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Attachment loss in Moroccan early onset periodontitis patients and infection with the JP2-type of *Actinobacillus actinomycetemcomitans*

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Abstract

Background: A clone of *Actinobacillus actinomycetemcomitans* (JP2) with increased leukotoxin production and characterized by a 530-bp deletion in the leukotoxin gene operon is endemically present in Morocco and strongly associated with the presence of early onset periodontitis (EOP).

Objectives: To compare patterns of attachment loss among EOP-patients with or without JP2-type of *A. actinomycetemcomitans* in dental plaque.

Material and methods: Among 45 Moroccan adolescents with EOP (i.e. one or more teeth with attachment loss ≥3 mm) 39 had cultivable plaque samples. Fifteen (38.5%) were culture-positive for *A. actinomycetemcomitans* of the JP2-type as determined by PCR, and 24 (61.5%) were not (mean age 16.5 years in both groups).

Results: EOP-patients culture-positive for *A. actinomycetemcomitans* of the JP2-type had significantly more teeth with attachment loss (mean 5.1, median 4.0) than EOP-patients not culture-positive for *A. actinomycetemcomitans* of the JP2-type (mean 2.8 teeth, median 1.0) ($p = 0.02$), and higher attachment loss (mean 4.3 mm vs. 3.4 mm; median 4.0 mm vs. 3.0 mm) ($p = 0.01$). No major differences could be detected between the two groups in the pattern of affected teeth in the dentition.

Conclusions: The study demonstrates increased periodontal destruction among EOP-patients culture-positive for *A. actinomycetemcomitans* of the JP2-type compared with EOP-patients without the JP2-clone.

Key words: Actinobacillus actinomycetemcomitans; attachment loss; early onset periodontitis; JP2; leukotoxin

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In 1994, Brogan and coworkers showed that *A. actinomycetemcomitans* strains characterized by a 530-bp deletion in the promoter region of the leukotoxin gene (*ltx*) operon, called JP2-type, had an increased production of leukotoxin in contrast to *A. actinomycetemcomitans* strains without this deletion (Brogan et al. 1994). Recently, other variations in the *ltx* operon among highly leukotoxic *A. actinomycetemcomitans* strains have been identified (He et al. 1999).

A. actinomycetemcomitans strains characterized by the 530-bp deletion has been isolated from African immigrants in European countries (Haubek et al. 1996, 1997). Recently, it was confirmed that *A. actinomycetemcomitans* of the JP2-type is endemically present in Morocco and strongly associated with presence of EOP (Haubek et al. 2001).

Due to the relatively high leukotoxic-

ity of *A. actinomycetemcomitans* with the 530 bp-deletion, the clinical manifestation of EOP in individuals with JP2-type of *A. actinomycetemcomitans* may differ from that of EOP in individuals without *A. actinomycetemcomitans* of the JP2-type.

The aim of the present study was to compare the extent, severity, and pattern of attachment loss among EOP-patients with or without the JP2-type of *A. actinomycetecomitans* in dental plaque.

Material and methods

This study includes 45 Moroccan adolescents with EOP (i.e. attachment loss ≥ 3 mm on one or more teeth) identified during a cross-sectional study of 301 Moroccan adolescents aged 14–19 years from eight public schools in Rabat (Haubek et al. 2001). After informed consent participants were given a full mouth periodontal examination including probing of attachment levels of mesial and distal sites from both the buccal and palatal/lingual aspect. Attachment level for each tooth was determined as the maximum score of the four registrations obtained per tooth. All periodontal recordings were done by the same examiner (O.-K.E.), who had demonstrated a high intraexaminer reliability (Haubek et al. 2001).

Plaque samples were collected as pooled samples from two sites selected on the basis of criterias previously described (Haubek et al. 2001). The majority of sites selected (90.0%) were on permanent first molars and incisors. On seven (15.6%) of 45 patients, one of the two sites selected per individual was on a premolar, the plaque sample from one individual (2.2%) was taken from periodontal pockets around a canine and a second molar, while the remaining 37 (82.2%) plaque samples were collected from pockets arround molars and incisors. Six of 45 plaque samples were excluded due to the primary cultures being either overgrown by yeast or showing no growth.

A. actinomycetecomitans was identified and analyzed for presence of the 530-bp deletion in the promoter region of the *ltx* operon by polymerase chain reaction (PCR) using methods previously described (Haubek et al. 2001). Briefly, plaque samples were grown on Slots selective medium (Slots 1982) and one isolate of *A. actinomycetecomitans* per individual, identified according to characteristic colony morphology and positive catalase reaction, was cultured and used for PCR (Haubek et al. 2001).

