Aggressive Periodontitis Associated With Fanconi's Anemia. A Case Report

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Background: *Fanconi's anemia is an autosomal recessive disease associated with chromosomal breakage as well as pancytopenia, skin pigmentation, renal hypoplasia, cardiac defects, microcephaly, congenital malformations of the skeleton, hypogonadism, and increased risk of leukemia. The present report describes the periodontal clinical and microbiological status of an 11-year old male having Fanconi's anemia.*

Methods: *Polymerase chain reaction analysis to detect human cytomegalovirus (HCMV), Epstein-Barr type 1 virus, and herpes simplex virus (HSV) was performed on paper-point samples pooled from either 3 periodontal sites with advanced attachment loss or 3 gingivitis sites with no clinical attachment loss. Anaerobic bacterial culture examination was performed on the pooled periodontitis sample.*

Results: *The patient suffered from pancytopenia, allergy, asthma, hearing impairment, and mental retardation. Dentition consisted of 7 primary teeth, 11 erupted permanent teeth, and 14 unerrupted permanent teeth. Most erupted teeth showed severe gingival inflammation with some gingival overgrowth and various degrees of periodontal attachment loss. Genomes of HCMV and HSV were detected in the pooled periodontitis sample and HCMV in the pooled gingivitis sample. The periodontitis sample but not the gingivitis sample revealed HCMV mRNA of major capsid protein, suggestive of active viral infection. The periodontitis sample also yielded* Actinobacillus actinomycetemcomitans *(1.1% of total isolates),* Fusobacterium *species (7.9%),* Campylobacter *species (2.2%),* Peptostreptococcus micros *(3.4%), and* Candida albicans *(0.3%).*

Conclusions: *Oral features of Fanconi's anemia may include increased susceptibility to periodontitis. It is likely that underlying host defense impairment coupled with periodontal infection by HCMV and* A. actinomycetemcomitans *contribute to the severe type of periodontitis associated with Fanconi's anemia.* J Periodontol 2001;72:1601-1606.

KEY WORDS

Fanconi's anemia; periodontitis/epidemiology; human cytomegalovirus; herpes simplex virus; *Actinobacillus actinomycetemcomitans*; polymerase chain reaction.

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Aplastic anemia can be acquired or genetic. Acquired aplastic anemia may be due to exposure to excessive radiation, toxic chemicals, certain drugs, infections, or a variety of environmental agents with ability to damage bone marrow. Fanconi's anemia is a rare autosomal recessive aplastic anemia that occurs in all ethnic groups and equally in males and females.[1,2] Characteristically, chromosomes break and rearrange easily. Patients with Fanconi's anemia may suffer from various abnormal physical conditions or have no visible disorders. They have a life-threatening anemia that is characterized by spontaneous hemorrhage; a marked reduction of erythrocytes, leukocytes, and platelets; and the inability to successfully combat infection and fatigue.[3] Elevated incidence of leukemia (10% to 15%) and cancer is associated with the disease.[4] Toxic environmental factors may contribute to the development of aplastic anemia.[5]

Fanconi's anemia is under-diagnosed, probably because of lack of knowledge about the signs and symptoms of the disease. The blood diepoxybutane analysis to detect chromosomal breakage and tendency of rearrangement can distinguish between Fanconi's anemia and diseases with similar presentations. Fanconi's anemia may be diagnosed before birth by analyzing chorionic villus samples, performed in the tenth to twelfth week of gestation, or by amniocentesis, performed in the fifteenth to seventeenth week of gestation.[6] Since normal-appearing brothers and sisters may also be affected by the condition, siblings of patients with Fanconi's anemia should receive the chromosome breakage test.[6]

Most Fanconi's anemia patients show birth defects that can involve any system of the body with little or no predilection or predictability. Approximately one-fourth of patients are born with structural renal malformations associated with dermal hyper-pigmentation. Some patients are born with heart defects, usually in the tissues separating cardiac chambers. Bone marrow failure usually begins at 7 years of age with a range of 3 to 12 years.[1]

