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Abstract: We report a patient with Japanese minor β thalassemia and HIV-1 infection.
The patient showed prolonged anemia, which was originally attributed to chronic parvovirus B19 infection. Twelve years later, the patient presented with exacerbation of microcytic anemia following cessation of anti-retroviral therapy; the exacerbation resolved when anti-retroviral therapy was resumed. Sequencing of the β globin gene revealed heterozygosity for a four-nucleotides deletion at codon 41/42 and minor β thalassemia was confirmed.
Because HIV-1-infected patients frequently show anemia due to nutritional deficiencies, opportunistic infections, AIDS-related malignancies, drug treatment and a direct effect of HIV-1 on the bone marrow, it is likely to overlook other causes of anemia.
Thalassemia should be considered in the differential diagnosis of anemia even in HIV-1 infected patients, when microcytic anemia without iron deficiency is observed.
Our case suggested that active HIV infection may have worsened β thalassemia, and early introduction of anti-retroviral therapy is beneficial for the recovery of anemia.

Response to Reviewer and Editorial office

Response to Reviewer

Major point

According to the reviewer's suggestion, I discussed possible causes of the temporal correlation between HIV-1 viremia and exacerbation of microcytic anemia and summarized current literatures. I added two references.

Interestingly, I also found description in Hattori's paper that some type of minor thalassemia (including -4 codons 41/42) tend to show acute exacerbation by acquired factors, such as pregnancy and infection.

I also discussed multifactorial pathogenesis of anemia in HIV-positive patients such as Erythrocyte differentiation impairment, HIV-1 effect on stromal cells with cytokine dysregulation in the last part of the paper.

Minor point

According to the reviewer's suggestion, I corrected English usage "slight anemia" to "moderate anemia" which appeared in lines 8, 10 and 11 in the Discussion paragraph.

Response to Editorial Office

According to the suggestion, I provided brief descriptive legends in the text part and located after "References" section.

According to this alteration, I deleted legends which originally appeared with each Figures.

# Exacerbation of microcytic anemia associated with cessation of anti-retroviral therapy in an HIV-1-infected patient with beta thalassemia

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Abstract

We report a patient with Japanese minor β thalassemia and HIV-1 infection.

The patient showed prolonged anemia, which was originally attributed to chronic parvovirus B19 infection. Twelve years later, the patient presented with exacerbation of microcytic anemia following cessation of anti-retroviral therapy; the exacerbation resolved when anti-retroviral therapy was resumed. Sequencing of the β globin gene revealed heterozygosity for a four-nucleotides deletion at codon 41/42 and minor β thalassemia was confirmed.

Because HIV-1-infected patients frequently show anemia due to nutritional deficiencies, opportunistic infections, AIDS-related malignancies, drug treatment and a direct effect of HIV-1 on the bone marrow, it is likely to overlook other causes of anemia.

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## Introduction

HIV-1 infected patient frequently manifest anemia [1]. Anemia prior to anti-retroviral therapy (ART) is often caused by amebic or cytomegalovirus colitis, parvovirus B19 infection, and HIV-1 infection itself [2]. After anti-retroviral therapy, anemia is mainly due to ART therapy itself, especially using Zidovudine (ZDV or AZT), which resulting in macrocytic changes.

In this paper, we present an HIV-1-infected β-thalassemia patient who showed exacerbation of microcytic anemia along with the cessation of ART, and the anemia resolved when ART was resumed. Hemoglobinopathy should be considered in the differential diagnosis of anemia even in HIV-1 infected patient, especially where there is microcytic anemia without iron deficiency. Early re-introduction of anti-retroviral therapy is beneficial for the recovery of anemia in β-thalassemia patient with HIV infection.

