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The highly leukotoxic JP2 clone of *Aggregatibacter actinomycetemcomitans* in localized and generalized forms of aggressive periodontitis

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Abstract

Objective: To investigate the presence of *A. actinomycetemcomitans,* including the highly virulent JP2 clone, in young adult patients with aggressive periodontitis, and associate the findings with the two forms of the disease. ***Materials and methods:*** Seventy Moroccan subjects with aggressive periodontitis, aged less than 35 years, were recruited. Among these, 41 had LAgP and 29 had GAgP. Plaque samples were collected from periodontal pockets and examined using a PCR that detects the presence of *A. actinomycetemcomitans* and which differentiates between JP2 and non-JP2 genotypes of the bacterium. ***Results :*** total of 58 (83%) from the 70 AgP patients were positive for *A. actinomycetemcomitans,* among whom 77% were positives for the JP2 clone. The JP2 clone was detected in 34 (83%) of the LAgP patients compared to 20 (69%) of the GAgP patients ($p = 0.17$). Fourteen (20%) of the patients harbored non-JP2 genotypes of *A. actinomycetemcomitans,* although most of these patients (10/14) also harbored the JP2 clone. ***Conclusions:*** The presence of the JP2 clone of *A. actinomycetemcomitans* is strongly associated with both LAgP and GAgP in young adults in Morocco. This implies that treatment of AgP in this population should include microbiological screening and aim at eradication of the bacterium when present.

Key Words: *aggregatibacter actinomycetemcomitans*, aggressive periodontitis, highly leukotoxic, JP2 clone

Introduction

Aggressive periodontitis (AgP) is characterized by rapid and severe periodontal destruction in otherwise healthy individuals. Two forms of AgP, localized AgP (LAgP) and generalized AgP (GAgP), have been distinguished based on the number of teeth affected and the distribution of the lesions within the dentition [1,2]. The presence of *Aggregatibacter actinomycetemcomitans* (previously named *Actinobacillus actinomycetemcomitans*) has been correlated with AgP, including LAgP in particular [3–6]. However, conflicting results for the association between colonization with *A. actinomycetemcomitans* and LAgP compared to GAgP have been reported for different populations in, e.g. the UK, the US, Turkey, Korea, Japan, Colombia, Brazil and Chile [7–15].

A. actinomycetemcomitans expresses several factors that could play a role in the pathogenesis of periodontitis [16,17]. Among these are the leukotoxin, which is a cytotoxic protein that specifically kills a sub-set of leukocytes, including polymorphonuclear leukocytes and peripheral blood monocytes [18]. The production of leukotoxin differs among strains of *A. actinomycetemcomitans* and a particular clone, termed the JP2 clone after the first isolate of this type, has enhanced leukotoxic activity [19]. The JP2 clone of *A. actinomycetemcomitans* has repeatedly been isolated from patients with AgP [20–26]. A very strong association between the presence of the JP2 clone and periodontitis was demonstrated among adolescents in Morocco (OR 29.4; 95% CI = 8.3–104) and in a prospective longitudinal study we showed that infection with the JP2 clone is likely to be important in

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initiation of the disease (RR 18.0; 95% CI = 7.8–41.2) [27,28]. Notably, the JP2 clone of *A. actinomycetemcomitans* shows a pronounced racial tropism for North- and West-African descent [22–29]. Most likely, AgP depends on a number of risk factors, which together contribute to lowering the threshold for initiation of disease [30]. The impact of each of the risk factors may differ in different geographic areas and it may depend on the genetic make-up of both the microorganisms and the human host. Previously, we have suggested that juvenile periodontitis represents two different types of disease with distinct etiologies: one type found worldwide, in which a diversity of *A. actinomycetemcomitans* genotypes may act as an opportunistic pathogen; and another type, primarily found among adolescents of African origin, in which the JP2 clone of *A. actinomycetemcomitans* acts as an exogenous pathogen [20].

Previous reports on the association between presence of *A. actinomycetemcomitans* and the two forms of aggressive periodontitis, LAgP and GAgP, have not included specific detection of the highly virulent JP2 clone, and studies on the prevalence of the JP2 clone of *A. actinomycetemcomitans* in periodontitis have not discriminated between the two forms of the disease. In the present study we assay for presence of *A. actinomycetemcomitans*, including the JP2 clone, in young adult patients with AgP in Morocco and associate the findings to LAgP and GAgP.

Materials and methods

Study population

The study population included 70 young periodontitis patients, aged between 12–35, referred to the Department of Periodontology at the Dental School of Mohamed V Souissi University, Rabat, Morocco. All subjects were verbally informed about the investigation and they all signed informed consent. The study was approved by the Medical Ethical Committee of Mohamed V Souissi University, Rabat, Morocco. The inclusion criteria were: patients not medically compromised, not having received periodontal or antibiotic treatment within the preceding 6 months and not being under orthodontic treatment. Further, patients should have at least 20 teeth present in the oral cavity.

