Acta Scientific Dental Sciences (ISSN: 2581-4893)

Volume 6 Issue 6 June 2022

Research Article

Detection of JP2 and Non-JP2 Genotype Strains of *Aggregatibacter Actinomycetemcomitans* in Localized Aggressive Periodontitis Patients Among the Egyptian Population

Ibrahim Hammad Ibrahim[1], Alaa Moustafa Attia[1,2]*, Mohamed Fouad Edrees[1], Mohamed A. EL-Mokhtar[1] and Heba Ali Hammad[3]

[1]Department of Oral Medicine, Periodontolgy, Oral Diagnosis, and Dental Radiology, Faculty of Dental Medicine Al-Azhar University (Assiut), Egypt.

[2]Department of Basic and Clinical Oral Sciences, Division of Periodontology, Faculty of Dentistry, Umm Al-Qura University, Makkah, Saudi Arabia and Al-Azhar University (Assiut), Egypt.

[3]Department Microbiology and Immunology Faculty of Medicine, Assiut University, Egyot

***Corresponding Author:** Alaa Moustafa Attia, Department of Oral Medicine, Periodontolgy, Oral Diagnosis, and Dental Radiology, Faculty of Dental Medicine Al-Azhar University (Assiut), Egypt and Department of Basic and Clinical Oral Sciences, Division of Periodontology, Faculty of Dentistry, Umm Al-Qura University, Makkah, Saudi Arabia.

DOI: 10.31080/ASDS.2022.06.1394

Received: May 06, 2022

Published: May 25, 2022

Abstract

Background: JP2 clone of *Aggregatibacter actimycetemcomitans* has been mainly recovered from adolescents living in northwestern Africa or African descends. A very strong association between this and Aggressive periodontitis (AgP) specifically its localized form was found among adolescents.

Objectives: The purpose of the current study to evaluate the prevalence of *Aggregatibacter actimycetemcomitans* (Aa) genotype strains and to determine the effect of presence of these strains on the severity of localized aggressive periodontitis (LAP) patients at base line presentation among the Egyptian population.

Materials and Methods: This study was done on sixty patients (54 females and 6 males) aged (9-26) years diagnosed clinically and radiographically as a LAP patient were selected from examination of 270 periodontitis patients. The presence of Aa (JP2 and non-JP2) strains in the collected microbiological samples were identified by conventional PCR. All clinical parameters including plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment level (CAL) were taken for all the patients at base line.

Results: This study found that: *Aggregatibacter actinomycetemcomitans* (JP2 and non-JP2) was identified by PCR in 20/60 patients of the total participants in this study. Presence of Aa specifically its JP2 strain was associated with a significant increase in the estimated clinical parameters, which appeared clinically in the form of more destructive LAP cases at base line presentation.

Conclusion: The identification of Aa in minority of cases excludes the role of Aa as a major bacterial risk factor in LAP. JP2 and non-JP2 strain infection at baseline diagnosis can be predictive of potentially periodontal destruction in LAP patients. Further research are needed to clarify the role of JP2 in both severity and outcomes of periodontal management in people of LAP.

Keywords: *Aggregatibacter; Actinomycetemcomitans;* JP2 Strain

Abbreviation

AgP: Aggressive Periodontitis; LAP: Localized Aggressive Periodontitis; GI: Gingival Index; PI: Plaque Index; PD: Pocket Depth; CAL: Clinical Attachment Level; RTX: Repeats-in-Toxin; LTX: leukotoxin; LPS: lipopolysaccharide; PCR: Polymerase Chain Reaction; SPSS: Statistical Package of Social Science

Introduction

Aggressive periodontitis has received considerable attention due to its peculiar clinical presentation, with rapid attachment loss and bone destruction, with an apparent lack of the local factors, in patients with a reasonably good oral hygiene. A variety of factors such as microbial, environmental, genetic, behavioral factors and systemic diseases have been suggested to influence the risk of aggressive periodontitis [1].