## Case report

In March 2000, the patient, in his early forties, was admitted to the Kyushu Medical Center with Pneumocystis pneumonia. Since two months before the admission, severe anemia had continued. The HIV-1 RNA copy number in the plasma was 90000 copies/ml (Fig. 1) and CD4 positive T cell count was 70/μ l. Acquired immune deficiency syndrome (AIDS) was diagnosed. At the time of admission (March 2000), the hemoglobin concentration [Hb] was 5.8g/dl and mean corpuscular volume (MCV) was 72.4fl. On April 7, following the administration of antibiotics, [Hb] declined to 4.7g/dl without any hemorrhagic lesion, and the white blood cell (WBC) count declined to 900/μl. On April 13, bone marrow aspiration showed hypoplasia, and antiretroviral therapy [Sanilvudine (d4T), Lamivudine (3TC), Indinavir (IDV), and Ritonavir (RTV); later switched to d4T, 3TC, and Efavirenz (EFV)] was started on the same day. PCR of the bone marrow fluid revealed parvovirus B19 infection which suggested that the pancytopenia was caused by the bone marrow suppression due to antibiotics administration, or by HIV-1 infection itself, and parvovirus B19 infection-accelerated severe anemia. On April 21, HIV RNA was reduced to 1000 copies/ml and [Hb] was 7.6g/dl. He discharged on May 2000 and his [Hb] continued to recover. On July 2000, his [Hb] was 12.5g/dl: however, MCV was 82.7fl and remained microcytic. The cause of continued anemia of the patient was attributed to chronic parvovirus B19 infection at that time and was reported elsewhere [3].

Five years later in June 2005, he attended Kagoshima University Hospital. At that

time, the CD4 positive T cell count was 465/μ l and the HIV-1 RNA copy number in the plasma was undetectable (<50 copies/ml). RBC was 5 million/μl, [Hb] was 13g/dl, MCV was 79fl and the reticulocyte count was 15‰. For three years he continued the same antiretroviral therapy (d4T, 3TC, EFV) during which the HIV-1 RNA copy number was always undetectable and the CD4 count ranged between 441 and 790/μl. At that time, his [Hb] level ranged between 11.4 and 13.9g/dl and MCV ranged between 77 and 79fl.

In Feb 2008, he moved to another prefecture. Four years later, in March 2012, he again visited Kagoshima University Hospital due to job re-relocation, and complained of easy fatigue. He had discontinued antiretroviral therapy of his own will eight months prior to this visit. His HIV-1 RNA copy number in the plasma was 920000 copies/ml, CD4 count was 101/μl, [Hb] was 7.9g/dl, MCV was 64fl and reticulocyte count was 19 ‰. He showed no evident opportunistic infection at this time. Four days later, antiretroviral therapy [3TC, Abacavir (ABC), Darunavir (DRV), RTV] was resumed. Ten days after re-administration of ART, his [Hb] was 7g/dl, but 24days after re-administration of ART (April 2000), his [Hb] increased to 8.4g/dl and MCV was 68fl, HIV-1 RNA copy number decreased to 3300 copies/ml and CD4 count recovered to 308/μl. In May 2012 (52days after re-administration of ART), his [Hb] increased to 11g/dl, HIV-1 RNA copy number decreased to 1300 copies/ml and CD4 count recovered to 422/μl. One year later in May 2013, his [Hb] increased to 13.6g/dl along with the complete inhibition of HIV RNA copy number in the plasma (Fig. 1). The serum iron in March 2012 was 37μg/dl (normal range 44-192) and UIBC was 161μg/dl (normal range 111-255). However, 24days after re-administration of ART, serum iron was 92μg/dl and UIBC was 112μg/dl without iron administration, which suggested his microcytic anemia was not from iron deficiency. Because the [Hb] in March 2012 was so low with microcytic change and there was no hemorrhagic lesion or opportunistic infection, another reason for the anemia was suspected.

Target cells were observed in the peripheral blood (Fig 2). Hemoglobin analysis revealed a HbA2 of 9% and HbF of 4%, which suggested the existence of a hemoglobinopathy. Further tests for hemoglobinopathies showed a prolongation of the glycerol lysis time (107 sec, compared to the normal control of 22-55sec) which implies elevated osmotic resistance. Finally, DNA sequencing revealed heterozygocity in the β globin gene, with the deletion of 4 nucleotides at codon 41/42 (TTCTTT to TT) in one allele (Fig 3), and β-thalassemia minor was diagnosed.

## Discussion

Anemia is a common clinical finding in HIV-1-infected patients. Many factors may contribute to the development of anemia in HIV-1-infected patients including nutritional deficiencies, opportunistic infections, AIDS-related malignancies, drug treatment and a direct effect of HIV-1 on the bone marrow [2].