Clinical and radiographic examination

The inflammation was measured using the gingival index [31] and presence of plaque was recorded according to the plaque index by Silness and Löe [32]. The probing depth was measured at six sites around each tooth (mesio-buccal, mesio-lingual, mid-buccal, mid-lingual, disto-buccal, disto-lingual) to the nearest millimeter using a standard periodontal probe (Roenvig, Denmark). Attachment loss was measured to the nearest millimeter from the cemento-enamel junction to the bottom of the periodontal pocket. A full mouth periapical radiographic examination was performed and, together with the clinical recordings, it provided the basis for diagnosing LAgP and GAgP.

The clinical diagnosis of AgP and the classification into LAgP and GAgP was based upon criteria defined by the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions arranged by American Academy of Periodontology [2]. LAgP was defined as a 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. GAgP was defined as generalized interproximal attachment loss affecting at least three teeth other than first molars and incisors.

Bacterial sampling and analysis

From each patient two subgingival plaque samples were collected from periodontal pockets with increased depth (PPD >4 mm), preferentially from first molars. A sterile, absorbent paper point was gently inserted apically into the periodontal pocket. After 20 s, the paper was removed and placed in a tube containing 1 ml of 0.9% (wt/vol) NaCl. All plaque samples were collected by the same examiner (O.-K.E.).

Samples were sent to the Department of Medical Microbiology and Immunology, Aarhus University, Denmark, for PCR analysis. Samples were processed and analyzed by PCR for the presence of *A. actinomycetemcomitans* as described previously [33]. Notably, the PCR detects the presence of the bacterium and distinguishes between the JP2 clone and non-JP2 genotypes based on distinct sizes of the amplicons, because members of the JP2 clone have a characteristic deletion in the promoter of the *ltx* operon.

Data analysis

The statistical analyses were carried out using the SPSS.13.0 program (SPSS® 13.0, Chicago, IL). The subject was taken as the statistical unit for all analyses. Gender was expressed by percentage. Demographic variable (age) and clinical parameters (plaque index and gingival index) were expressed as mean values and standard deviations. Clinical parameters were calculated as the mean value of recordings of teeth present in the oral cavity. The detection of the JP2 clone and non-JP2 genotypes of *A. actinomycetemcomitans* in the various groups were compared using Chi-2 test. $p < 0.05$ was used as the significance level. A subject was designated positive for *A. actinomycetemcomitans* of the JP2 clone and/or non-JP2

genotypes if at least one of the two samples was positive in the PCR and negative if both samples were negative.

Results

Among the 70 patients with AgP, 41 were classified as LAgP and 29 as GAgP, respectively. The mean age was similar in the two disease categories (Table I). Females were predominant in the study population. GAgP patients had a slightly higher plaque and gingival index than those with LAgP (Table I).

Among the 70 AgP patients, 58 (83%) were positive for *A. actinomycetemcomitans* and 54 (77%) carried the JP2 clone. The presence of *A. actinomycetemcomitans* was significantly associated with LAgP compared to GAgP (OR 0.17; 95% confidence interval 0.04–0.72) ($p = 0.009$) (Table II).

In the LAgP and the GAgP patients the JP2 clone, either alone or with non-JP2 clone, was detected in 83% and 69%, respectively, and the difference between the two groups of patients is not statistically significant ($p = 0.17$). However, the presence of the JP2 clone alone was associated with LAgP ($p = 0.034$). The presence of the JP2 clone together with a non-JP2 genotype of *A. actinomycetemcomitans* was detected in 10 (14%) of the patients, whereas only four (5.7%) patients carried non-JP2 genotypes alone and there was no significant difference between the two disease categories (Table II).

Discussion

This study included a large group of young adults diagnosed as having AgP. Subgingival presence of *A. actinomycetemcomitans*, including sub-division into JP2 and non-JP2 genotypes, was related to periodontal disease status. The participants were recruited from the Dental School, Rabat, Morocco, and patients were divided into sub-categories of disease in terms of GAgP and LAgP based on clinical parameters.

In all groups there was an uneven distribution by gender with a majority of women (Table 1), which is likely to reflect a bias in the study population in that Moroccan women rather than men tend to turn to the dentist for cosmetic reasons. In a previous cross-sectional study on school children in the same population we found no difference according to gender in development of clinical attachment loss [28].

Table I. Characteristics of the study population according to periodontal status groups. Values are presented as means, with standard deviations in parentheses.

	Localized aggressive periodontitis ($n = 41$)	Generalized aggressive periodontitis ($n = 29$)
Age (years), *M* (SD)	19.3 (4.4)	21.6 (3.4)
Gender (% female)	79%	89%
Plaque Index, *M* (SD)	1.5 (0.5)	1.9 (0.6)
Gingival Index, *M* (SD)	1.5 (0.6)	1.8 (0.5)

Table II. Presence of JP2 and non-JP2 genotypes of *A. actinomycetemcomitans* (*Aa*).