The median life expectancy of patients with Fanconi's anemia is 25 years, although many patients survive into their 30s and 40s.[7,8] Alter et al.[9] reported that 110 females reached the age of 16 years or more

and 15% became pregnant. There were 26 pregnancies resulting in 19 births and 18 surviving, normal children. Most women with Fanconi's anemia experienced decreased blood counts during pregnancy and often required transfusions; however, no maternal deaths occurred during pregnancy. Males appear to have reduced fertility, and the literature reports only 3 Fanconi's anemia men who have fathered children.[9]

Therapy falls into 4 categories: bone marrow transplantation, androgen therapy, synthetic growth factors, and gene therapy. A successful bone marrow transplant or umbilical cord blood infusion can correct problems related to anemia, neutropenia, thrombocytopenia, myelodysplasia or leukemia; however, patients can still suffer from problems related to other organs or systems.[10] The prognosis for transplant is best for young patients with uncomplicated aplastic anemia who are in otherwise good clinical condition.[6]

Between 50% and 75% of patients respond to oxymetholone or other types of androgen therapy that entail artificial male hormones to stimulate production of one or more types of blood cells. Androgens prolong the lives of many patients but are not a cure and most patients eventually fail to respond to androgens.[7] In the last few years, hematopoietic growth factors have been used to further stimulate the production of cells that are vital parts of the blood system. Although gene therapy is not yet a reality, gene therapists have been studying the introduction of the healthy gene into the patient's body at the right cellular location. In the future it may be possible to accomplish autologous transplantation of bone marrow that has been genetically corrected in vitro.[11]

Oral conditions associated with Fanconi's anemia include generalized microdontia,[12] squamous cell carcinoma,[13] and advanced periodontitis.[14,15] One report describes treatment of an edentulous Fanconi's anemia patient with osseous grafting and dental implant placement.[15] No information is available on the infectious agents involved in Fanconi's anemia-associated periodontitis. In this paper, we report a case of aggressive periodontitis in a patient with Fanconi's anemia and present an analysis of microbiota and human herpesviruses present in periodontitis and gingivitis sites.

CASE REPORT

Medical and Oral Examination

An 11-year-old Caucasian boy was referred to the University of Southern California School of Dentistry, Department of Periodontology, for treatment of severe gingivitis and periodontitis characterized by pain and discomfort, gingival redness, bleeding upon tooth brushing, and loss of periodontal attachment around several maxillary and mandibular teeth.

The diagnosis of Fanconi's anemia was made at birth. The newborn revealed bifid thumbs, incompletely developed radial bones, spinal malformations, and rib abnormalities. The child experienced jaundice at birth and several episodes of mild asthmatic crisis during infancy.

Medical examination showed developmental disability, learning disability, hearing impairment, anemia, and bleeding disorders. The patient's height and weight were below normal. Short stature was striking and below the third percentile in height.

While the patient's chronological age was 11 years, functional age was judged to be 3 years. Microcephaly and microphthalmia were noticeable. Hematologic findings showed pancytopenia (abnormally low counts in all 3 major lineages of blood cells (erythrocytes, leukocytes, and platelets) and defective hematopoiesis associated with bleeding and bruising tendency. Gastrointestinal disorders included frequent minor aphthae and recurrent herpes labialis. Polydypsia and polyphagia were reported, and the patient was recently diagnosed having insulin-dependent diabetes mellitus.

The parents denied being cousins or having any other familial relationship. Their other son, who was 14 years old, showed no birth defects and is presently healthy with normal blood counts.

Patient management difficulties allowed only for limited examination and treatment on an outpatient basis. More definitive dental treatment was accomplished in a hospital setting under general anesthesia.

Oral examination revealed several stainless steel crowns and generalized gingival inflammation (Fig. 1). The dentition consisted of teeth 3, A, C, 8, 9, H, J, 14, 19, L, M, 23, 24, 25, 26, 27, T, and 30. Unerupted teeth included 4, 5, 6, 10, 11, 12, 13, 18, 20, 21, 22, 28, 29, and 31. Radiographic examination revealed localized areas of advanced alveolar bone loss (Fig. 2). The pediatric dentist had placed the patient on 0.12% chlorhexidine gluconate rinse, 0.05 ounce twice daily, but otherwise there was no previous periodontal therapy.

Herpesvirus Examination

The viral examination was performed without knowledge of the clinical features of the sample sites.

