Clinical and Microbiologic Study of Periodontitis Associated With Kindler Syndrome

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Background: Little is known about the onset and prevalence of periodontal disease in patients with the rare Kindler syndrome, a genodermatological disorder. This study investigated the level of clinical periodontal attachment in relation to age and presence of putative periodontopathogenic bacteria in individuals with Kindler syndrome.

Methods: Eighteen individuals diagnosed with Kindler syndrome and 13 control subjects, aged 4 to 37 years, from rural Panama received a limited clinical periodontal examination. Subgingival samples were collected for identification of putative periodontal pathogens by polymerase chain reaction.

Results: Mild to severe gingivitis was a common finding in all adults of the study population. Seventy-two percent (13/18) of the Kindler patients and 46% (6/13) of the control subjects showed mild to severe periodontal disease (P = 0.001, chi-square test). The onset of periodontitis was earlier and the progression occurred at a faster rate in the Kindler group. There was a strong correlation (r = 0.83) between the level of attachment loss and age in the Kindler group and a weaker correlation (r = 0.66) in the control group. The appearance of gingival tissues suggested atypical periodontitis with spontaneous bleeding and fragile, often desquamative, gingiva. In periodontitis patients, Porphyromonas gingivalis and Dialister pneumosintes tended to occur more frequently in control individuals compared to those with Kindler syndrome.

Conclusions: In the Kindler group, periodontitis had an onset in early teenage years and progressed more rapidly compared to non-Kindler individuals of the same geographic and ethnic group. Clinical and microbiological findings suggest atypical periodontitis in Kindler patients. We propose to include Kindler syndrome in the category of medical disorders predisposing to destructive periodontal disease. J Periodontol 2003;74:25-31.

KEY WORDS
Disease progression; Kindler syndrome; periodontal attachment loss; periodontal diseases/pathogenesis.

Kindler syndrome is a rare vesiculobulbular dermatological disorder characterized by acral bullae in infancy and early childhood, generalized progressive poikiloderma, and diffuse cutaneous atrophy,1 first described in a single patient by Kindler in 1954. Other features that vary between cases include photosensitivity,2-4 acral hyperkeratosis; nail dystrophy;3,5 webbing and contractures of the fingers and toes;4,6,7 alopecia;5,6,7 actinic changes;8 and mucosal involvement including urethral,5,7,9 vaginal, anal,5,7 esophageal, and oral commissure10 stenosis as well as ectropion of the eyelids,5,9,10 pigmentation of the lips,5 and onychodystrophy.4,6,9

Kindler1 questioned whether her patient might actually have had a combination of epidermolysis bullosa and poikiloderma congenitale rather than a new disease entity. Although there is overlap of the clinical findings between Kindler syndrome and epidermolysis bullosa, and the genetic mutation(s) responsible for Kindler syndrome is yet to be determined, it is generally agreed that the ultrastructural findings of Kindler syndrome are unique and are not observed in inherited epidermolysis bullosa.6,11,12 In Kindler syndrome, there is an unusual interruption and reduplication of the basement membrane11-14 and a broad reticular pattern of type VII collagen staining deep into the connective tissue beneath the basement membrane.6,14 It has also been suggested that Kindler syndrome and Weary’s hereditary acrokeratotic poikiloderma (HAP)
are variants of the same disease.\textsuperscript{2,5,15} However, there seem to be differences in mode of inheritance, photosensitivity, onset of blistering, and the presence of eczema in these 2 syndromes. The level of ultrastructural cleavage for blistering appears to be junctional in Kindler syndrome and intraepidermal in HAP.\textsuperscript{2,6,14} Accordingly, existing evidence suggests that HAP and Kindler syndrome are separate entities, although the disease-related mutations will have to be determined to confirm this. Approximately 70 cases of Kindler/HAP have been reported, of which 26 seem to fulfill the present consensus clinical description of Kindler syndrome. This paper considers only cases that are consistent with the current understanding of the clinical presentation of Kindler syndrome.