Our case showed severe anemia ([Hb] 5.8g/dl) when AIDS was first diagnosed, when he had a high HIV-1 RNA in the plasma. The patient's anemia improved after anti-retroviral therapy, but moderate anemia continued. At this time the anemia was attributed to chronic parvovirus B19 infection [3, 4]. However, even after the recovery of the $CD4^+$ cell count, moderate anemia with microcytic change continued for years. Because his anemia was moderate ([Hb] 13g/dl), it was not investigated further at that time. Twelve years later, when he ceased ART, he again showed moderate microcytic anemia (Hb 7.9g/dl), and this anemia resolved when ART was resumed. Because there was no hemorrhagic lesion, or opportunistic infection, we sought another cause of anemia. First, the hemoglobin fraction was measured, and both Hb-A2 and Hb-F were elevated, suggesting a hemoglobinopathy. Finally, sequencing of the β globin gene revealed a four-nucleotide deletion at codon 41/42 in one allele of the β globin gene, leading to the diagnosis of β thalassemia minor.

Even in non-thalassemic HIV-1 carriers, higher values of Hb-A2 have been observed during ART, especially with Zidovudine (ZDV) [5-7]: that is increased HbA2 alone is not a sufficient reason to suspect thalassemia in HIV-1 patients receiving ART. However, treatment with anti-retroviral drugs such as ZDV often results in macrocytosis [8]. It has also been reported that mean corpuscular volume (MCV) of HIV-1 patients with thalassemia after ART increased from microcytic levels to normocytic levels, and ART did not worsen anemia in patients with thalassemia [9, 10]. Therefore, HIV-1-infected patients with non-iron-deficient microcytic anemia, in whom a hemoglobinopathy is suspected from abnormal hemoglobin fractions should be subjected to gene analysis to make a concrete diagnosis of thalassemia.

Thalassemia is relatively rare in Japan, where malaria is uncommon. The frequency of β-thalassemia in Japan is one in 600 to 1,000 of the general population [11].

Most β-thalassemia patients in Japan are heteorozygote and present with thalassemia minor. They are prone to be misdiagnosed as having iron deficiency anemia.

The four-nucleotide deletion at codon 41/42 in β globin gene found in this patient is the fourth most frequent mutation found in Japanese β thalassemia patients [11]. It is not known whether active HIV-1-infection (i.e. not controlled by ART) exacerbates all types

of β thalassemia, but in the present case there was a strong temporal association between exacerbation of microcytic anemia when HIV-1 infection worsened following cessation of ART, and resolution of the anemia when ART was resumed (Fig. 1). Although most β-thalassemia in Japanese is heterozygous and shows no overt hemolysis but mild anemia with macrocytosis, it is reported that some of the mutant including four-nucleotide deletion at codon 41/42 observed in our case occasionally do have acute exacerbation by acquired factors such as pregnancy and infection [11].

Effect of HIV replication on erythropoiesis is not well understood. The pathogenesis of anemia in HIV-positive patients could be multifactorial [2]. Dysfunction of erythroid differnetiation related to bone marrow (BM) microenvironment damage and stromal cell impairment by HIV-1 infection is reported [12]. It is also reported that IL-1β, IFN-γ, TGFβ1 and TNFα, which are elevated in BM as a result of chronic infalmmation that may be associated with HIV-1 viremia, suppress the growth of progenitor cell *in vitro* and may play an important role in the induction of HIV-associated anemia [13]. Moreover, unbalanced hemoglobin chain synthesis during HIV-1 infection has been reported [14]. Thsese multiple factors may be involved in the temporal correlation between HIV-1 viremia and exacerbation of microcytic anemia observed in the present case.

In conclusion, thalassemia should be considered in the differential diagnosis in an HIV-1-infected patient who presents with microcytic anemia without iron deficiency. And early introduction of anti-retroviral therapy is beneficial for the recovery of anemia in β thalassemia.

Conflict of interest The authors declare that there have no conflict of interest

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Legend to figures

Fig. 1

Clinical course of Hemoglobin (Hb) and HIV RNA copy number along with the cessation of anti-retrovirus therapy (ART) and re-administration of ART.

Correlation between HIV-1 viremia and exacerbation of anemia was observed.

d4T sanilvudine, 3TC lamivudine, IDV indinavir, RTV ritonavir, EFV efavirenz, ABC abacavir, DRV darunavir

Fig.2

Target cells were observed in the blood film. (arrows)

Fig.3

DNA sequencing revealed four-nucleotide deletion at codon 41/42 in one allele of the β-globin gene.