Aggregatibacter actinomycetemcomitans is a Gram-negative species that was suggested to be highly implicated in the etiology of localized aggressive periodontitis [2]. It possesses many virulence factors, such as RTX (repeats-in-toxin) and leukotoxin (LTX), which received much attention which induces cell lysis, degranulation and induce an inflammatory response in human leukocytes [3].

Population genetic analysis of *Aa,* has identified a mainly clonal structure but exhibit significant genetic population diversity and seven serotypes of *Aa*, have been identified based on the antigenicity of the oligo- polysaccharide (O-polysaccharide) component of the lipopolysaccharide (LPS), among them, serotypes a, b, and c are globally dominant, whereby type c is the most prevalent, and serotype b is the most frequently associated with periodontitis [4].

Aggregatibacter actinomycetemcomitans isolated from AgP cases in adolescents of African descent living in different parts of the world are genetically homogeneous and belong to a single clone called JP2 [5]. The JP2 clone belongs to *Aa* serotype b and, among other unique characteristics, has a 530-base-pair deletion in the promoter region of the leukotoxin gene operon. This deletion leads to increased leukotoxic activity with a theoretically improved potential to interfere with innate immune defense [4]. It was demonstrated that adolescents colonized with *Aa* have a significantly increased risk of the development of periodontal attachment when compared to those that are not and carriers of the JP2 genotype strain are at higher risk of development of periodontal destruction compared to carriers of a non-JP2 genotype [6].

Molecular assays for *Aa* detection are based on DNA-probes; PCR and real time PCR methods are faster and more sensitive than cultural systems [7]. The present study used conventional PCR to detect the presence of *Aa* (JP2 and non JP2) strains in LAP patients and relates the presence of these strains with severity of the disease among the Egyptian population.

Materials and Methods

Patient selection

This study was carried out on sixty patients (54 females and 6 males) aged (9-26) years with mean age 22.7 ± 5.142 diagnosed as LAP (Figure 1). The patients were selected by history taking, clinical as well as radiographic examination of 270 periodontitis patients attending at the Outpatient Clinic of Oral Medicine, Periodontology, Oral Diagnosis and Dental Radiology Department, Faculty of Dental Medicine, Al-Azhar University, Assiut branch.

According to the criteria of American Academy of Periodontology, patients diagnosed as LAP when the following features were included [8]: Patients were clinically healthy except for the presence of periodontitis, rapid attachment loss and bone destruction, familial aggregation, the amounts of microbial deposits are inconsistent with the severity of periodontal tissue destruction, circumpubertal onset and localized first molar/incisor presentation with interproximal attachment loss on at least two permanent teeth (one of which is a first molar) and involving no more than two teeth other than first molars and incisors.

Periodontal parameters

All clinical parameters including plaque index (PI) [9], gingival index (GI) [10], pocket depth (PD) and clinical attachment level (CAL) [11] were taken.

Plaque sample collection

The selected sites and adjacent teeth were isolated with cotton rolls, supragingival plaque was careful removed with a sterile scaler to prevent the contamination of the samples [12], then paper point ISO #40 taper 0.02 mm/mm® (Co. Roeko, Langenau, Germany) was inserted slowly with a sterile dental tweezer into the pocket until tissue resistance felt or the paper points bent [13]. The paper point was left for 20 sec, then carefully removed without touching the adjacent unrelated tissues and then transferred to a sterile Eppendorf tube containing a phosphate buffer saline (PBS) and frozen at-80 ◦ C until further microbiological analysis.

Screening for *Aggregatibacter Actinomycetemcomitans*

DNA was extracted from collected samples using Gene JET Genomic DNA purification kit® (Thermo scientific, EU). Screening for Aa was carried out by conventional polymerase chain reaction (PCR) performed using the T100 thermal cycler ® (Bio Rad, USA). The reaction was done using the following primer sequence: OG155-CATTCTCGGCGAAAAAACTA-3', OG156 5'-CCCATAACCAAGCCACATAC-3', that distinguishes between JP2 strains (amplicon size 195 bp) and non-JP2 strains (amplicon size 696 bp) [14].