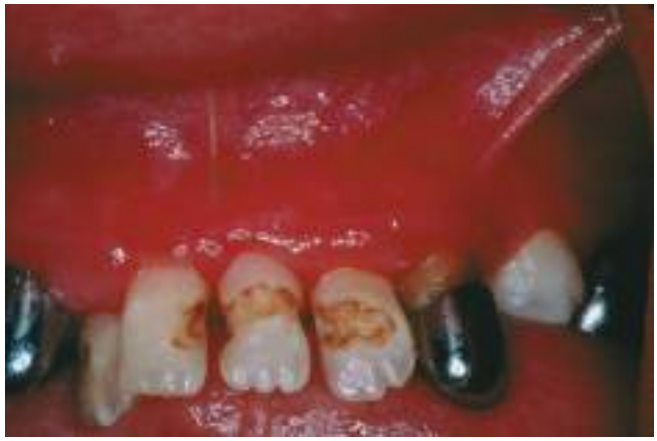

Figure 1.
Eleven-year old patient with Fanconi's anemia and aggressive periodontitis.

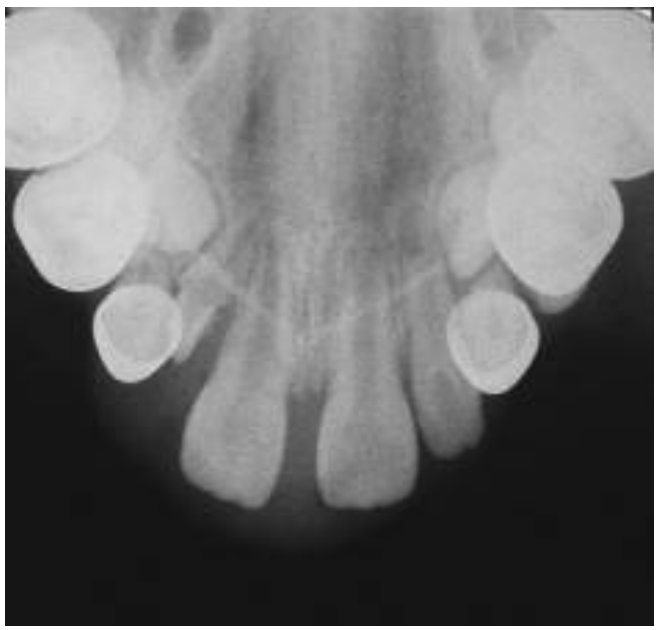

Figure 2.
Radiographic evidence of advanced bone loss on the distal aspect of the maxillary right permanent central incisor.

Nested polymerase chain reaction (PCR) was employed to detect human cytomegalovirus (HCMV), Epstein-Barr virus type 1 (EBV-1), and herpes simplex virus (HSV)

The primers and the sensitivity and specificity of the PCR techniques used to detect genomes of HCMV, EBV-1, and HSV are described elsewhere.[16,17]

Briefly, subgingival plaque samples were obtained from sites demonstrating advanced attachment loss and from gingivitis sites showing no loss of clinical attachment. After removing supragingival plaque with sterile cotton pellets, one endodontic fine paper point was inserted to the depth of each of 3 periodontitis sites and 3 gingivitis sites and retained for 30 seconds. Subgingival specimens pooled from the periodontitis sites and the gingivitis sites were sequentially collected for herpesvirus DNA extraction, for HCMV mRNA extraction, and for microbial culture. Paper points for nucleic acid extraction were placed into empty sterile silicon plastic vials and cooled on ice to retard RNA degradation. Paper points for microbial culture were placed into a screw-capped 2 ml vial containing VMGA III anaerobic transport medium.[18] Nucleic acid extraction and culture procedures took place within 1 hour of sampling.

For herpesvirus DNA extraction, periodontal specimens were suspended in 500 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and homogenized by vigorous vortex mixing. Nucleic acid extraction was based on preferential binding of DNA to silica particles in the presence of a high concentration of guanidium thiocyanate (GuSCN).[19] The details of the extraction procedure are described elsewhere.[16]

Optimal conditions for each nested PCR reaction were determined using positive and negative DNA controls.[16] Each PCR test tube (final volume of 40 µl) contained 2 to 5 µl of DNA sample, 20 to 50 pmol of outer primers, 0.2 mM of a mixture of deoxynucleoside triphosphates (dATP, dCTP, dGTP, dTTP)‡ 1.25 U of *Thermus aquaticus (Taq)* polymerase,§ 4 µl of 10× *Taq* buffer, various concentrations of $MgCl_2$, and double distilled water.