Dental findings have been briefly reported for Kindler patients in dermatologic and pediatric publications including oral lesions,\textsuperscript{3} atrophy of the buccal mucosa,\textsuperscript{3,10} limited oral opening,\textsuperscript{3,13,16,17} malocclusion,\textsuperscript{3} caries,\textsuperscript{6,17} dystrophic teeth,\textsuperscript{7,9} ankyloglossia,\textsuperscript{3} bleeding gingiva,\textsuperscript{3,10,17-20} lip erosions and leukokeratosis,\textsuperscript{3,4,19} geographic tongue,\textsuperscript{18} atrophy of the gingiva,\textsuperscript{3,17} erosion of the hard palate,\textsuperscript{5} gingival swelling,\textsuperscript{10,18,19} and desquamative gingivitis.\textsuperscript{10}

We and others have reported on isolated cases of Kindler syndrome with aggressive periodontitis.\textsuperscript{10,21} With a single affected case,\textsuperscript{21} it remained uncertain whether periodontal disease is associated with this syndrome or is just a coincidence with no link to the genetic defects affecting these individuals. Recently, more than 20 cases of Kindler syndrome have been identified in a rural area of the Bocas del Toro province of Panama. The purpose of this study was to determine in this large group of subjects with Kindler syndrome the extent to which periodontal disease is associated with Kindler syndrome. In addition, a polymerase chain reaction (PCR)-based microbial identification system was used to determine whether subjects with Kindler syndrome harbored specific subgingival putative periodontal pathogens.

**MATERIALS AND METHODS**

Subjects were examined at the Centro De Salud de Punta Peña, Ministry of Health, Panama. Eighteen individuals with Kindler syndrome volunteered to receive limited oral examination including oral photography, cancer screening, charting of missing teeth and tooth mobility, half-mouth periodontal probing, and clinical attachment level determination. A similar protocol was carried out in 13 non-Kindler individuals from the same ethnic group and geographical location, most of whom were related to at least one of the Kindler patients. The study was approved by the Clinical Research Ethics Board of the University of British Columbia. All patients signed consent forms prior to the examinations. It was not possible to perform a complete periodontal examination with diagnostic radiographs due to the limited time available and the lack of radiographic equipment. The greatest clinical attachment loss from the half-mouth probing examination was used for comparison between the Kindler and control groups.

A subgingival sample was obtained from the opposite side of the mouth in areas that corresponded to the deeper sites from the contralateral side. Three sites were sampled in each patient. Prior to sampling, supragingival plaque was removed with a cotton gauze, and the sample site was isolated with cotton rolls. Three medium endodontic paper points were placed in the sulcus/pocket for 20 seconds and then transferred to an empty sterile plastic vial for bacterial PCR analysis.

PCR analysis was performed without knowledge of the clinical features of the subjects or sites.

DNA extraction was performed as previously described.\textsuperscript{22} In brief, the subgingival plaque specimens were diluted with distilled water to a final volume of 0.5 ml and homogenized by vortex mixing for 1 minute. The paper points were then removed, and the bacterial cells collected by centrifugation at 10,000 × g for 5 minutes and washed twice with distilled water. After the final wash, the bacterial pellet was resuspended in 0.3 ml distilled water, boiled for 10 minutes, quickly chilled on ice for 2 minutes, and centrifuged at 10,000 × g for 10 minutes to remove unbroken cells and large debris. Five µl of the sample was used as the nucleic acid template in bacterial PCR amplification.

The bacterial PCR detection was based on the amplification of signature sequences of bacterial 16S rRNA genes, as previously described.\textsuperscript{22,23} A total of 50 µl of PCR reaction mixture contained 1 × PCR buffer, 1.25 units of *Thermus aquaticus* (Taq) DNA polymerase,\textsuperscript{¶} 0.2 mM each of deoxynucleotidetriphosphates,\textsuperscript{¶} 1.0 µM of each primer, and 1 mM MgCl\textsubscript{2} for *Prevotella intermedia*, *Prevotella nigrescens*, and *Actinobacillus actinomycetemcomitans* or 1.5 mM of MgCl\textsubscript{2} for *Porphyromonas gingivalis*, *Bacteroides forsythus*, *Dialister pneumosintes*, and *Treponema denticola*.