A total of 15 (38.5%) out of the 39 EOP-patients were culture-positive for *A. actinomycetemcomitans* of the JP2-type. Individuals without JP2-type of *A. actinomycetecomitans* could be colonized either by other clonal types of *A. actinomycetemcomitans* or without detectable *A. actinomycetemcomitans.* There was no difference in age between EOP-patients with and without JP2-type of *A. actinomycetemcomitans* (mean 16.5 years, SD 1.2 and 16.5 years, SD 1.4).

Only 27 teeth (2.1% of all teeth) were missing among the 45 individuals.

Mean attachment loss per individual was calculated as mean attachment loss on affected teeth (i.e. teeth with attachment loss ≥ 3 mm).

Results

Of a total of 180 teeth with attachment loss ≥ 3 mm among 45 adolescents participating in the study, 90 teeth (50.0%) had loss of attachment of ≥ 3 mm on both oral and buccal aspects, while 60 teeth (33.3%) had loss of attachment of ≥ 3 mm from the buccal aspect only, and 30 teeth (16.7%) from the oral aspect only.

Table 1 gives the distribution of EOP-patients (n = 39) with and without JP2-type of *A. actinomycetemcomitans* in dental plaque according to number of teeth with attachment loss ≥ 3 mm. Six EOP-patients without cultivable plaque samples had a total of 37 teeth with attachment loss ≥ 3 mm.

EOP-patients culture-positive for *A. actinomycetemcomitans* of the JP2-type had more teeth with attachment loss ≥ 3 mm than EOP-patients not culture-positive for *A. actinomycetemcomitans* of the JP2-type (Mann–Whitney; $U = 101.5$; $p = 0.02$) (Table 2). In EOP-patients culture-positive for *A. actinomycetemcomitans* of the JP2-type, teeth with attachment loss had a statistical significantly higher attachment loss than teeth in EOP-patients with no detectable *A. actinomycetemcomitans* of the JP2-type (Mann–Whitney; $U = 76.5$; $p = 0.01$) (Table 2).

The proportion of teeth with attachment loss for each tooth in the dentition in EOP-patients with and without *A. actinomycetemcomitans* of the JP2-type is depicted in Fig. 1. Of the 28 teeth in the dentition, 20 were affected in both carriers and non-carriers of the JP2 clone, three were affected only in individuals without the JP2-clone, one was affected only in individuals with the JP2-clone and four were not affected at all. Thus, the overall pattern of attachment loss within the dentition among individuals with and without *A. actinomycetemcomitans* of the JP2-type was very similar. It should, however, be noted that not only the classical sites for EOP (i.e. incisors and molars), but also premolars were affected in both groups.

Table 1. Distribution of 39 EOP-patients with and without JP2-type of *A. actinomycetemcomitans* (*A.a*) in dental plaque according to number of teeth with attachment loss ≥ 3 mm. Numbers in parentheses are percentages

Number of teeth with attachment loss ≥ 3 mm	Frequency of EOP-patients with JP2-type of *A. a* (n = 15)	Frequency of EOP-patients without JP2-type of *A. a* (n = 24)
1	3 (20.0)	14 (58.3)
2	2 (13.3)	2 (8.3)
3	1 (6.7)	1 (4.2)
4	3 (20.0)	3 (12.5)
5	1 (6.7)	2 (8.3)
7	1 (6.7)	1 (4.2)
8	1 (6.7)	0
9	1 (6.7)	0
11	1 (6.7)	0
15	1 (6.7)	0
16	0	1 (4.2)

Discussion

The present clinical epidemiological study has demonstrated that EOP-patients infected with the JP2-type of *A. actinomycetemcomitans* have more teeth with attachment loss and that attachment loss on affected teeth is more pronounced compared to EOP-patients without the JP2-type of *A. actinomycetemcomitans* in dental plaque. The location of attachment loss in the dentition

