



A Case Report on Recurrent Oral Ulcers Associated with Cyclic Neutropenia

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Abstract

A 12-year-old boy had recurrent oral ulcer and gingival necrosis accompanied with fever, pharyngalgia and painful neck lymph nodes since six months of age. Laboratory examinations revealed an oscillation of peripheral blood neutrophils from normal to severely low levels, with 21-day periodicity. A mutation in exon 4 of neutrophil elastase 2 (ELA2) was identified. The symptoms and lab examination led to the diagnosis of cyclic neutropenia. After the patient received recombinant human granulocyte colony-stimulating factor (rhG-CSF) treatment around the early neutropenic phase, the symptoms abated and peripheral blood neutrophil counts returned to normal levels. This case demonstrates the need for oral medicine clinicians to perform testing for cyclic neutropenia when patients are afflicted with recurrent oral ulcers.

Keywords: Oral ulcer; Cyclic neutropenia; Neutrophil elastase 2

Introduction

Cyclic Neutropenia (CyN) is a rare blood disorder characterized by regular oscillations in the value of peripheral blood neutrophils, generally with 21-day periodicity [1]. CyN was first reported in 1910, and usually presents early in childhood [2]. An estimated incidence of CyN was one to two per million [3]. Absolute neutrophil count is nadir, and always below $0.2 \times 10^9/L$ [4]. During phases of neutropenia, patients frequently suffer from fever, necrotic stomatitis, pharyngalgia, lymphadenopathy and more serious infections. With increasing neutrophil count, the infections disappear [5,6]. CyN is responsive to granulocyte colony-stimulating factor (G-CSF), whereby G-CSF administration leads to shortened nadir duration, increased mean neutrophil count and alleviated clinical symptoms, but does not prevent recurrence [4]. Recently the locus for autosomal dominant CyN was mapped to chromosome 19p13.3, and CyN is now attributable to mutations found in the gene encoding neutrophil elastase (the ELA2 gene) [5]. Eighty percent of CyN patients are reported as having an ELA2 mutation. Several studies reported that CyN ELA2 mutations tend to be located in intron 4 and exons 2, 3 and 4 [4,5]. Here, we report a case of CyN in a 12-year-old boy as determined through clinical and lab examinations and by demonstration of a mutation in exon 4 of the ELA2 gene. There is an association of oral ulcers with cyclic neutropenia and this should be taken into consideration by examining physicians.

Case Presentation

A 12-year-old Chinese boy experienced year-long signs of oral ulcers and fever, with a cycle time of 3 weeks. The patient's medical history indicated that he had suffered from oral ulcers, gingival necrosis and fever accompanied with pharyngalgia and painful neck lymph nodes since he was six months of age. Oral examination revealed oral ulcers and gingival necrosis. Four necrotic ulcers were found on the tongue mucosa. The ulcers were covered by a yellow and thick pseudomembrane (Figure 1A). Gingival margin and papilla were necrosed, common in upper and mandibular anterior teeth. The necroses were covered by yellow pseudomembrane, with easily hemorrhage and special septic halitosis. The gingival around necroses were congestion (Figure 1B). Poor oral hygiene was also observed, along with accumulation of bacterial plaque and food debris. In addition, the patient's neck lymph nodes were pain and enlargement, pharynx mucosa was congestion. By this time, laboratory examination revealed a neutrophil count of $0.14 \times 10^9/L$. His neck lymph nodes ultrasonography demonstrated bilateral neck lymphadenopathy. Around 21 days later, the signs, including oral ulcers, gingival necrosis, lymphadenopathy and congestion of pharynx mucosa, were dissipated (Figure 1C and D). At this moment, his neutrophil count returned

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Figure 1: Oral symptoms (12-year-old, male). (a) Several major ulcers on tongue mucosa with a yellow pseudomembrane; (b) Generalized gingival necrosis with gingival congestion; (c-d) The ulcers on tongue mucosa and gingival necrosis abated, when the patient's neutrophil counts returned to normal.