Amplification program was performed with the following steps: an initial denaturation step at 95° C for 5 min, followed by 35 cycles of DNA denaturation at 95° C for 45 sec, primer annealing at 50° C for 1 min, primer extension at 72° C for 40 sec and final extension steps was done at 72° C for 5 minutes.

Statistical analysis

Data were written and calculate to be analyzed through the Statistical Package for Social Science (SPSS), version 26 (IBM Inc., United States). Descriptive data of both groups were presented as mean, standard deviation (SD). The comparison between values (Mean + SD) by using compared t-test. p-Value was considered significant at level ($P \leq 0.05$).

Figure 1: Panoramic radiograph of 22 years old LAP case with severe interproximal attachment loss localized to the first molar and incisor area.

Results

Prevalence of Aa genotype strains

Aggregatibacter actinomycetemcomitans was detected in 20/60 (33.3%) patients of the total participants in this study (Figure 2) Among the *Aa*-positive ten patients, the JP2 strain alone was identified in 4/20 (20%), 12/20 patients (60%) showed mixed genotypes (jp2 and non jp2). Non-JP2 strain was observed in 4/20 patients (20%) (Figure 3).

Figure 2: Pie chart showing Prevalence of Aa among the participants in the study.

Figure 3: Pie chart showing distribution of JP2 and non-JP2 strains among Aa-positive patients.

Comparisons of disease severity

Between negative and positive detectable Aa LAP patients*:* There was no statistically significant difference in means of plaque index scores but there were statistically significant differences in means of gingival index scores, clinical attachment level and probing pocket depth between the two groups (Table 1).

Between positive JP2 and non JP2 strain: There was a statistically significant difference in means of plaque index scores, gingival in-

dex scores and clinical attachment level and no statistically significant difference in means of probing pocket depth between the two groups (Table 2). *Between positive JP2 and positive mixed strains:* There was no statistically significant difference in means of plaque index scores, gingival index scores and probing pocket depth but a statistically significant difference in means of clinical attachment level was found between the two groups. (Table 2).

	Negative *Aa*	Positive *Aa*	T value	P Value
PI	(0.81 ± 0.75)	(1.12 ± 0.71)	-1.30	0.200
GI	(0.67 ± 0.60)	(1.27 ± 0.64)	-3.41	0.002**
PD	(6.30 ± 0.73)	(7.11 ± 1.18)	-2.57	0.014*
CAL	(5.00 ± 1.12)	(6.11 ± 1.56)	-2.53	0.016*

Table 1: Statistical comparisons (Mean ± SD) of clinical prompters between negative and positive detectable *Aa* LAP patients.

	Comparisons		T Value	P Value
PI	JP2 (0.75 ± 0.82)	Non JP2 (1.62 ± 0.41)	-2.33	0.042*
GI	JP2 (1.50 ± 0.54)	Non JP2 (1.00 ± 0.00)	2.23	0.049*
PD	JP2 (7.50 ± 1.64)	Non JP2 (7.00 ± 1.09)	0.62	0.549
CAL	JP2 (8.00 ± 0.00)	Non JP2 (4.50 ± 0.54)	15.65	0.000**
PI	JP2 (0.75 ± 0.82)	Mixed (1.00 ± 0.63)	-0.591	0.568
GI	JP2 (1.50 ± 0.54)	Mixed (1.33 ± 0.51)	0.542	0.599
PD	JP2 (7.50 ± 1.64)	Mixed (6.83 ± 0.75)	0.90	0.388
CAL	JP2 (8.00 ± 0.00)	Mixed (5.83 ± 0.75)	7.050	0.000**

Table 2: Statistical comparisons (Mean ± SD) of clinical parameters between positive *Aa* LAP cases (JP2, Non JP2 and Mixed (JP2 and Non JP2).

Discussion

Aggressive periodontitis is a severe and rapidly destructive form of periodontitis, characterized by early onset; familial aggregation and affect individuals are otherwise clinically healthy. It is a multifactorial process results from a combination of genetic, environmental, host and microbial factors and present in a localized or generalized form. In the present study 60 patients with age ranged between (9-26), with mean age (22.7 ± 5.142) diagnosed as having LAP according to criteria of Armitage 1999-classification system were selected [8].