Amplification was performed in a DNA thermal cycler.\textsuperscript{§} Temperature profile for *P. gingivalis*, *B. forsythus*, and *T. denticola* included an initial step of 95°C for 2 minutes, followed by 36 cycles of 95°C for 30 seconds, 60°C for 1 minute, 72°C for 1 minute, and a final step of 72°C for 2 minutes. Temperature profile for *D. pneumosintes*, *P. intermedia*, *P. nigrescens*, and *A. actinomycetemcomitans* included an initial step of 95°C for 2 minutes, followed by 36 cycles of 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 2 minutes, and a final step of 72°C for 10 minutes. Positive and negative controls were routinely included.

\textsuperscript{¶} Promega, Madison, WI.

\textsuperscript{¶} Pharmacia LKB, Piscataway, NJ.

\textsuperscript{§} PTC-100, MJ Research, Boston, MA.
PCR products were analyzed by 1.5% agarose gel electrophoresis conducted at 4 V/cm in Tri-acetate EDTA buffer. Gels were stained with 0.5 µg/ml of ethidium bromide and photographed under 300 nm UV light. One-kb DNA ladder digest** served as the molecular size marker.

RESULTS
The skin of the Kindler patients demonstrated the typical atrophic changes of wrinkling and xeroderma, particularly of the hands and feet (Fig. 1A). Open lesions on the skin were apparent especially in younger patients (Fig. 1B).

Screening for oral and perioral cancer in the Kindler and the non-Kindler patients revealed no signs of premalignant or malignant lesions. The basic jaw movements, including the range of oral motion and opening, demonstrated no abnormalities. Oral hygiene in all subjects was poor, and visible plaque scores were close to 100% (Fig. 2). In addition, dental calculus was frequently present in both groups. Gingivitis was moderate to severe in most individuals. Clinical views of a control subject and 5 subjects with Kindler syndrome (aged 12 to 37 years) are presented in Figure 2. Typical clinical changes of gingiva in individuals with Kindler syndrome included spontaneous bleeding, redness, transparent appearance of attached gingiva, pus excretion, and severe attachment loss at early adulthood.

Definition criteria for periodontitis in this study was loss of clinical attachment of at least 4 mm. Based on this criterion, 13 of the 18 (72%) Kindler subjects and 6 of the 13 (46%) control subjects had periodontitis (Table 1). The median age for Kindler patients with periodontitis was 17 years, and the only Kindler individuals who were periodontitis-free were younger than 10 years of age. In the control group, individuals with periodontitis had a mean age of 35 years, and only one subject under age 20 showed periodontitis. Although both groups demonstrated a positive correlation

Figure 1.
Typical clinical presentations of skin in Kindler patients. A. Atrophic changes to skin associated with feet of 13-year-old female; B. an open lesion on forearm of 10-year-old male.

Figure 2.
Typical clinical appearance of gingival tissues in 32-year-old control individual (A) and patients with Kindler syndrome (B, 12-year-old; C, 13-year-old; D, 19-year-old; E, 26-year-old; and F, 37-year-old).

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between age and the level of attachment loss, the subjects with Kindler syndrome showed earlier and accelerated attachment loss over time (\(P<0.001\), chi-square test, Fig. 3). Kindler subjects had lost an average of about 6 mm of clinical attachment by age 20, while it was estimated to take 60 years to accumulate the same level of destruction in the control groups (Fig. 3). There was equal representation of both females and males in periodontitis groups with or without Kindler syndrome (Table 1).

Bacterial samples were collected from 17 Kindler and 11 non-Kindler subjects (Table 2). On average, 2 pathogens were detected in Kindler patients who had periodontitis versus only 1 periodontal pathogen in the periodontally healthy Kindler subjects (\(P=0.05\), chi-square test). The chronic periodontitis patients showed an average of 3 periodontal pathogens, and the periodontally healthy patients an average of 1 pathogen (\(P=0.001\), chi-square test). The prevalence of both \(P.\) gingivalis and \(D.\) pneumosintes in individuals with Kindler syndrome and periodontitis was relatively low (31%). In contrast, the prevalence of \(P.\) gingivalis was high in the control group with periodontitis (80%), followed by \(T.\) denticola (60%), \(D.\) pneumosintes (60%), and \(P.\) nigrescens (40%). All other putative periodontal pathogens studied were present in similar frequency in both periodontitis groups (Table 2). Control subjects with no periodontitis harbored only a few pathogens. Interestingly, \(A.\) actinomycetemcomitans was present in 50% of control subjects who did not have periodontitis, while it was infrequently present in both groups with periodontitis (Table 2).

