Case Report

Oral findings and microbiological evaluation in a case of triple-X syndrome

Kazuhiko Nakano, Kiyoko Tamura, Tomohiro Ogawa,
Keiko Kawabata and Takashi Ooshima

Department of Pediatric Dentistry, Osaka University Graduate School of Dentistry
1-8 Yamada-oka, Suita, Osaka 565-0871, JAPAN

Abstract We present a case of a triple-X syndrome in a Japanese girl who was treated from 5Y9M to 14Y6M of age at the Pedodontic Clinic of Osaka University Dental Hospital. At the first examination, multiple dental caries were identified, and a pulpectomy and extraction of the affected teeth were carried out. There were no congenitally absent teeth, including third molars, though the development of permanent teeth was approximately 2 years behind chronological age. At 14Y6M, a PCR analysis of 10 putative periodontopathic bacteria was performed, and all 10 species were found in saliva samples. At the same time, an oral examination revealed that the gingival tissues were highly inflamed with an extremely large amount of calculus, suggesting a risk for the early onset of periodontitis.

Key words
PCR,
Periodontitis,
Periodontopathic bacteria,
Retarded eruption,
Triple-X syndrome

Introduction

Triple-X syndrome with premature ovarian failure was first described by Jacobs *et al.*¹⁾ In 1991, a cytogenetic survey of 14,835 consecutive live births (7,608 males and 7,227 females) in Japan showed that 6.27 per 1,000 had a major chromosomal abnormality, and 0.98 per 1,000 females were diagnosed as having triple-X syndrome²⁾. There were 12 newborns with triple-X syndrome born between 1964 and 1974 and then longitudinally monitored until 15 to 22 years of age for whom medical manifestations until adolescence were recorded in detail³⁾. It is thought that problems with triple-X syndrome are more likely to have verbal processing deficits, lower IQ scores, and a global delay⁴⁾. However, there have been few reports on oral manifestations associated with this syndrome; the only reported findings are that tooth enamel in subjects with this syndrome was thicker than that in normal subjects without a systemic disorder⁵⁾ and that bilateral maxillary second

molars were congenitally missing in one case⁶⁾.

Periodontitis is rarely seen in children and adolescents except in association with systemic diseases such as neutropenia, Chediak-Higashi syndrome, Papillon-Lefevre syndrome, Down's syndrome, diabetes mellitus, hypophosphatasia, Histiocytosis syndrome, Ehlers-Danlos syndrome, and acquired immunodeficiency syndrome⁷⁾. We previously reported the detection of 10 putative periodontopathic bacteria found in subgingival plaque samples from 144 systemically healthy children without periodontitis using a PCR method⁸⁾. *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Campylobacter rectus*, *Eikenella corrodens*, and *Actinobacillus actinomycetemcomitans* were found in approximately half of the samples from children of all ages. *Prevotella nigrescens* was also frequently detected in samples from older children, whereas *Tannerella forsythensis* and *Prevotella intermedia* were detected less frequently, and *Porphyromonas gingivalis* and *Treponema denticola* were not found in any of the samples. In a series of studies on Down's syndrome patients, the distribution of periodontal bacteria was evaluated using the same methods, and

Received on March 31, 2005

Accepted on July 7, 2005

it was revealed that the Down's syndrome patients possessed significantly larger numbers of those species than did generally healthy subjects⁹⁾. In this report, we describe oral manifestations in a Japanese girl with triple-X syndrome and results of microbiological analysis.

Case Report

Case description and oral findings

A girl aged 5 years (Y) and 9 months (M) was referred to the Pedodontic Clinic of Osaka University Dental Hospital by her pediatrician for treatment of multiple dental caries. She was reported to be complicated with triple-X syndrome, with develop-

mental retardation. Our initial intraoral examination revealed 11 primary teeth; 9 other primary teeth had been extracted due to dental caries prior to visiting our clinic. According to her mother, she was born after a 40-week gestation without any abnormalities or delivery complications. The first primary tooth had erupted at the age of 12 months. According to the record, her height was 48.0 cm (approximately 25th percentile) and body weight was 2,720 g (approximately 50th percentile) at the time of birth, whereas those at 6 months, and at 1, 2, and 5 years of age showed her to be lower than the 10th percentile. At 6Y1M and 6Y3M, we performed pulpectomies on the maxillary left and right second primary molars, respectively. The maxillary right primary lateral incisor and canine, mandibular right and left primary central incisors, and right primary lateral incisor were also extracted, followed by the application of partial dentures.