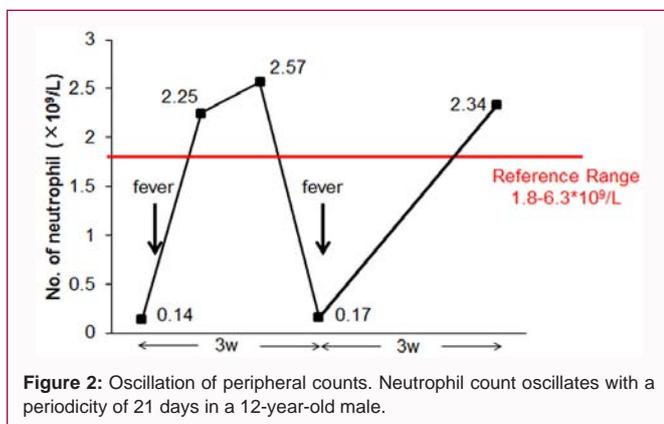


Figure 2: Oscillation of peripheral counts. Neutrophil count oscillates with a periodicity of 21 days in a 12-year-old male.

to $2.25 \times 10^9/L$. Several blood routine tests have been carried out on the patient, showed severely low peripheral blood neutrophils count with 21-day periodicity (Figure 2). The patient was diagnosed with cyclic neutropenia based on his oral manifestations, cyclical decreased neutrophil count with 21-day periodicity. However, CyN need to differential diagnose with other diseases, especially agranulocytosis and periodic fever aphthous-stomatitis pharyngitis cervical-adenitis syndrome (PFADA syndrome). Agranulocytosis clinic was mainly included necrotic ulcers, gingival necrosis. While neutrophil count was below $0.5 \times 10^9/L$, but without periodicity. PFADA syndrome was characterized by periodic fever, aphthous stomatitis, pharyngitis and adenitis, those were similar with CyN. However, the patient's neutrophil count was not decreased.

Blood was collected from the patient and both parents. All procedures and consents were approved by human subjects of committees of Beijing Stomatological Hospital, and they signed informed consent forms. DNA was isolated from the peripheral blood using the DNeasy Blood Tissue Kit (Qiagen, Germany) according to the manufacturer's recommended procedures. PCR amplification of the affected sequence in the ELA2 gene was performed with two primers 5'-TGGCAGGCACTCAGCA-3' (F)/5'-GGGGTTCG TAGCCGTTTTTC-3' (R) and 5'-GCACTCAAGCCACATCC-3' (F)/5'-TCAACACCCAATCACACAG-3' (R). DNA regions found between these two primers contain 13 mutation locations (Table 1). The resulting amplicons were sequenced using a sequencing kit (Applied Biosystems, Foster City, CA) and ABI PRISM[®]3730XL

Table 1: Characteristics of mutations tested in CyN patient.

Location	Nucleotide change	CyN patient	Father	Mother
Exon 2	1847C>A	C/C	C/C	C/C
Exon 2	1855C>T	C/C	C/C	C/C
Exon 2	1900C>T	C/C	C/C	C/C
Exon 3	2192G>A	G/G	G/G	G/G
Exon 4	4495C>T	C/T	C/C	C/C
Exon 4	4534C>T	C/C	C/C	C/C
Exon 4	4675-4715del	-	-	-
Intron 4	IVS4 + 1G>A	G/G	G/G	G/G
Intron 4	IVS4 + 5G>A	G/G	G/G	G/G
Exon 5	4902G>C	G/G	G/G	G/G
Exon 5	4939G>A	G/G	G/G	G/G
Exon 5	4943G>A	G/G	G/G	G/G
Exon 5	5069C>T	C/C	C/C	C/C

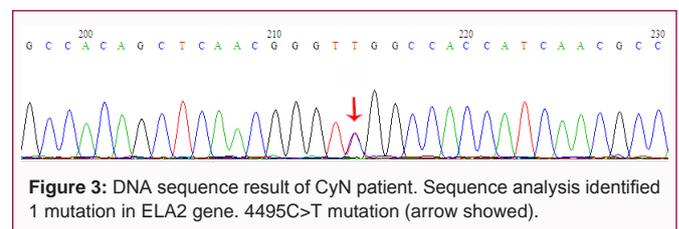


Figure 3: DNA sequence result of CyN patient. Sequence analysis identified 1 mutation in ELA2 gene. 4495C>T mutation (arrow showed).

Genetic analyzer. The sequence examination identified the patient's ELA2 gene had a mutation in exon 4, with a single nucleotide change (4495C>T), however, as this mutation was not found in either of his parents it was considered a *de novo* mutation (Figure 3 and Table 1). The patient was advised to receive subcutaneous injections of recombinant human granulocyte colony-stimulating factor (rhG-CSF) (150 µg) at the early neutropenic phase, until neutrophil counts returned to normal. The patient was also prescribed pidotimod (800 mg per day) along with tinidazole (1g per day) for gingival necrosis. In addition, the patient was advised to use 1% povidone iodine solution as a mouth wash three times per day while he presented oral ulcers. Two year following treatments, the patient's symptoms relieved and his peripheral blood neutrophils counts returned to normal levels.