**DISCUSSION**

Previous publications describing the Kindler syndrome included only a few subjects, as the disease is a rare genetic disorder. Only 2 publications have included periodontal examinations.\(^{10,21}\) Although a link between periodontal disease and Kindler syndrome has been suggested in these case reports, comprehensive analysis has been impossible given the low number of study subjects. Discovery of a relatively large group of individuals with Kindler syndrome in a remote, geographically isolated area of Panama allowed us to assess the link between Kindler syndrome and destructive periodontal disease.

For practical reasons, the present study used half-mouth clinical attachment level measurements. Par-
Partial-mouth scores are often employed in epidemiologic studies to estimate the prevalence and severity of periodontal disease. Partial-mouth periodontal recordings tend to underestimate the prevalence of disease, but the half-mouth examination design is believed to produce relatively unbiased estimates of disease severity in comparison to using selected teeth or only facial probing measurements. Because of severe gingivitis in most individuals, it was not possible to obtain bacterial samples from the areas that were probed, due to profuse bleeding. It was decided, therefore, to identify the deepest probing sites on one side and then use them as a predictor of deep sites on the contralateral teeth. Preliminary examination suggested that the Kindler subjects exhibited largely similar patterns of periodontal destruction on both sides of the mouth.

The major finding of this study was that individuals with Kindler syndrome develop periodontitis at an earlier age and the disease progresses rapidly. Within limitations of this cross-sectional study, it appears that periodontitis debuts at the same time as the permanent dentition emerges. Individuals under 10 years of age were either periodontally healthy or exhibited minimal attachment loss. This is in agreement with a case study of Patrizi et al. who did not find any oral or mucosal abnormalities in their Kindler patient at age 6. By age 20 years, most individuals with Kindler syndrome (72%) in the Panama cohort had advanced attachment loss. This agrees with our previous case report of a patient who was 17 years of age at the time of examination and presented with aggressive periodontitis. The prevalence of aggressive periodontitis in Kindler subjects is much higher than one would expect based on the prevalence of juvenile periodontitis in the general population. In Kindler patients with periodontitis, the appearance of the periodontal tissues differed from that of chronic periodontitis, supporting the notion that these individuals suffer from periodontitis that is associated with their systemic disease. Spontaneous gingival bleeding was often present and gingiva appeared thin, confirming the findings of our study and other previously reported single cases. The non-Kindler subjects studied seemed to develop periodontitis with relatively high incidence but slow progression, findings that are comparable to other epidemiological surveys of large numbers of individuals without access to professional dental care. In the control group, the attachment loss tended to accumulate slowly, which is typical for common, plaque-induced chronic periodontitis. Older adults generally have more attachment loss than younger individuals, probably due to an accumulation of periodontal damage over time, but advanced periodontitis remains a relatively rare finding. In multivariate models, age does not remain a significant variable for periodontitis when recognized primary risk factors (smoking, diminished immune factors, depression, etc.) are accounted for. In the Panama control cohort, the increase in attachment loss with increased age is expected, particularly given the poor level of oral hygiene.

Because of practical limitations at the study location, it was not possible to perform an optimal microbiological examination. The present PCR identification method has high sensitivity and specificity but can only detect microorganisms for which primers are employed and does not provide information about the total number of microorganisms. To determine the