At 7Y6M, the mandibular central incisors, lateral incisors, and first permanent molars began to emerge into the oral cavity, and eruption of maxillary central incisors was confirmed at 9Y1M. Since retarded development of all permanent teeth was suspected, orthopantomograph images were routinely taken to evaluate tooth development at 10Y2M, 13Y1M, and 14Y5M (Fig. 1). Although there were no congenitally absent teeth, all teeth showed retarded development. Table 1 shows a comparison of chronological age and dental age, which was calculated by the developmental stage of the permanent teeth in orthopantomograph images by a method described previously¹⁰⁾. After analyzing 5 images taken from 10Y2M to 14Y5M, dental age was determined to be approximately 2 years behind chronological age.

Fig. 1 Orthopantomographs images taken at 10Y2M (a), 13Y1M (b), and 14Y5M (c)

Table 1 Comparison of chronological and dental ages of the patient

Chronological age (years)	Dental age [mean $\pm$ SD (years)]
10Y 2M	7Y9M $\pm$ 1Y 1M
11Y11M	10Y3M $\pm$ 9M
13Y 1M	11Y2M $\pm$ 11M
13Y 7M	12Y0M $\pm$ 9M
14Y 5M	12Y8M $\pm$ 1Y 3M

Dental age was calculated by evaluating the developmental stages of the permanent teeth by the method described previously¹⁰⁾.

Microbiological evaluation

An intraoral examination at 13Y9M revealed that the gingival tissues were highly inflamed with severe deposition of dental calculus on nearly all of the teeth (Fig. 2), and microbiological examination for periodontopathic bacteria using a PCR assay, as described previously¹¹, was therefore initiated at the next visit. Expecterated whole saliva was collected from the patient and kept in sterile plastic tubes. The samples were mixed with Chelex 100 (Bio-Rad Laboratories, Hercules, CA, USA) and incubated at 56°C for 30 min, followed by boiling at 100°C for 10 min. The saliva samples were then centrifuged at 15,000 rpm for 20 min, and the supernatants used as templates for the PCR assays. Subgingival plaque was also collected from all teeth with sterile Gracey curettes and, after gently removing supragingival plaque, the samples were suspended in 1 ml of sterile saline. The plaque samples in 1 ml of saline were then centrifuged at 15,000 rpm for 5 min to pellet the bacterial cells, and bacterial genomic DNA was

isolated from the pellets using a DNA isolation kit (Puregene, Gentra Systems, Minneapolis, MN, USA).

PCR was carried out using species-specific primers for the following 10 species of putative periodontopathic pathogens: *P. gingivalis*¹², *T. forsythensis*¹³, *P. intermedia*¹³, *P. nigrescens*¹³, *C. rectus*¹³, *E. corrodens*¹³, *A. actinomycetemcomitans*¹⁴, *C. ochracea*¹⁴, *C. sputigena*¹⁴, and *T. denticola*¹⁵ (Table 2). A ubiquitous primer set that matches nearly all bacterial 16S rRNA genes was used as a positive control¹⁶. PCR amplification was performed in a reaction mixture containing PCR beads (Ready-To-Go; Amersham Pharmacia Biotech, Piscataway, NJ, USA).

Table 3 summarizes detection of the 10 periodontopathic bacteria in saliva and dental plaque samples taken from 13Y9M to 14Y6M. Almost all of the bacterial species were detected in both saliva and plaque samples at all 4 examinations. Removal of calculus as well as tooth brushing instruction were performed at each visit. The period between periodical examinations was made shorter

Fig. 2 Intraoral photograph taken at 13Y9M

than usual to closely follow the gingival condition.

Clinical examinations and bacterial detection

At 14Y6M, subgingival plaque samples were taken from 4 representative teeth and bacterial genomic DNA was extracted. PCR analysis was then carried out to determine the presence of the 10 periodontal

bacteria, as described above. Clinical parameters, including probing depth, bleeding on probing, pus discharge, tooth mobility, plaque index¹⁷⁾ and gingival index¹⁸⁾, of 4 representative teeth were also examined. The periodontal pocket depth was measured to the nearest millimeter at 6 points around the circumference of each tooth (mesio-,

Table 2 PCR primers used for detection of 10 putative periodontopathic bacterial species

