Aggregatibacter Actinomycetemcomitans in Down´s Syndrome Children

El Housseiny AA*

Objectives: *To investigate the presence of Aggregatibacter actinomycetemcomitans (Aa) in Down's syndrome (DS) children in comparison with age-matched normal children and to determine if Aa was acquired during childhood in DS individuals.* ***Study design:*** *60 DS children and 60 healthy children participated in this cross-sectional study. Both groups were matched regarding gender and age and were equally selected from public schools/non-fee paying centers and private schools. Subgingival plaque sample were obtained and analyzed for Aggregatibacter actinomycetemcomitans (Aa). Traditional cultivation method was used on a selective medium "modified malachite green vancomycin agar."* ***Results:*** *A significantly higher percentage of DS children had Aa in their subgingival plaque compared to their healthy counterparts (Chi square = 8.78, p=0.003). Although Aa was acquired by children as early as 5 years of age in both groups, no association was found between the occurrence of Aa and age.* ***Conclusions:*** *higher proportion of DS children acquired Aa compared to their healthy controls and Aa is acquired by children as early as 5 years of age.*

Keywords: *Aggregatibacter actinomycetemcomitans, Down's syndrome, children, periodontal disease, prevalence.*

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INTRODUCTION

Down's syndrome (DS), is an autosomal chromosomal anomaly resulting from trisomy of all or a critical part of chromosome 21.[1] The prevalence of DS in the United States and Europe varies from approximately 9-12.5 *per* 10000 live births[2]. In Sweden, the prevalence is one in 600-1000 live births,[3] while in Japan, the overall prevalence is 5.82 *per* 10000 live births.[4] In Egypt, the prevalence of DS has been reported to be 1 *per* 1000 births.[5] The frequency of occurrence increases with maternal age, and there is no racial, social, economic or gender correlation.[6]

It has been reported that individuals with DS often develop severe periodontal inflammation similar to the condition of the localized aggressive periodontitis.[7,8] Its basis in patients with DS is largely due to immunological deficiency[9] and its evolution to poor masticatory function and poor oral hygiene.[1] However, the etiology of the disease in DS is not yet fully understood.

Recently, the prevalence of periodontal disease among children and adolescents with DS aged between 6-20 years was evaluated.[10] The prevalence of gingivitis was reported in 91%, whereas periodontitis was found in 33% of the individuals.[10] Periodontal disease in DS has been reported to begin as early as 5 years of age, and by adulthood nearly all persons with the syndrome are affected.[11,12]

One of the important lines of investigations concerning the pathogenesis of DS periodontitis has focused on differences in the subgingival microflora. During the last two decades, a lot of attention has been diverted to the study of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* (Aa) as one of several microorganisms in the pathogenesis of periodontal disease.[13,14]

Aa is a Gram-negative, small rod (1–1.5mm), capnophilic facultative anaerobe.[15] It has been easily isolated and recovered on a special selective culture media.[16,17] On culture, colonies grow in small flat, circular colonies that have slightly blurred borders and a translucent outlook in solid media, sometimes with a typical inner star-like morphology.[16]

It is well known that Aa produces a number of virulence factors involved in the periodontal disease process.[18] Thus, Aa has been suggested to be an important pathogen associated with periodontitis in DS.[19]

The baseline data concerning the pathogenesis of DS periodontal disease is still deficient. Thus, further investigations were needed to assess the occurrence of Aa as one of

* Azza A El Housseiny, BDS, MSc,PhD., Professor of Pediatric Dentistry, Faculty of Dentistry. University of Alexandria. Egypt and King Abdulaziz University, Saudi Arabia.

Send all correspondence to: Azza A. El Housseiny, Faculty of Dentistry, King Abdulaziz University, Saudi Arabia. POBox: 80209 Jeddah 21589, Saudi Arabia.

Tel: (9662) 6403443
Fax: (9662) 6403316

E-mail: ahussini@hotmail.com

the widely suspected periodontopathogens. The aim of this study was to investigate the presence of *Aggregatibacter actinomycetemcomitans* (Aa) in DS children in comparison with age-matched normal children and to determine if (Aa) was acquired during childhood in DS individuals.