Subgingival plaque samples were taken from all patients in this study using sterile paper point sampling method, this technique has been used for microbiological sampling of periodontal lesions because the ability of reproducibility obtained by paper point samples as recommend by Mombelli., *et al.* [15]. Also, difficulties in standardizing curette sampling method which considered effective quantitively were encountered by Sixu., *et al.* which failed to achieve reproducible results [16].

Jervøe-Storm., *et al.* [12] compared curette and paper point sampling techniques, subgingival bacteria samples were analyzed by qPCR and found that; although curette samples harvested significantly more total bacteria, the composition of the plaque samples with respect to selected target pathogens was quite similar for both sampling techniques.

PCR technology, when used optimally offers high sensitivity and specificity in detection *Aa* than culture techniques and comparison between culture and PCR methods in detection of five putative periodontal pathogenic bacteria in subgingival plaque samples was performed by Jervøe-Storm., *et al.* [17] and reported that; *Aa* has been found in 48.7% of the patients by using PCR and only in 7.7% of the patients by using the culture method.

Based on these findings, the present research used molecular based technology (PCR) to detect *Aa* in the plaque samples in LAP patients and to differentiate between the JP2 with 530-base pair deletion at promoter region of leukotoxin operon and other non JP2 genotypes without deletion based on differences in size of the amplified products and allows simultaneous detection of the two genotypes.

Results of the present study showed that only 20/60 cases were *Aa* positive with prevalence about 33.3%. By contrast, most studies found that the prevalence of *Aa* in adolescents with aggressive periodontitis is very high (73-100%) as reviewed by Slots and Ting [18], but these results are comparable with the results of Chile study by Lopez., *et al.* [19] which found a lower isolation frequency (39-44%) were detected in similar localized juvenile periodontitis (LJP) patients. Also, Chahboun., *et al.* [20] in Morocco studied the bacterial profile of aggressive periodontitis found that; only (46.2%) of LAP patients were *Aa* positive.

The present study was performed to detect the presence of JP2 strain of *Aa* in Egypt as part of North African countries in which JP2

clone of *Aa* is believed to be endemically present as by Haubek., *et al.* [21] and hoped to complete picture of the geographic dissemination of the JP2 strain because the entire picture of the dissemination of the JP2 clone, even within the African continent, is still not completely known.

Results of this study found that; the JP2 Strain of *Aa* alone was present in 4/20 (20%) of LAP *Aa*-positive patients and 12/20 patients (60%) were found to harbor mixed (jp2 and non jp2) and in agreement with results of Bandhaya., *et al.* [22] which concluded that; although the majority *Aa* positive patients carry only one clonal type, some positive subjects harbor multiple clones.

The present study found that most of LAP-*Aa* positive patients, about (80%) harbor JP2 (alone or with non JP2) strain and this support the evidence proposed by Haubek., *et al.* [23] which concluded that; JP2 strain may be one of the etiologic factors of LAP and found that clonal types other than JP2 can be isolated from healthy as well as periodontally diseased individuals, whereas the JP2 clone has been isolated primarily from periodontally diseased individuals. Also, Cortelli., *et al.* [24] found that; the probability of having aggressive periodontitis in adolescents infected with (JP2) clone was close to 100%, whereas being colonized with other clonal types was associated with a lower prevalence rate of the disease.

The presence of LAP (40/60) 66.7% patients being -ve *Aa* (JP2 and non JP2) in this study is in contradict with previous results in different studies that for long time considered *Aa* the primary etiologic factor of AgP especially its localized form, and support the recent findings of Haubek and Johansson [25] which found that; the JP2 clone of *Aa* is not the only factor involved in and cannot explain all cases of aggressive periodontitis in the adolescent population and another factors other than microbial such as environmental, genetic and defect in host response also implicated.