Primer set	Sequence (5' to 3')	Size (bp)	Annealing temperature (°C)	Reference
Universal primer (positive control)	AGA GTT TGA TCM TGG CTC AG CTG CTG CSY CCC GTA G	315	55	16
<i>P. gingivalis</i>	TGT AGA TGA CTG ATG GTG AAA ACC ACG TCA TCC CCA CCT TCC TC	197	60	12
<i>T. denticola</i>	AAG GCG GTA GAG CCG CTC A AGC CGC TGT CGA AAA GCC CA	311	55	15
<i>C. ochracea</i>	AGA GTT TGA TCC TGG CTC AG GAT GCC GTC CCT ATA TAC GGG G	185	55	14
<i>C. sputigena</i>	AGA GTT TGA TCC TGG CTC AG GAT GCC GCT CCT ATA TAC CAT TAG G	185	55	14
<i>P. intermedia</i>	TTT GTT GGG GAG TAA AGC GGG TCA ACA TCT CTG TAT CCT GCG T	575	55	13
<i>P. nigrescens</i>	ATG AAA CAA AGG TTT TCC GGT AAG CCC ACG TCT CTG TGG GCT GCG A	804	55	13
<i>A. actinomycetemcomitans</i>	AGA GTT TGA TCC TGG CTC AG CAC TTA AAG GTC CGC CTA CGT GCC	593	60	14
<i>T. forsythensis</i>	GCG TAT GTA ACC TGC CCG CA TGC TTC AGT GTC AGT TAT ACC T	641	60	13
<i>C. rectus</i>	TTT CGG AGC GTA AAC TCC TTT TC TTT CTG CAA GCA GAC ACT CTT	598	60	13
<i>E. corrodens</i>	CTA ATA CCG CAT ACG TCC TAA G CTA CTA AGC AAT CAA GTT GCC C	688	60	13

Table 3 Periodontal bacteria identified by PCR from saliva and plaque samples

Bacterial species	Chronological age			
	13Y9M	14Y2M	14Y5M	14Y6M
<i>P. gingivalis</i>	+	-(+)	+(+)	+(+)
<i>T. denticola</i>	-	-(+)	-(-)	-(+)
<i>C. ochracea</i>	-	+(+)	+(+)	+(+)
<i>C. sputigena</i>	+	+(+)	+(+)	+(+)
<i>P. intermedia</i>	+	-(+)	-(+)	+(+)
<i>P. nigrescens</i>	-	-(+)	+(+)	+(+)
<i>A. actinomycetemcomitans</i>	+	+(+)	+(+)	+(+)
<i>T. forsythensis</i>	+	+(+)	+(+)	+(+)
<i>C. rectus</i>	+	+(+)	+(+)	+(+)
<i>E. corrodens</i>	+	-(+)	-(+)	+(+)

Symbols in parenthesis note the absence (-) or presence (+) of periodontal bacteria in dental plaque. Dental plaque was not collected from the subject at the age of 13Y9M.

mid-, and disto-buccal points and disto-, mid-, and mesio-lingual points) from the gingival margin to the deepest probing point, using a round-ended probe tip of 0.4 mm in diameter. Bleeding on probing was scored as follows: (+) immediate bleeding on probing or (−) no bleeding. Tooth mobility was scored as follows: (2) moderate mobility (1–2 mm) in a bucco-lingual direction and (1) slight mobility (0.2–1 mm) in a bucco-lingual direction or (0) physiological mobility within 0.2 mm. Pus discharge was scored as follows: (+) spontaneous pus discharge or (−) no pus discharge. Table 4 shows the detection of periodontal bacteria and clinical conditions at the 4 representative sites. There were no differences in periodontal pocket depth, bleeding on probing, pus discharge, mobility and plaque index among the 4 representative sites. Three and 8 of the 10 tested bacterial species were identified in samples from the mandibular left canine and maxillary left first molar, respectively, both of which showed a gingival index of 1, while all 10 species were identified in samples from the maxillary right first molar and mandibular right first premolar, both of which had a gingival score of 2.

Discussion

There have been few reports on oral manifestations of triple-X syndrome, and the associated common dental features remain to be elucidated. We know of only one case report describing oral findings of a patient with this syndrome, a 3-year-6-month-old Japanese female who had a single maxillary central incisor and congenitally missing bilateral maxillary second molars⁶⁾. In the present case, there were no congenitally missing permanent teeth, including third molars (Fig. 1), though retarded tooth development was prominent. We speculated that tooth development might be retarded in subjects with this syndrome; however, accumulation of oral findings is required to clarify this speculation.