Discussion

CyN was diagnosis by regular oscillations of peripheral blood neutrophils from normal to severely low levers, generally with 21-days periodicity [4]. The diagnosis depends on serial measurements of absolute nertrophil counts over a period of several weeks [5,6]. This patient was diagnosed with CyN as he was determined to have severely low peripheral blood neutrophils count with 21-day periodicity associated with fever, oral ulcers, gingival necrosis, pharyngalgia and neck lymphadenopathy, followed by a recovery phase during where his peripheral neutrophil levels returned to normal. CyN occurs both as a childhood-onset and adult-onset disease by taking account of age. While Cyn generally first presents in childhood, for nearly 25% of patients', the first symptoms present after age 20 [1]. Affected individuals typically experience recurrent aphthous stomatitis, fever, malaise, pharyngitis, sinusitis, or more serious infections (such as colitis with gram negative sepsis) [4,7]. The mechanisms driving CyN is still unknown. A possible mechanism for childhood-onset CyN is associated with an underlying disturbance in the granulocyte-macrophage colony-stimulating factor (GM-CSF) responsive growth

of myeloid progenitors committed to neutrophilic differentiation [8]. Interestingly, rhGM-CSF treatment is not effective at increasing neutrophil counts in all CyN patients suggesting that a lack of GM-CSF in patients with CyN does not entirely explain the mechanisms of CyN development [9]. Recently, several studies reported positional cloning studies that mapped the locus for autosomal dominant CyN to chromosome 19p13.3, the locus for several serine proteases, and this disease is now attributable to multiple mutations found in the gene encoding ELA2 [5]. ELA2 gene is activated primarily at the promyelocytic stage during neutrophil development [4,10]. Mutations found in this gene result in accelerated apoptosis in differentiating myeloid cells [4,11]. Additionally, *Ela2*^{-/-} mice do not have deficient neutrophil counts, but do have impaired intracellular killing of bacteria by neutrophils and are susceptible to death following intraperitoneal infection with Gram-negative bacteria [12]. ELA2 gene mutations have been found in 80-100% of CyN cases [4]. Most of splice-site mutations cluster in the splice-donor region of intron 4, and others were located in exons 2, 3, 4 and 5 [3]. The mutation type were missense, splicing defect and in-frame deletions, such as 1847C>A in exon2, 4495C>T and 4675-4715 del in exon4 and IVS4+1 G>A in intron4. At the protein level, these mutations resulted in amino acid substitutions, such as F14L, S17F and S97L [3,4]. In our study, sequencing of the patient's ELA2 gene demonstrated a mutation in exon 4 (4495C>T). This missense mutation 4495C>T is responsible for the S97L substitution. Genotype-phenotype analysis strongly suggests that ELA2 mutations are correlated with more severe presentation of neutropenia [3]. Colony stimulating factor 3 receptor is another gene associated with congenital neutropenia and CyN [13]. These studies elucidate several genetic disruption and molecular mechanisms leading to CyN, thus providing important insights into potential mechanisms of CyN development. Those new insights could improve diagnosis and treatment for CyN patients. Adult-onset CyN can be treated with corticosteroids or immunosuppressants, whereas childhood-onset CyN is not responsive to these treatments. Recently, a positive outcome reported in patients treated through systemic administration of G-CSF suggests that G-CSF is a potentially effective treatment of childhood-onset CyN patients [14]. RhG-CSF treatment has been shown to result in substantially increased average neutrophil counts in patients with childhood-onset CyN [15]. Contrasting effects of G-CSF and GM-CSF treatment on CyN found that G-CSF increased average neutrophil counts more than 20-fold, and GM-CSF increased neutrophil counts only modestly. Another study showed that G-CSF treatment significantly increased the patient's average neutrophil counts while at the same time amplifying neutrophil cycling and reducing the duration of neutropenic nadir and the frequency of skin and pulmonary infections [16]. Recently, researchers demonstrated that intramuscular administration of G-CSF-lentivirus to a normal dog and a gray collie elevated the neutrophil levels for more than 5 months, significantly increased neutrophil counts over the pretreatment level, with no adverse effects. G-CSF delivery by gene therapy using lentiviral vectors could also be a mode of delivery for a long-term treatment of patients with CyN [17]. However, more studies need to be performed to determine its efficacy and safety as a clinical treatment.

In summary, this report presents a rare case of childhood-onset CyN. A thorough review of the patient's medical history and monitoring of neutrophil counts were essential to make this diagnosis. Treatment with rhG-CSF resulted in a significant increase in neutrophil counts and reduction in associated symptoms. Since oral ulcer occurs in all CyN patients, dentist should be aware of the

association between recurrent oral ulcer and cyclic neutropenia in order to make give early diagnosis and treatment.

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