MATERIALS AND METHOD

A total of 120 subjects selected randomly, aged between 5–14 years, participated in this study. The present sample was classified into two distinct groups for comparative purposes:

The DS Group

It comprised 60 children with DS. They were non-institutionalized; attending day care centers in Alexandria, Egypt, and were randomly drawn from:

- College of Saint Marc (includes training and learning center for children with special needs).
- Fair Heaven School for children with special needs
- Caritas-Egypt Seti Center for Training and Studies on Disability (DS children were drawn from two of Caritas centers that provide unpaid services for children with special needs).

The Control Group

This group included 60 systemically healthy children, who were chosen to match DS children, with respect to age and gender, enrolled in private & public schools in Alexandria.

The inclusion criteria, for both DS and healthy children, were as follows:

- No periodontal or antibiotic treatment for one month before enrollment in the study.
- No history of any medical condition that requires preoperative prophylactic antibiotics.
- For DS children, those with at least moderate grade of mental deficiency were included in the study (according to the schools/centers records).

The University of Alexandria Research Board approved this study. Permissions to carry out the present study were first obtained from the education and health authorities in Alexandria (Ministry of Education and the Health Insurance Organization in Alexandria).

Schools authorities and centers for children with special needs were then contacted and the purpose of the study was explained to them in order to ensure their approval and full cooperation. Informed consent was taken from the parents or caregiver.

Microbiological analysis

Microbiological sampling was carried out one hour after the children had received their breakfast meal in the presence of the children's parents/ medical or dental care givers in different schools/centers

Subgingival Plaque Sampling

In each individual, one subgingival plaque sample was obtained from mesio-buccal aspect of the tooth with the highest periodontal screening and recording (PSR)™ score.[20] The PSR score (ranged from code 0 to 4) was determined according to the criteria described in the PSR teaching manual recommended by the American Dental Association.[20] All measurements were performed using a specifically designed metal ball-ended PSR probe (WHO-621). The left upper first molar was chosen if no difference in clinical parameters were found.[19]

Sampling site was gently isolated with cotton rolls and the supragingival plaque was removed with sterile cotton pellets. Subgingival plaque was obtained by inserting one fine sterile endodontic paper point #40 (Kerr color coded absorbent paper point, Kerr®, Germany) to the depth of the mesio-buccal aspect until resistance was met or the paper point bent and kept in place for 60 seconds.[21] It was then transferred to 1ml of one-quarter strength pre reduced sterilized Ringer's solution.[17] Samples were kept on ice until transferred to the laboratory where the processing procedure was performed within the next two hours.

Anaerobic Microbiological Processing and Culturing

The samples were mixed well by vortex at the maximal setting for 60s. Of each sample, 100µl was plated on a selective medium "modified malachite green vancomycin agar" (MGVA). The medium consisted of 8µg/ml malachite green Loba Chemie (malachite green for microscopy), PVT. LTD, Mumbai, India) and 10µg/ ml vancomycin (Culture Media Supplements, Oxoid, LTD, Basingstoke, Hampshire, England) in a Tryptic Soy Agar (TSA) (Tryptic Soy Agar, Oxoid, LTD, Basingstoke, Hampshire, England) base supplemented with 5% blood. The plates were incubated for 5 days at 37˚c in 5-10% CO_2 anaerobic conditions (Gas Generating Kit Carbon Dioxide System, Oxoid, LTD, Wade Road, Basingstoke, Harts, RG24, 8PW, UK).

After five days, colonies of Aa were identified by their characteristic morphology in light microscopy (power of the lens x40). Small, round, convex, translucent, adherent colonies, 0.5 to 1.0 mm in diameter with slightly irregular edges and star-like inner structure were considered positive.[16]

All microbiological determinations were performed at the Department of Microbiology, Medical Research Institute, Alexandria University, Egypt.

Statistical Analysis

Descriptive statistics were displayed as frequencies and percents for qualitative variables (gender and type of school) and mean ± standard deviations for quantitative variables (age). Comparison was done between the two study groups (children with DS and healthy children) according to presence of Aa or type of school. The tests used for the comparison were Chi square for qualitative nominal variables (presence of Aa) and t test for mean age of the two groups.

Significance level was set at the 5% level, where p≤0.05. Statistical analysis was done using SPSS version 13.

RESULTS

The 120 subjects participated in the present study were classified equally into two groups; Down's syndrome (DS) group (47.8% males, 52.2% females) with mean age 10.20 ± 3.06 years and healthy group (55.6% males and 44.4% females) with mean age 9.39 ± 2.89. Both groups were matched regarding gender and age (P=0.30, P=0.07 respectively) and were equally recruited from public schools/non-fee paying centers and private schools.