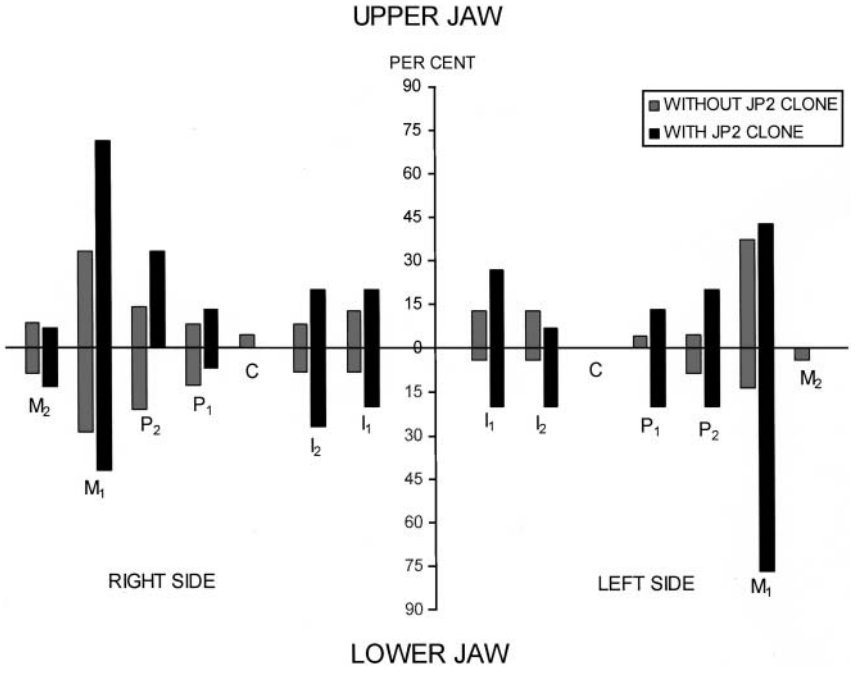

Fig. 1. Proportion of individual teeth in the dentition with attachment loss ≥3 mm among EOP-patients with or without the JP2-type of *A. actinomycetemcomitans* in dental plaque

according to tooth types is, however, the same in EOP-patients with and without JP2-type of *A. actinomycetemcomitans.* Thus, infection with the JP2 clone is not only a risk factor of EOP (Haubek et al. 2001), but patients harboring this clone also have more advanced stages of disease than patients without the clone.

In spite of the small number of individuals in the two groups, the present study has been able to demonstrate statistically significant differences in disease level in relation to infection with the JP2 clone. This is indicative of the high virulence of the clonal type of *A. actinomycetemcomitans* characterized by the 530-bp deletion in the *ltx* operon.

Among the 39 individuals with cultivable plaque samples, 10 showed no detectable *A. actinomycetemcomitans,* 14 were colonized by clonal types of *A. actinomycetemcomitans* without 530-bp deletion, and four were culture-positive for *A. actinomycetemcomitans* both with and without the 530-bp deletion. Only one isolate per sample was cultivated. However, due to the sticky nature of *A. actinomycetemcomitans,* two different clonal types can be found by the sensitive PCR technique even after several cultivations (Haubek et al. 1997). As *A. actinomycetemcomitans*, in general, is considered an opportunistic pathogen in periodontal disease (Haubek et al. 1995), our analyses of the clinical findings have been focusing only on *A. actinomycetemcomitans* strains with the 530-bp deletion, having properties as a traditional pathogen.

In studies on EOP, patients are usually classified as having localized or generalized attachment loss according to criteria such as those of Baer (1971), Genco et al. (1986) and Löe & Brown 1991). Since it has been shown that attachment loss may also progress in patients with incidental attachment loss (Albandar et al. 1997), we decided not to use any of the previously suggested classification systems, but simply to define EOP-patients as individuals with loss of attachment loss ≥3 mm on one or more teeth. In our study, three (17.6%) out of 17 individuals with cultivable plaque samples and only one tooth with attachment loss, of which one was a very severe, localized defect on a first molar with a pocket depth of 11 mm, were culture-positive for *A. actinomycetemcomitans* of the JP2-type. Thus, relatively rapid progression of EOP in these individuals is expected, and our study supports the importance of considering even individuals with only one site with attachment loss ≥3 mm in the diagnosis of EOP.

Although the JP2-type of *A. actinomycetemcomitans* has the properties of a traditional pathogen, it remains obscure why periodontal destruction apparently follows a distinct pattern in the dentition. In the present study, attachment loss was found not only on the classical sites for EOP (first molars and incisors), but also on premolars. This might indicate that disease progression is faster in this population than in other populations. Longitudinal data are necessary in order to gain a better understanding of the progression of periodontal destruction in this population.