The other prominent oral manifestations in the present case were severe gingival inflammation and large amounts of calculus on the smooth surfaces of almost all teeth (Fig. 2). The frequency of periodontitis in children and adolescents is known to be extremely low, while gingivitis is often encountered¹⁹⁾. The amount of calculus deposition in the present case was significantly greater than that

Table 4 Site-specific clinical gingival condition and detection of periodontal bacteria in subgingival plaque samples

	Location			
	Mandibular left canine	Maxillary right first molar	Maxillary left first molar	Mandibular right first premolar
Gram-negative anaerobic bacteria				
<i>P. gingivalis</i>	−	+	+	+
<i>T. denticola</i>	−	+	+	+
<i>C. ochracea</i>	+	+	+	+
<i>C. sputigena</i>	+	+	+	+
<i>P. intermedia</i>	−	+	−	+
<i>P. nigrescens</i>	−	+	+	+
<i>A. actinomycetemcomitans</i>	−	+	+	+
<i>T. forsythensis</i>	−	+	+	+
<i>C. rectus</i>	+	+	+	+
<i>E. corrodens</i>	−	+	−	+
Periodontal condition				
Pocket depth (mm)	3	3	3	3
Bleeding on probing	+	+	+	+
Gingival index	1	2	1	2
Plaque index	3	3	3	3
Mobility	0	0	0	0
Pus exudate	−	−	−	−

in usual cases of gingivitis. It has been shown that several syndromes are associated with periodontitis in children and adolescents; therefore, we speculated that triple-X syndrome might be associated with periodontitis. Our clinical examination revealed that the subject had gingivitis; however, early onset of periodontitis is anticipated unless gingival conditions change.

The results of our microbiological evaluations revealed the presence of all 10 tested periodontal bacteria in saliva samples taken at 14Y2M and 14Y6M (Table 3). Furthermore, the total number of detected bacteria was extremely high as compared to systemically healthy subjects examined in our previous study, in which the average number of periodontal bacteria detected in children aged 7 to 13 using the same methods as those used in the present study was 3.64⁸⁾. At 14Y6M, plaque samples were taken from 4 representative teeth of the present patient. The number of bacterial species in the sample from the mandibular left canine, which showed slight inflammation, was 3, while all 10 species were detected in samples from the maxillary right first molar and mandibular right first premolar, which showed severe gingival inflammation (Table 4). We speculated that the number of periodontal bacteria detected might have been correlated with the degree of inflammation in the tested area.

P. intermedia is regarded as a major virulent species for periodontitis, as was indicated by a high detection rate in samples from adults with periodontitis in an earlier study²⁰⁾. Furthermore, *P. intermedia* has been shown to be strongly associated with periodontitis in childhood²¹⁾, while it is rarely isolated from healthy children¹¹⁾. As for the onset of periodontitis, analysis of the prevalence and proportion of 7 selected periodontal pathogens in 107 fifteen-year-old Japanese boys and girls led to the conclusion that *T. forsythensis* and *C. rectus* are risk markers of the disease²²⁾. In addition, *P. gingivalis*, *T. denticola*, and *T. forsythensis* have been named the red complex microbial consortium, and their presence together has been shown to be associated with periodontal disease and particularly with deep pocket depths and bleeding on probing^{23,24)}. In contrast, analysis of the distribution of the 10 periodontal bacteria that we examined in the present study in systemically healthy children aged 11 to 13 years old showed that *P. gingivalis* and *T. denticola* were not present in any of the subjects⁹⁾, while both were detected at 13Y9M in the present patient. In

another study, the detection profiles of the same 10 periodontal bacteria in Down's syndrome subjects were classified into 5 clusters, in which cluster 5, used to designate subjects with all species detected, occurred in most of those with severe gingival inflammation as well as those with the highest plaque scores and gingival index⁹⁾. The bacterial detection profile in the present patient resembled cluster 5, indicating that the subject possesses a high risk for the development of periodontitis. At the time of writing, there has been no demonstration of a correlation between periodontitis and triple-X syndrome. However, we believe that it is necessary to monitor the patient clinically, radiographically, and microbiologically as well as give appropriate instruction and therapy to reduce the risk of onset of the disease.

Acknowledgments

This study was supported by a Grant-in-Aid for Scientific Research (B) 16390605 from Japan Society for Promotion of Science, and was a part of 21st Century COE entitled "Origination of Frontier BioDentistry" at Osaka University Graduate School of Dentistry supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- 1) Jacobs, P.A., Baikie, A.G., Brown, W.M., Macgregor, T.N., Maclean, N. and Harnden, D.G.: Evidence for the existence of the human "super female". *Lancet* **2**: 423–425, 1959.
- 2) Maeda, T., Ohno, M., Matsunobu, A., Yoshihara, K. and Yabe, N.: A cytogenetic survey of 14,835 consecutive liveborns. *Jinrui Idengaku Zasshi* **36**: 117–129, 1991.
- 3) Linden, M.G., Bender, B.G., Harmon, R.J., Mrazek, D. and Robinson, A.: 47, XXX What is the prognosis? *Pediatrics* **82**: 619–630, 1988.
- 4) Sybert, V.P.: Phenotypic effects of mosaicism for a 47, XXX cell line in Turner syndrome. *J Med Genet* **39**: 217–220, 2002.
- 5) Alvesalo, L., Tammisalo, E. and Therman, E.: 47, XXX females, sex chromosomes, and tooth crown structure. *Hum Genet* **77**: 345–348, 1987.
- 6) Miura, M., Kato, N., Kojima, H. and Oguchi, H.: Triple-X syndrome accompanied by single maxillary central incisor: case report. *Pediatr Dent* **15**: 214–217, 1993.
- 7) Meyle, J. and Gonzales, J.R.: Influences of systemic diseases on periodontitis in children and adolescents. *Periodontol 2000* **26**: 92–112, 2001.
- 8) Kimura, S., Ooshima, T., Takiguchi, M., Sasaki,