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Kindler Periodontitis (N = 13)</th>
<th>Kindler No Periodontitis (N = 4)</th>
<th>Control Periodontitis (N = 5)</th>
<th>Control No Periodontitis (N = 6)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
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<td>P. gingivalis</td>
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<td>B. forsythus</td>
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<td>15</td>
<td>1</td>
<td>25</td>
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<td>D. pneumosintes</td>
<td>4</td>
<td>31</td>
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<tr>
<td>T. denticola</td>
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<td>P. nigescens</td>
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<td>46</td>
<td>1</td>
<td>25</td>
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<td>A. actinomyctemcomitans</td>
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<td>23</td>
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<td>Average number of pathogens</td>
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<td>1.0</td>
<td>2.8</td>
<td>1.0</td>
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presence and number of several periodontal microorganisms in Kindler periodontitis lesions, a culture analysis would have been desirable but was excluded because of potential loss of bacterial viability due to long transport times of microbiological samples. Although only a limited number of control non-Kindler individuals with periodontitis were studied, they seem to carry microorganisms typical for chronic periodontitis including P. gingivalis, D. pneumosintes, T. denti-cola, and P. nigrescens. In contrast, only a small portion of Kindler patients with periodontitis harbored P. gingivalis and D. pneumosintes, while T. denti-cola and P. nigrescens were present as often as in the control group. In addition, periodontitis patients in the Kindler group were not commonly infected with A. actinomycetemcomitans, which is interesting considering their early-onset disease that typically is associated with A. actinomycetemcomitans. The highest prevalence of A. actinomycetemcomitans was surprisingly found in the control group with no periodontitis. The most likely explanation is that different populations vary in terms of virulence of infecting A. actinomycetemcomitans strains. The microbiological findings support our previous observations with a single case of Kindler syndrome who tested negative for A. actinomycetemcomitans and showed only low levels of P. gingivalis and B. forsythus. These observations suggest that periodontitis in individuals with Kindler syndrome does not have to be associated with the presence of especially virulent periodontopathogens, and that the major reason for rapid loss of periodontal attachment in Kindler syndrome patients might be mainly due to an increased susceptibility to periodontal disease.

It is possible that there is more than one genetic defect in Kindler syndrome as reported for the related skin disorder epidermolysis bullosa. Kindler syndrome is poorly defined and is presently diagnosed by the appearance of the skin (epidermolysis and poikilo-derma congenitale). Previous case reports suggest autosomal recessive inheritance, but dominant inheritance has also been described and, therefore, more than one mode of inheritance seems to exist. Preliminary genotyping suggests an autosomal recessive inheritance pattern and a genetically homogeneous disease in our cohort and in other cases of the typical clinical appearance gathered from other parts of the world (unpublished data). The molecular defect in Kindler syndrome affects the basement membrane zone, and blistersthat form appear to be junctional. In addition, the distribution of type VII collagen that normally forms the anchoring fibers and is found in a linear distribution along the basement membrane zone in healthy individuals is altered to be streaking into the deeper connective tissue both in the skin and gingiva. It is currently not known how these defects contribute to the fast breakdown of periodontal tissues.

One possible scenario could involve microblistering at the gingival margin due to minor trauma from normal occlusal function and leading to local invasion of bacteria into the tissue. Spontaneous bleeding from gingiva supports this speculative pathological mechanism.

Other clinical dental and oral findings have been reported for cases with Kindler syndrome. Shimamoto et al. suggested that Kindler patients have defective dentitions, and Ban et al. referred to fragile teeth susceptible to fractures. In our study group, the dental examination did not show any significant differences between Kindler patients and non-Kindler patients from the same geographical area. Both actinic keratoses and squamous cell carcinoma of the lower lip have been reported in Kindler syndrome patients. Although no cases of actinic changes or squamous cell carcinoma were observed in the perioral region in our study population, the young age of the patients must be taken into consideration. It is possible that actinic-related malignancies may develop with age given the high level of sun exposure in Panama.

Systemic diseases that affect immune function, inflammatory responses, and weaken barrier defenses can modify the onset and progression of all forms of periodontal disease. The 1999 Classification of Periodontal Diseases and Conditions was expanded to include categories for hematological disorders (acquired neutropenia, leukemias, and other), genetic disorders (familial and cyclic neutropenia, Down syndrome, leukocyte adhesion deficiency syndrome, Papillon-Lefèvre syndrome, Chediak-Higashi syndrome, glyco- gen storage disease, infantile genetic agranulocytosis, Cohen syndrome, Ehlers-Danlos syndrome types IV and VIII, hypophosphatasia, and other) and “not otherwise specified.” We propose that Kindler syndrome should be added to the list of genetic disorders that predisposes to periodontal tissue breakdown.

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