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Table 2. Number of affected teeth per individual and attachment loss in millimeter (mm) per affected tooth in individuals with and without JP2-type of *A. actinomycetemcomitans*

Microbiological findings	Number of individuals, n	Number of affected teeth		Attachment loss (mm)	
		Mean (SD)	Median (1st and 3rd quartile)	Mean (SD)	Median (1st and 3rd quartile)
Plaque samples with JP2-type of *A. a*	15	5.1 (4.1)	4.0 (2.0 and 8.0)	4.3 (1.2)	4.0 (3.3 and 5.4)
Plaque samples without JP2-type of *A. a*	24	2.8 (3.3)	1.0 (1.0 and 4.0)	3.4 (0.8)	3.0 (3.0 and 3.3)

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Zusammenfassung

Attachmentverlust bei marokkanischen Patienten mit aggressiver Parodontitis und Infektion mit dem JP2-Klon von Actinobacillus actinomycetemcomitans

Hintergrund: Ein Klon von *Actinobacillus actinomycetemcomitans* (JP2) mit erhöhter Leukotoxinproduktion, der durch eine 530-bp Deletion im Leukotoxingen-Operon charakterisiert ist, kommt in Marokko endemisch vor und ist stark mit dem Auftreten aggressiver Parodontitis (AP) assoziiert.

Zielsetzung: Vergleich der Attachmentverlustmuster bei AP-Patienten mit bzw. ohne den JP2-Klon von *A. actinomycetemcomitans* in bakterieller Plaque.

Material und Methoden: Von 45 marokkanischen Jugendlichen mit AP (d.h., ein oder mehr Zähne mit Attachmentverlust ≥ 3 mm) konnten bei 39 kultivierbare Plaqueproben gewonnen werden. Bei 15 AP-Patienten (38,5%) konnte der JP2-Klon mittels Polymerasekettenreaktion aus der Kultur nachgewiesen werden und bei 24 (61,5%) nicht. Das durchschnittliche Alter in beiden Gruppen lag bei 16,5 Jahren.

Ergebnisse: AP-Patienten, bei denen der JP2-Klon von *A. actinomycetemcomitans* nachweisbar war, wiesen statistisch signifikant mehr Zähne mit Attachmentverlust auf (Mittelwert 5,1; Median 4,0) als AP-Patienten, ohne JP2-Klon (Mittelwert 2,8 Zähne; Median 1,0) (p=0,02), und hatten höhere Attachmentverluste (Mittelwert 4,3 mm/3,4 mm; Median 4,0 mm/3,0 mm) (p=0,01). Es konnten keine deutlichen Unterschiede zwischen beiden Gruppen hinsichtlich der Verteilung der erkrankten Zähne innerhalb der Dentition festgestellt werden.

Schlussfolgerungen: Die Untersuchung zeigte stärkere parodontale Destruktionen bei AP-Patienten, in deren Plaqueproben der JP2-Klon von *A. actinomycetemcomitans* nachgewiesen werden konnte, im Vergleich zu AP-Patienten ohne JP2-Klon.

Résumé

Mots clés: Parodontite précoce; perte d'attache; leucotoxine; JP2; Actinobacillus actinomycetemcomitans

Origine: Un clone d'*Actinobacillus actinomycetemcomitans* (JP2) avec production accrue de leucotoxines et caractérisé par la délétion 530-bp dans l'opéron du gène de la leucotoxine est endémique au Maroc et étroitement associé à l'existence de parodontites précoces (*early-onset periodontitis,* EOP).

Buts: Comparer les modèles de perte d'attache parmi les patients EOP, avec ou sans *A. actinomycetemcomitans* de type JP2 dans la plaque dentaire.

Matériaux et méthodes: Parmi 45 adolescents marocains atteints d'EOP (c'est-à-dire ayant une ou plusieurs dents avec une perte d'attache ≥3 mm), 39 présentaient des échantillons de plaque cultivables. La PCR a montré que quinze d'entre eux (38,5%) étaient positifs à la culture pour *A. actinomycetemcomitans* de type JP2, alors que 24 (61,5%) ne l'étaient pas (moyenne d'âge: 16,5 ans pour les deux groupes).

Résultats: Les patients EOP positifs à la culture pour *A. actinomycetemcomitans* de type JP2 avaient significativement plus de dents avec perte d'attache (moyenne: 5,1; médiane: 4,0) que les patients EOP non positifs à la culture pour *A. actinomycetemcomitans* de type JP2 (moyenne: 2,8; médiane: 1,0) (p=0,02), ainsi qu'une perte d'attache plus élevée (moyenne: 4,3 mm contre 3,4 mm; médiane: 4,0 mm contre 3,0 mm) (p=0,01). Aucune différence majeure n'a pu être mise en évidence entre les deux groupes au niveau de la répartition des dents affectées dans la dentition.

Conclusions: Cette étude met en évidence une destruction parodontale accrue chez les patients EOP positifs à la culture pour *A. actinomycetemcomitans* de type JP2 par rapport aux patients EOP sans le clone JP2.

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