- Y., Amano, A., Morisaki, I. and Hamada, S.: Periodontopathic bacterial infection in childhood. *J Periodontol* **73**: 20–26, 2002.
- 9) Amano, A., Kishima, T., Kimura, S., Takiguchi, M., Ooshima, T., Hamada, S. and Morisaki, I.: Periodontopathic bacteria in children with Down syndrome. *J Periodontol* **71**: 249–255, 2000.
 - 10) Nakano, K., Matsuoka, T., Takahashi, A., Matsumura, M., Sobue, S. and Ooshima, T.: Delayed development or congenital absence of a single first permanent molar in Japanese child patients. *Int J Paediatr Dent* **9**: 271–276, 1999.
 - 11) Ooshima, T., Nishiyama, N., Hou, B., Tamura, K., Amano, A., Kusumoto, A. and Kimura, S.: Occurrence of periodontal bacteria in healthy children: a 2-year longitudinal study. *Community Dent Oral Epidemiol* **31**: 417–425, 2003.
 - 12) Tran, S.D. and Rudney, J.D.: Multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *J Clin Microbiol* **34**: 2674–2678, 1996.
 - 13) Ashimoto, A., Chen, C., Bakker, I. and Slots, J.: Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol* **11**: 266–273, 1996.
 - 14) Conrads, G., Mutters, R., Fischer, J., Brauner, A., Luttkien, R. and Lampert, F.: PCR reaction and dot-blot hybridization to monitor the distribution of oral pathogens within plaque samples of periodontally healthy individuals. *J Periodontol* **67**: 994–1003, 1996.
 - 15) Watanabe, K. and Frommel, T.O.: *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* and *Treponema denticola* detection in oral plaque samples using the polymerase chain reaction. *J Clin Periodontol* **23**: 212–219, 1996.
 - 16) Doungudomdacha, S., Rawlinson, A. and Douglas, C.W.: Enumeration of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* in subgingival plaque samples by a quantitative-competitive PCR method. *J Med Microbiol* **49**: 861–874, 2000.
 - 17) Silness, J. and Loe, H.: Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand* **22**: 121–135, 1964.
 - 18) Loe, H. and Silness, J.: Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* **21**: 533–551, 1963.
 - 19) Jenkins, W.M. and Papapanou, P.N.: Epidemiology of periodontal disease in children and adolescents. *Periodontol 2000* **26**: 16–32, 2001.
 - 20) Matto, J., Saarela, M., von Troil-Linden, B., Kononen, E., Jousimies-Somer, H., Torkko, H., Alaluusua, S. and Asikainen, S.: Distribution and genetic analysis of oral *Prevotella intermedia* and *Prevotella nigrescens*. *Oral Microbiol Immunol* **11**: 96–102, 1996.
 - 21) Okada, M., Hayashi, F. and Nagasaka, N.: PCR detection of 5 putative periodontal pathogens in dental plaque samples from children 2 to 12 years of age. *J Clin Periodontol* **28**: 576–582, 2001.
 - 22) Suda, R., Kobayashi, M., Nanba, R., Iwamaru, M., Hayashi, Y., Lai, C.H. and Hasegawa, K.: Possible periodontal pathogens associated with clinical symptoms of periodontal disease in Japanese high school students. *J Periodontol* **75**: 1084–1089, 2004.
 - 23) Socransky, S.S., Haffajee, A.D., Cugini, M.A., Smith, C. and Kent, R.L. Jr.: Microbial complexes in subgingival plaque. *J Clin Periodontol* **25**: 134–144, 1998.
 - 24) Rocas, I.N., Siqueira, J.F. Jr., Santos, K.R. and Coelho, A.M.: “Red complex” (*Bacteroides forsythus*, *Porphyromonas gingivalis*, and *Treponema denticola*) in endodontic infections: a molecular approach. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **91**: 468–471, 2001.