PCR amplification was performed in a DNA thermal cycler.‖ The first amplification round included an initial denaturation at 95°C for 1 minute, followed by 30 cycles of denaturation at 94°C for 1 minute, primer annealing at 50 to 60°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 1 minute.

The second amplification round was performed using 2 to 5 µl of the first round product, 40 to 100 mmol of each of the inner primers, 10× *Taq* buffer, a mixture of deoxynucleoside triphosphates, *Taq* polymerase, and various concentrations of $MgCl_2$. The

‡ Pharmacia LKB, Piscataway, NJ.
§ Promega Co., Madison, WI.
‖ MJ Research, Watertown, MA.

second amplification round consisted of 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute extension at 72°C for 1 minute, and final extension at 72°C for 2 minutes.

The acid guanidium-phenol-chloroform method was used for HCMV RNA extraction.[20] The details of the extraction procedure are described elsewhere.[17] For amplification of the HCMV specific mRNA major capsid protein, 10 µl of the total RNA extraction mixture was transcribed using 100 U murine moloney reverse transcriptase,¶ 20 U RNAsin,# 50 pM of primer complementary to the positive strand (MCP2), and 0.2 µM of each dNTP. The procedure was carried out at 42°C for 60 minutes in a final volume of 25 µl. The cDNA obtained was amplified using the entire transcription mixture, 50 pM of the other primer (MCP1), 1.25 U *Taq* polymerase, and PCR buffer as described for direct PCR. To confirm the absence of contaminating DNA in the extracted RNA, PCR was performed on the remaining RNA sample without the reverse transcription step.

Table 1 shows that the periodontitis sample contained dual infection of HCMV and HSV. It also shows transcription of the HCMV specific major capsid protein, an indication of active HCMV infection.[17] In contrast, the gingivitis sample revealed only HCMV that occurred in the latent rather than in the productive phase.

Microbial Examination

For microbial culture, microorganisms were mechanically dispersed from the paper points with a Vortex mixer at the maximal setting for 45 seconds, and were then 10-fold serially diluted in VMG I anaerobic dispersion solution.[18] Using a sterile bent glass rod, 0.1 ml aliquots from 10^3 to 10^5 dilutions were plated onto non-selective 4.3% brucella agar** supplemented with 0.3% bactoagar, 5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells, 0.0005% hemin, and 0.00005% menadione. Total viable counts and proportions of specific bacteria in relationship to the total viable counts were determined. Aliquots diluted in VMGA III medium were plated onto TSBV medium for culture of *Actinobacillus actinomycetemcomitans,* enteric Gram-negative rods, and yeasts.[21]

The non-selective blood agar was incubated at 35°C in an anaerobic chamber†† containing 85% N_2-10% H_2-5% CO_2 for 10 days. TSBV medium was incubated in 10% CO_2 in air at 35°C for 4 days. Presumptive identification of representative colonies of each group of organisms that morphologically resembled the study species was performed according to methods described by Slots[22] and by use of a micromethod system.‡‡ Organisms examined included *A. actinomycetemcomitans, Prevotella intermedia/Prevotella nigrescens, Porphyromonas gingivalis, Bacteroides forsythus, Campylobacter* species, *Fusobacterium* species, *Peptostreptococcus micros,* enteric Gram-negative rods, and *Candida* species.

Table 2 shows that the pooled periodontitis sample contained *A. actinomycetemcomitans* as well as *Campylobacter* species, *Fusobacterium* species, and *P. micros. Candida albicans* was also recovered from the periodontitis lesions.

Table 1.