Figure1

Fig. 1

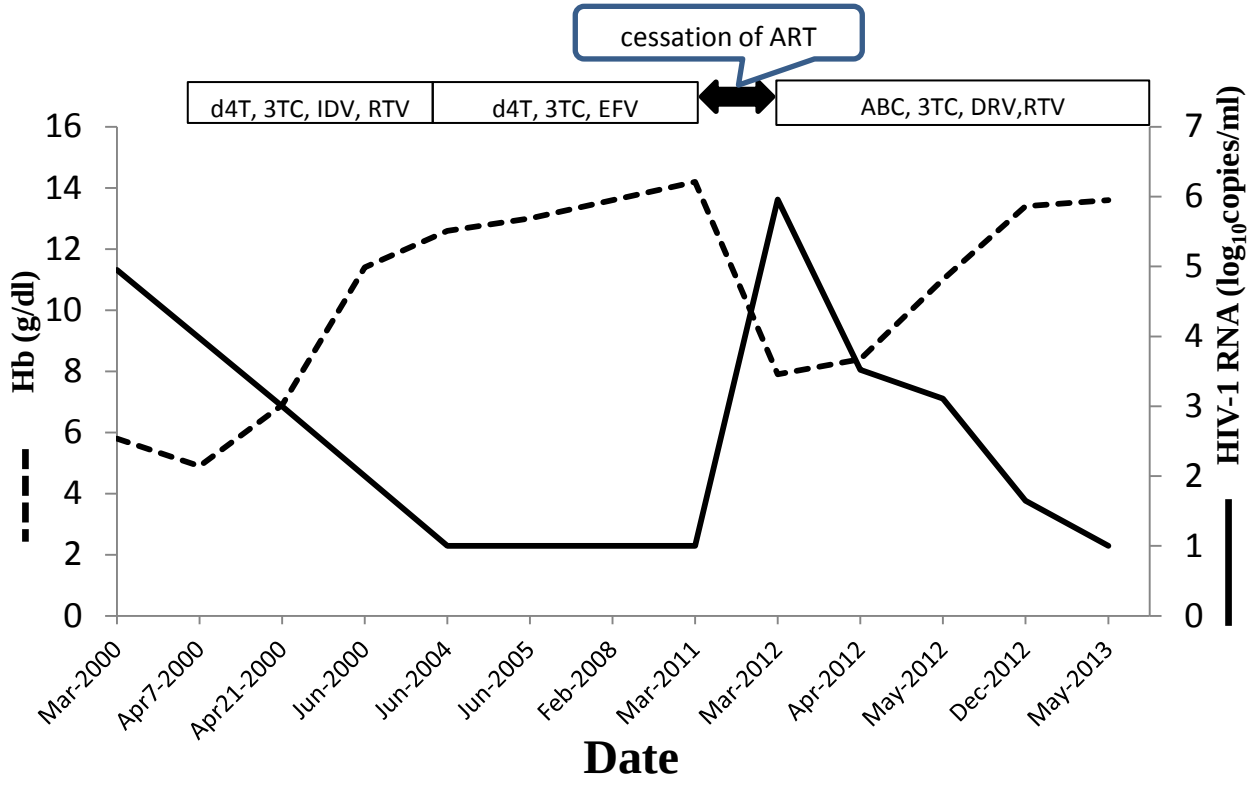

**Figure2**

Fig. 2

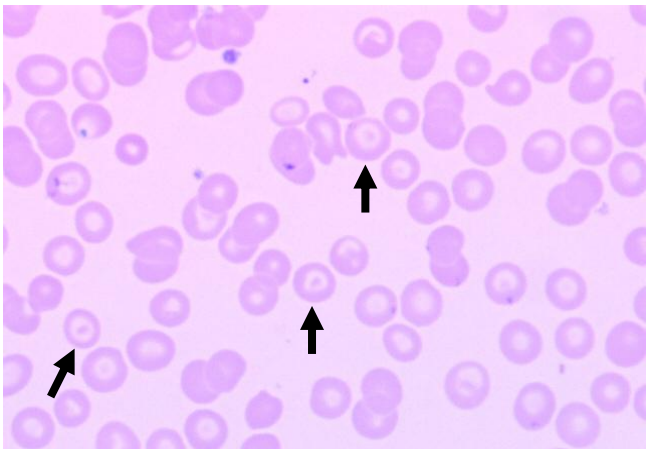

**Figure3**

Fig. 3