Microbiological findings	Localized aggressive periodontitis	Generalized aggressive periodontitis	*p*
Presence of Aa	38 (83%)	20 (69%)	0.0095
JP2 clone alone	30 (73%)	14 (48%)	0.034
Co-presence of JP2 and non-JP genotypes of *Aa*	4 (9.8%)	6 (21%)	0.198
Non-JP2 genotypes of *Aa* alone	4 (9.8%)	0 (0%)	0.083

The presence of *A. actinomycetemcomitans* in subgingival plaque samples was assayed by PCR. The used primers flank the deletion in the *ltx* promoter region, which is characteristic for strains of the JP2 clone and thereby it was possible to discriminate between colonization with JP2 strains and other genotypes of the species. We have previously found that this PCR is highly specific and superior to cultivation in demonstrating the presence of *A. actinomycetemcomitans* in such samples [33].

A. actinomycetemcomitans, including the JP2 clone, has been associated with the initiation of AgP in adolescents [23,28,34,35]. In the present study a very strong association between AgP in the young adults and the presence of JP2 clone of *A. actinomycetemcomitans* was found. Among the 70 AgP patients, 54 (77%) were infected with the JP2 clone. Thus, the JP2 clone is also associated with the more advanced stages of the disease. In the same population we have previously found that, among 39 adolescents, aged 14–19 years and diagnosed as having early-onset periodontitis, 15 (38%) were positive for the JP2 clone by cultivation [27]. The population included in the present study is slightly older and the method used for detection of the bacterium is more sensitive. Thus, the difference could be partly explained by these differences between the two studies. Alternatively, some of the adolescents may have developed mild disease due to transient infection with the JP2 clone, as it has been found that over a 2-year period approximately half of this age group was temporarily colonized [36], whereas those persistently infected are supposed to develop more severe disease over time, as presumed for the slightly older patient sample included in the present study. In support of this, elimination of the JP2 clone has been shown to correlate with diminishing disease activity [37]. An association between the presence of the JP2 clone of *A. actinomycetemcomitans* and severity of disease among young adults with AgP is in agreement with results from a previous study on a Brazilian

population, where the JP2 clone is also endemically present [26].

Non-JP2 genotypes of *A. actinomycetemcomitans* were detected in 14 (20%) of the 70 AgP patients and among these 10 were also positive for the JP2 clone. Thus, in agreement with previous conclusions for Moroccan adolescents, colonization with non-JP2 genotypes alone shows a weak or no association with AgP in this population [27,28]. Among the LAgP patients there was a significantly higher frequency of the presence of *A. actinomycetemcomitans* compared to those with GAgP (OR 0.17; 95% confidence interval 0.04–0.72) ($p = 0.009$) (Table II). Previously, a significant increase in presence of *A. actinomycetemcomitans* in shallow and intermediate but not in deep periodontal pockets has been found in subjects with LAgP in comparison with those with GAgP [15]. These observations may be explained by the deepening of the pockets and that increase in the anaerobic environment may favor the growth of other periodontal pathogens, such as the strict anaerobic species. There was a tendency of co-presence of JP2 and non-JP2 genotypes to be more prevalent in GAgP, suggesting interference between the different genotypes of *A. actinomycetemcomitans*. However, these groups are rather small in the present study and the PCR used is not quantitative, which precludes firm conclusions on this aspect.

Previously, studies on the association between the presence of *A. actinomycetemcomitans* and the two forms of disease, LAgP and GAgP, were based on populations where only non-JP2 genotypes of the bacterium are found and conflicting results for different populations have been reported. The present study includes a very high number of AgP patients from an area where the JP2 clone of *A. actinomycetemcomitans* is endemically present. We show that among young adults in Morocco the JP2 clone is very strongly associated with both LAgP and GAgP and there is a tendency of this particular bacterium to be more prevalent in LAgP. Accordingly, we suggest that the two clinical diagnoses may merely represent different stages of the same disease and changes in the ecological niche in GAgP representing the more advanced stages of the disease may be disadvantageous to *A. actinomycetemcomitans*. Infection with the JP2 clone was found in 83% and 69% of the LAgP and GAgP patients, respectively.

The present study shows an association between infection with the JP2 clone of *A. actinomycetemcomitans* and the two forms of AgP. However, as periodontitis are not mono-infectious diseases, looking for other periopathogens is necessary to clarify the microbial etiology of this disease in our Morocco. Nevertheless, with the limits of our data, and with regard of previous studies, the presence of a JP2 clone of *A. actinomycetemcomitans* in aggressive periodontitis is a fact. Thus, eradication of such a virulent bacterium should be considered for treatment in this population.

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