gingival inflammation (El-Qaderi S and Quteish Ta'ani D., 2006; Kostadinović LB et al.,
 200 2011). Calculus had been implicated as a risk factor for the development of periodontal
 201 disease in children and adults in other studies (Ashkenazi M, Miller R, Levin L (Ashkenazi M
 202 et al., 2012).

203 Out of the 10 other bacterial species apart from *Actinomyces naeslundii* that were detected
 204 in this study, *Actinomyces viscosus*, *Propionibacterium acnes*, and *Actinomyces israelii* were
 205 found in gingivitis while *Capnocytophaga* sp. and *Bacteroides ureolyticus* were found in

206 juvenile periodontitis in other studies (Moore L et al., 1987; Loesche W and Syed S., 1978;
207 Baker J et al., 1976; Rummens J et al., 1986; Duerden B et al., 1987).

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210 5. CONCLUSION

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212 The autistic group had significantly more calculus than control but the prevalence of plaque
213 and gingivitis were similar between the two groups. The prevalence of *Actinomyces*
214 *naeslundii* was significantly higher in the autistic group. *Prevotella intermedia* and
215 *Porphyromonas gingivalis* were not detected in any of the samples that were tested. As
216 dental plaques and calculi are considered as risk factors for the development of gingivitis,
217 good personal oral hygiene habits along with professional treatments are important
218 measures to prevent gingivitis. A limitation of this study is the small sample size as it was
219 aimed as a pilot study. Larger multi-centric studies are needed to validate the findings from
220 this study.

237 ETHICAL APPROVAL (WHERE EVER APPLICABLE)

238 Ethical approval taken by institution research and ethics committee

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