Microbiological Analysis

Aggregatibacter actinomycetemcomitans was detected in subgingival plaque in a statistically significant higher percentage among DS children compared to healthy children 55% versus 28.3% respectively, (Chi square = 8.78, P= 0.003).

The relation between the occurrence of Aa and age was analyzed. Table 1 shows that according to age, no significant difference in the number of children showed presence or absence of Aa in both groups. It also shows that Aa was acquired by the children as early as 5 years of age in both groups. When children were pooled in two age groups; less than and more than 10 years, Chi square analysis showed no statistically significant difference in the number of children showed presence or absence of Aa in both groups (Chi square = 2.99, P = 0.09)

Table 2 shows no significant differences between children with Aa and children without Aa as regards mean age, either in the DS group (P=0.63) or the healthy group (P=0.29).

Table 1. Number and percentage of children with or without *Aggregatibacter actinomycetemcomitans* (Aa) distributed according to age in both groups

	Aa in DS children		Total	Aa in healthy children		Total N (%)
	Absent N (%)	Present N (%)		Absent N (%)	Present N (%)	
5- 6 yrs	1 (3.7)	5 (15.2)	6 (10)	16 (37.2)	5 (29.4)	21 (35)
7- 8 yrs	8 (29.6)	8 (24.2)	16 (26.7)	12 (27.9)	7 (41.2)	19 (31.7)
9- 10 yrs	6 (22.2)	3 (9.1)	9 (15)	13 (30.2)	2 (11.8)	15 (25)
11-12 yrs	2 (7.4)	8 (24.2)	10 (16.7)	1 (2.3)	1 (5.9)	2 (3.3)
13-14 yrs	10 (37)	9 (27.3)	19 (31.7)	1 (2.3)	2 (11.8)	3 (5.0)
Total	27 (100)	33 (100)	60 (100)	43 (100)	17 (100)	60 (100)
Chi square P value	6.79 0.15 NS			5.19 0.27 NS		

NS: Not statistically significant

Table 2. Mean age of DS and healthy children with and without *Aggregatibacter actinomycetemcomitans* (Aa)

	AA		T - TEST	P VALUE
	Absent	Present		
DS	10.52 ± 2.71	10.15 ± 3.13	0.48	0.63 NS
Healthy	7.60 ± 1.97	8.29 ± 2.82	1.08	0.29 NS

NS: Not statistically significant

DISCUSSION

Among several differences in the oral conditions of DS patients, periodontal disease appears with an increased prevalence and severity.[22] This is believed to have negative effects on the quality of life of DS individuals, which is aggravated by the seriousness of the disease.[10]

Half of the DS group was drawn from schools/centers where the children receive paid training and learning services. The rest of the DS group was selected from non-fee paying centers that provide unpaid services for children with disabilities. Similarly, in an attempt to eliminate the differences in social level, healthy children were equally drawn from both public and private schools. Both DS and healthy children were matched as regards to gender and age.

For both DS and healthy children, those who received periodontal or antibiotic treatment for at least one month before examination were excluded from the study so as not to affect clinical and microbiological conditions. In addition, those who had any medical condition that requires pre-operative prophylactic antibiotics were excluded from the study since periodontal probing needs pre-operative prophylaxis for these patients.[23]

The present microbiological analysis investigated the occurrence of Aa, since it is listed among the suspected putative periodontopathogens involved in DS periodontal disease.[19,22] In addition, because of being one of the facultative, capnophilic anaerobes, it is easily sampled and cultured on a specific media,[16,17] compared to the rest of strict anaerobic pathogens that require fastidious and difficult culture techniques.[24]

Subgingival plaque is commonly used to detect and quantify bacterial species since it is considered the principle etiological factor in the onset and progression of periodontitis.[17] It was suggested that the subgingival prevalence of Aa is directly related to the initiation and progression of an inflammatory process.[25,26] In addition, an increase in periodontal probing depth seems to be associated with a higher probability of subgingival detection of AA.[25,26] Based on these suggestions, subgingival plaque samples were taken from mesio-buccal aspect of the tooth with the highest periodontal conditions in the mouth according to the PSR scoring system. The left upper first molar was chosen, because of easier isolation, if no difference in clinical parameters were found.[19]

Traditional cultivation method on a selective medium "modified malachite green vancomycin agar" (MGVA) was used in the present study, since this culture method is still the gold standard for detecting and characterizing human pathogens.[27]