Periodontal Herpesviral Presence in a Patient With Fanconi's Anemia

Study Sites	HCMV Genome	Productive HCMV	Latent HCMV	HSV Genome
Periodontitis (advanced attachment loss)	X	X		X
Gingivitis (no attachment loss)	X		X	

DISCUSSION

Two cases of severe periodontitis have previously been reported in Fanconi's anemia patients. Engel et al.[15] described a patient who was completely edentulous by the age of 22 years, but provided no detailed information of the previous periodontal condition. Opinya et al.[14] reported on a 24-year old male with spontaneous exfoliation of 3 first molars and all incisors. Remaining teeth showed advanced attachment loss with suppuration, bleeding upon probing, extreme mobility, and radiographic evidence of severe horizontal alveolar bone loss.

The case reported here describes a much younger (11 years-old) patient than those in previous publications. Periodontal disease occurred with a markedly localized pattern of destruction. The maxillary right central incisor exhibited advanced periodontal attachment loss, whereas the maxillary left central incisor demonstrated only gingivitis and no clinical or radi-

¶ Bethesda Research Laboratories, Bethesda, MD.
# Sigma Chemical Co., St. Louis, MO.
** BBL Microbiology Systems, Cockeysville, MD.
†† Coy Laboratory Products, Ann Arbor, MI.
‡‡ API 20A, bioMérieux, Marcy l'Etoile, France.

Table 2.

Microbial Analysis of Periodontitis Lesions in a Patient With Fanconi's Anemia

Organisms	% of Cultivable Microbiota
A. actinomycetemcomitans	1.1
Campylobacter species	2.2
Fusobacterium species	7.9
P. micros	3.4
C. albicans	0.3

ographic evidence of periodontal attachment loss. Several teeth showed deep periodontal pockets and radiographic evidence of advanced vertical bone loss but other teeth again demonstrated only gingivitis with no attachment loss.

Microbiologic analysis performed on subgingival samples from periodontitis sites which included the maxillary right central incisor revealed the presence of a dual infection of HCMV and HSV. Herpesviruses have been linked to various types of destructive periodontal disease.[23] In particular, HCMV seems strongly associated with aggressive periodontal disease in young individuals, including patients suffering from various forms of aggressive periodontitis[24-26] and Papillon-Lefèvre syndrome-associated periodontitis.[27] Moreover, HCMV and HSV dual infection occurs with especially high frequency in advanced periodontitis lesions of adults[23] and exhibits particularly high pathogenicity in immunocompromised patients.[28,29] As discussed elsewhere,[23] herpesvirus periodontal infections may cause local immune suppression, induce pro-inflammatory cytokine production, alter the structural integrity of the periodontium, and lead to overgrowth of periodontopathic bacteria. Periodontal herpesvirus infection may impair the already deficient leukocytes and help set the stage for severe periodontal breakdown.

The presence of HCMV active infection in the study of periodontitis lesions may be of pathogenic significance. Similar to the present findings, Ting et al.[24] showed that HCMV activation, at a given time, might occur in some but not in other herpesvirus-positive periodontal sites of an infected individual. Active herpesvirus infections are generally more deleterious than latent herpesviruses.[30] Ting et al.[24] presented evidence for active HCMV periodontal infection being associated with the initiation and progression of localized juvenile periodontitis. HCMV activation has also been detected in advanced periodontitis lesions of adult patients.[17]

A. actinomycetemcomitans is an important periodontal pathogen in young individuals[31] and may contribute to the development of periodontitis associated with Fanconi's anemia. In the present study, periodontitis lesions with HCMV active infection revealed 1.1% *A. actinomycetemcomitans;* that is more than 100-fold higher than the estimated critical periodontopathic level of 0.01% for the organism.[32] Ting et al.[24] also found that progressing localized juvenile periodontitis lesions with HCMV-active infection harbored elevated levels of *A. actinomycetemcomitans.* HCMV-mediated impairment of host defenses may predispose to periodontal overgrowth by *A. actinomycetemcomitans* and other periodontopathic bacteria.[33] However, the interaction between HCMV and bacteria is probably bi-directional, with bacterial enzymes or other inflammation-inducing products activating herpesviruses, which subsequently might increase bacterial pathogen counts. In any case, we suggest that the combination of active HCMV and *A. actinomycetemcomitans* periodontal infection together with inherent leukocyte defects may play an important role in the etiopathogenesis of periodontitis associated with Fanconi's anemia, at least in the patient described here. Further research involving additional patients is needed to test this hypothesis.

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