The current study found that: in *Aa* positive LAP (JP2, non JP2 and mixed) cases was associated with higher rate of periodontal destruction in the form of a significant increase the clinical parameters (PD and CAL) when compared with negative *Aa* cases, at the same time a significant increase in these parameters in the presence of JP2 strain when compared with cases which harbor *Aa* non JP2. These findings in agreement with Haubek., *et al.* [26], which found a higher rate of progression of periodontal disease among JP2 strain positive patients.

The explanation for these results may be related to the fact that the deletion of this 530-bp at promoter region of leukotoxin (LTX) gene operon, which is found in JP2 strain has critical role in faster expression of the leukotoxin (LTX) gene, leading to the production of a larger amount of LTX than in non JP2 strains (termed minimally leukotoxic strain) where this promoter region remains intact and appear to be associated with a lesser severity of periodontal disease [27] but evidence supporting these findings is scanty and inconclusive because *Aa* is not only universally detected in AgP sites and cases, it is also found in healthy individuals and in healthy sites of diseased individuals. Furthermore, a similar prevalence of detection in aggressive and chronic periodontitis cases was found and although highly prevalence of *Aa* in most cases of localized aggressive periodontitis, it is not present in all cases [28].

Recently, Haubek., *et al.* (2021) [29] investigated the prevalence JP2 and Non-JP2 Genotypes of *Aggregatibacter actinomycetemcomitans* in Kenyen population. They concluded that "the prevalence of the JP2 genotype of *A. actinomycetemcomitans* is low, a possible indicator that spreading through human migration from North and West Africa to East Africa is a rare occasion". This finding is considered a supporting to conclusions of the present research work.

Conclusion

The present study concluded that: The identification of *Aa* has a low prevalence of cases excludes the role of *Aa* as a major bacterial risk factor in LAP. JP2 and non-JP2 strain infection at baseline diagnosis can be predictive of potentially periodontal destruction in LAP patients. More investigations are required to clarify the role of JP2 in both severity and outcomes of periodontal management in people of LAP.

Funding

None.

Conflict of Interest

None declared.

Bibliography

1. Sharn Pal Sandhu., *et al*. "Unilateral gingival fibromatosis with localized aggressive periodontitis (involving first molars): An unusual case report". *Journal of Indian Society of Periodontology* 13.2 (2009): 109-113.

2. Nissen L., *et al*. "*Lactobacillus salivarius* down-regulate *Aggregatibacter actinomycetemcomitans* exotoxins expression". *Annals of Microbiology* 64.2 (2014): 611-617.

3. Balashova, N., *et al*. "*Aggregatibacter actinomycetemcomitans* Leukotoxin Induces Cytosol Acidification in LFA 1 Expressing Immune Cells". *Molecular Oral Microbiology* 31.1 (2016): 106-114.

4. Stähli A., *et al*. "JP2 Genotype of *Aggregatibacter actinomycetemcomitans* in Caucasian Patients: A Presentation of Two Cases". *Pathogens* 9.3 (2020): 178-186.

5. Yoshida A., *et al*. "Quantitative discrimination of *Aggregatibacter actinomycetemcomitans* highly leukotoxic JP2 clone from non-JP2 clones in diagnosis of aggressive periodontitis". *BMC Infectious Diseases* 12.10 (2012): 253-263.

6. Johansson A., *et al*. "Genetic Profiling of *Aggregatibacter actinomycetemcomitans* Serotype B Isolated from Periodontitis Patients Living in Sweden". *Pathogens* 8.3 (2019): 153.

7. Teughels W., *et al*. "Guiding periodontal pocket recolonization: a proof of concept". *Journal of Dental Research* 86.11 (2007): 1078-1082.

8. Armitage G. "Development of a classification system for periodontal diseases and conditions". *Annals of Periodontology* 4.12 (1999): 1-6.

9. Silness J and Lo¨e H. "Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition". *Acta Odontologica Scandinavica* 22.2 (1964): 112-135.

10. Lo¨e H and Silness J. "Periodontal disease in pregnancy I. Prevalence and severity". *Acta Odontologica Scandinavica* 21.12 (1963): 533-551.