The differences encountered in periodontal status of DS are explained by a multi-factorial etiology. The first factor to be considered, which might account for these differences, is the changes in the subgingival microflora toward more pathogenic species, which is reported among both DS children[28]

and DS adolescents as well.[22] It is documented that periodontal diseases are associated with a shift in the periodontal bacterial flora, from the healthy to the diseased state.[29] In the present study, Aa was detected in a significantly higher percentage among DS children compared to healthy children (55% vs. 28.3%). These findings suggested that early and heavy colonization with Aa occurs in DS children, which might play an important role in the initiation of gingival inflammation in these children compared to healthy children. These findings are comparable with previous studies on DS children that isolated Aa by using culture method.[30, 31] In one of these studies,[30] Aa was detected in 35% of 37 DS subjects compared to 5% in healthy subjects, while in the other study, Aa was found in 54% of 35 DS children compared to only 5.7% of the healthy subjects.[31]

A Polymerase Chain Reaction (PCR) assay could be more suitable for the detection of periodontopathogens, especially in cases of subgingival plaque in children where there are a limited number of pathogens present.[32] This might explain why an association between the occurrence of Aa and age could not be established in the present study, neither in DS nor in healthy children. This finding is in contrast to other studies noted that the occurrence of Aa in DS individuals gradually increases with age.[19,22] This might be explained by the differences in size and age of the examined sample, where one of these studies examined a group of 267 DS subjects aged between 8-28 years[22] compared to 60 DS children aged between 5-14 years in the present study. Thus, the microbial profile of children, adolescents and young adults was explored compared to the present study that only included children. However, in another study where PCR technique was used, Aa was detected in all age groups of 60 DS children aged 2-13 years and were reported to gradually increase with age.[19] These differences might be attributed to either sampling or culturing techniques used in the present study, where culture technique, though is still considered the reference method (gold standard) among new microbial diagnostics in periodontics, it has some shortcomings[17,27] as it could only grow viable bacteria; thus necessitates precise sampling which was difficult while dealing with children with special needs. Appropriate isolation was difficult to be attained as a result of increased salivation, enlarged tongue and excessive gagging reflex characterizing DS individuals. Additionally, limitations of paper point sampling, which have been reported in a previous study,[21] were encountered in the present study. Because of difficult isolation, it was noted that the absorbent material starts to absorb gingival fluid containing bacteria as soon as it has been inserted into the gingival crevice. The paper point may be saturated before it reaches the apical portion of the crevice, thus it might represent only the more coronal bacterial content of the site.

In light of the present results, the occurrence of Aa in children with DS and the potential virulence properties of this bacterium might contribute on one hand to the basic concepts of specificity in periodontal disease. However, on the other hand the presence of Aa in subgingival plaque does not necessarily imply an ongoing pathological process. It has been proposed that the higher prevalence of periodontal disease in DS individuals is probably related to the impaired host response and not to the occurrence of specific periodontopathogens.[19] As Aa was found in periodontally healthy children,[19,33] hence, the nature of the immune disorders encountered in the DS, may allow a specific periodontopathogen, such as Aa, to colonize and destroy gingival sites.[22]

It was reported that DS subjects had reduced ability to maintain adequate plaque control due to poor manual dexterity, joint laxity (including the carpal joints) and lack of comprehension of oral hygiene needs due to mental difficulties.[34] The inability of people with DS to effectively control supragingival plaque is of major importance in altering its composition and also affecting subgingival plaque composition.[35] Thus, the need to reinforce oral hygiene procedure in DS subjects was supported by previous studies.[22, 34]

It is well known that intervention of periodontal disease is based on the control of the most accessible risk factor, including the supra-and subgingival bacterial load. The dentist has an obligation to prevent and control oral diseases in DS individuals through appropriate preventive measures and comprehensive programs that take the special needs of these subjects into account.[34]

In the present study inclusion criteria included children who do not require pre-operative prophylactic antibiotics and those did not received antibiotic treatment for at least one month before examination. This is supported by other studies which exclude subjects require antibiotic prophylaxis for periodontal examination without giving any recommendations to exclude those who receives antibiotics.[36,37] However, other studies recommended no antibiotics for 3 or more months before enrolment.[38,39] Further investigations are needed to determine what is the suitable time needed to wash off the effect of antibiotics before a subject can be included in a similar study.

CONCLUSION

It can be concluded that: higher proportion of DS children acquired Aa compared to their healthy controls and children acquire Aa as early as 5 years of age.

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