11. Ramfjord S. "The periodontal disease index (PDI)". *Journal of Periodontology* 38.6 (1967): 602-610.

12. Jervøe-Storm PM., *et al*. "Comparison of curette and paper point sampling of subgingival bacteria as analyzed by real-time polymerase chain reaction". *Journal of Periodontology* 78.5 (2007): 909-917.

13. Stelzel M and Flores-de-Jacoby L. "Topical metronidazole application compared with subgingival scaling. A clinical and microbiological study on recall patients". *Journal of Clinical Periodontology* 23.1 (1996): 24-29.

14. Orru G., *et al*. "Usefulness of real time PCR for the differentiation and quantification of 652 and JP2 *Actinobacillus actinomycetemcomitans* genotypes in dental plaque and saliva". *BMC Infectious Diseases* 6 (2006): 98-108.

15. Mombelli A., *et al*. "The microbiota of osseointegrated implants in patients with a history of periodontal disease". *Journal of Clinical Periodontology* 22.2 (1995): 124-130.

16. Boutaga K., *et al*. "Comparison of subgingival bacterial sampling with oral lavage for detection and quantification of periodontal pathogens by real-time polymerase chain reaction". *Journal of Periodontology* 78.1 (2007): 79-86.

17. Jervøe-Storm PM., *et al*. "Comparison of culture and real-time PCR for detection and quantification of five putative periodontopathogenic bacteria in subgingival plaque samples". *Journal of Clinical Periodontology* 32.7 (2005): 778-783.

18. Slots J and Ting M. "*Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease: occurrence and treatment". *Periodontology* 20.1 (1999): 82-121.

19. Lopez NJ., *et al*. "Occurrence of A*ctinobacillus actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia* in juvenile periodontitis". *Journal of Clinical Periodontology* 23.2 (1996): 101-105.

20. Chahboun H., *et al*. "Bacterial profile of aggressive periodontitis in Morocco: a cross sectional study". *BMC Oral Health* 24.2 (2015): 15-25.

21. Haubek D., *et al*. "Early onset periodontitis in Morocco is associated with the highly leucotoxic clone of *Actinobacillus actinomycetemcomitans"*. *Journal of Dental Research* 80.6 (2001): 1580-1583.

22. Bandhaya P., *et al*. "*Aggregatibacter actinomycetemcomitans* serotypes, the JP2 clone and cytolethal distending toxin genes in a Thai population". *Journal of Clinical Periodontology* 39.6 (2012): 519-525.

23. Haubek D. "The highly leukotoxic JP2 clone of *Aggregatibacter actinomycetemcomitans:* evolutionary aspects, epidemiology and etiological role in aggressive periodontitis". *APMIS. Supplement* 130.9 (2010): 1-53.

24. Cortelli JR., *et al*. "Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis". *Journal of Clinical Periodontology* 32.8 (2005): 860-866.

25. Haubek D and Johansson A. "Pathogenicity of the highly leukotoxic JP2 clone of *Aggregatibacter actinomycetemcomitans* and its geographic dissemination and role in aggressive periodontitis". *Journal of Oral Microbiology* 14.8 (2014): 6-28.

26. Haubek D., *et al*. "The highly leukotoxic JP2 clone of Actinobacillus actinomycetemcomitans and progression of periodontal attachment loss". *Journal of Dental Research* 83.10 (2004): 767-770.

27. Cortelli SC., *et al*. "Diminished treatment response of periodontally diseased patients infected with the JP2 clone of *Aggregatibacter (Actinobacillus) actinomycetemcomitans". Journal of Clinical Microbiology* 47.7 (2009): 2018-2025.

28. Pihlstrom BC., *et al*. "Periodontal diseases". *Lancet* 19.366 (2005) :1809-1820.

29. Haubek D., *et al*. "Prevalence of JP2 and Non-JP2 Genotypes of *Aggregatibacter actinomycetemcomitans* and Oral Hygiene Practice of Kenyan Adolescents in Maasai Mara". Pathogens 10.4 (2021): 488-497.