Original Research Report

Significant Decrease in the Serum Haptoglobin Level after the Antiretroviral Therapy in Patients Infected with Human Immunodeficiency Virus-1

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Subjects : We examined the serum haptoglobin (Hp) levels in 52 HIV-1 infected patients at our hospital. Fifteen of them were hemophilic, and 37 of them had been infected by sexual contact.

Results : Twenty-two of the 52 cases (42.3%) demonstrated a lower Hp level than normal. Furthermore, the Hp level in 14 cases (26.9%) had decreased to levels lower than the measurement sensitivity. Only one of the patients, whose Hp values were less than normal, showed a weakly positive finding for Coombs direct test. We recognized neither an overt decrease of hemoglobin nor an overt increase in the number of reticulocytes in the patients whose Hp values showed less than normal values. We therefore concluded that hemolytic anemia including autoimmune hemolytic anemia was not related to a decrease in the Hp levels. We found a significant decrease with Hp levels in patients who were receiving antiretroviral therapy (ART). We also found a significant decrease in the Hp levels during ART even in patients with an HIV-1 infection and without hemophilia or hepatitis C. We measured the Hp levels before and during ART in 6 cases, and thus recognized a significant reduction in the Hp levels during ART. IL-4, which can affect Hp production, was not found to be related with the observed decrease in the Hp levels.

Conclusion : We concluded that ART induced the decrease of Hp. The cause of the decrease in the Hp level by ART is unknown, but it might promote arteriosclerosis by lipodystrophy because of breakdown in the Hp's defense mechanism for the vascular disorder.

Key words : haptoglobin, HIV therapy

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Introduction

Haptoglobin (Hp) is biosynthesized in either hepatic cells or mature granular leukocytes in lymph nodes (eosinophils in particular)[1]. Regarding the physiological function, it binds to oxidized hemoglobin (Hb/H_2O_2) generated by hemolysis, and forms an Hp-Hb complex[1]. Through receptors, reticuloendothelial system cells take and metabolize the complex. This mechanism neutralizes the vascular toxicity of oxidation by Hb/H_2O_2, and prevents a loss of Hb through glomerulus in the kidney[1].

The Hp value changes in various conditions, such as hemolytic anemia, inflammation, hormonal abnormality and immunological disorder. There have been some reports about autoimmune hemolytic anemia in HIV infected patients[2–4].

We herein report the changes observed in the Hp level in HIV-1 infected patients.

Patients and Methods

We examined 52 HIV-1 infected patients and 59

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samples in our hospital, consisting of 49 males and 3 females. The average age was 38.6±11.5 years old. Fifteen of them were hemophilic, and 37 of them were infected by sexual contacts (Table 1). The serum Hp was measured using nepherometry, and the serum interleukin-4 (IL-4) level was measured using an Enzyme-linked immunoabsorbant assay (Pharmingen©)[5].

Results were statistically analyzed using Student's t test. P-value < 0.05 was considered significant.

Results

We found a significant decrease in the serum Hp levels in HIV-1 infected patients. Twenty-two of 52 cases (42.3%) and 24 of 59 samples (40.6%) demonstrated lower Hp levels than normal. Furthermore, the Hp levels in 14 cases (26.9%) and 14 samples (23.7%) decreased to levels lower than the measurement sensitivity. However, there was neither any correlation between Hp and hemoglobin, nor between Hp and reticulocytes (Figure 1). Human Hp is classified into 3 phenotypes, namely 1–1, 2–1 and 2–2 according to polyacrylamide gel electrophoresis, and the normal ranges differ among types. Because the number of patients with the 1–1 type was small, we mainly evaluated patients with 2–1 and 2–2 types.

Table 1 Clinical profile of the subjects.

Total	52 cases
Male : Female	49 : 3
Average age	38.6±11.5
Hemophiliac : Infection by sexual contacts	15 : 37
Races	all Japanese

We found a weakly positive finding for Coombs direct test only with the IgG method in only one of the 12 patients whose Hp values were lower than normal. We recognized neither an overt decrease in hemoglobin (14.5±1.5 g/dl) nor an overt increase of reticulocytes (1.5±0.4%) in the patients whose Hp values were lower than normal. We therefore concluded that hemolytic anemia, including autoimmune hemolytic anemia, was not related to the decreased Hp levels.

We next examined the relationship between the decrease in the Hp levels and other various parameters. We found a significant decrease in the Hp in patients who received antiretroviral therapy (ART) (Figure 2 A). In patients with the 2–1 type, the Hp level in patients without ART was 240±99 mg/dl, which was within normal range (103–341 mg/dl), while the level during ART was 108±53 mg/dl ($p < 0.005$). In patients with the 2–2 type, the Hp level in patients during ART was 48±32 mg/dl, while the level without ART was 106±58 mg/dl ($p < 0.01$), which was also within normal range (41–273 mg/dl). We could not evaluate the Hp type in 9 patients because the Hp values were lower than the measurement sensitivity levels. These extremely low levels were all observed during ART. (The number of patients with the 1–1 type was too small to evaluate.)

Many HIV infected patients in Japan are hemophiliac, and many of the HIV infected patients have also been infected by the hepatitis C virus (HCV). The Hp levels have been reported to decrease in patients with

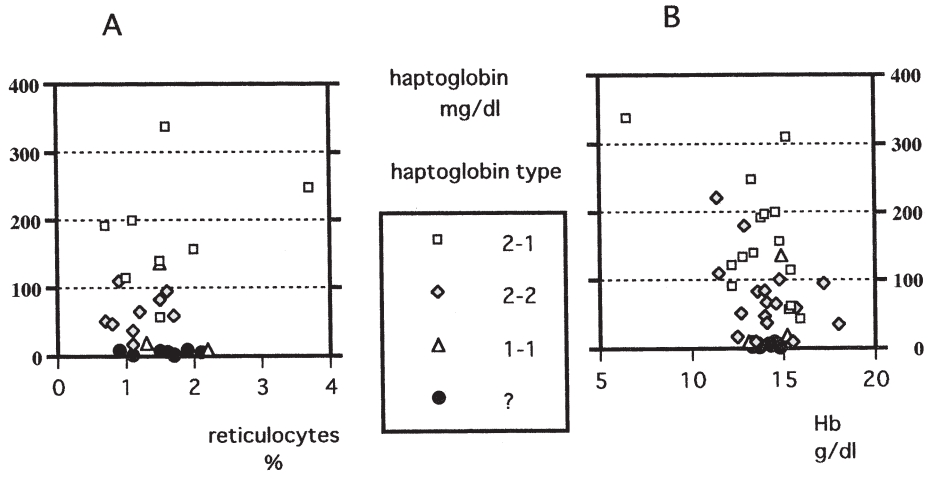

Figure 1 Correlation between haptoglobin and reticulocytes (A) or hemoglobin (B) in HIV-1 infected patients.
A : 2–1 type p=0.2451, 2–2 type p=0.4879
B : 2–1 type p=0.0458, 2–2 type p=0.2539

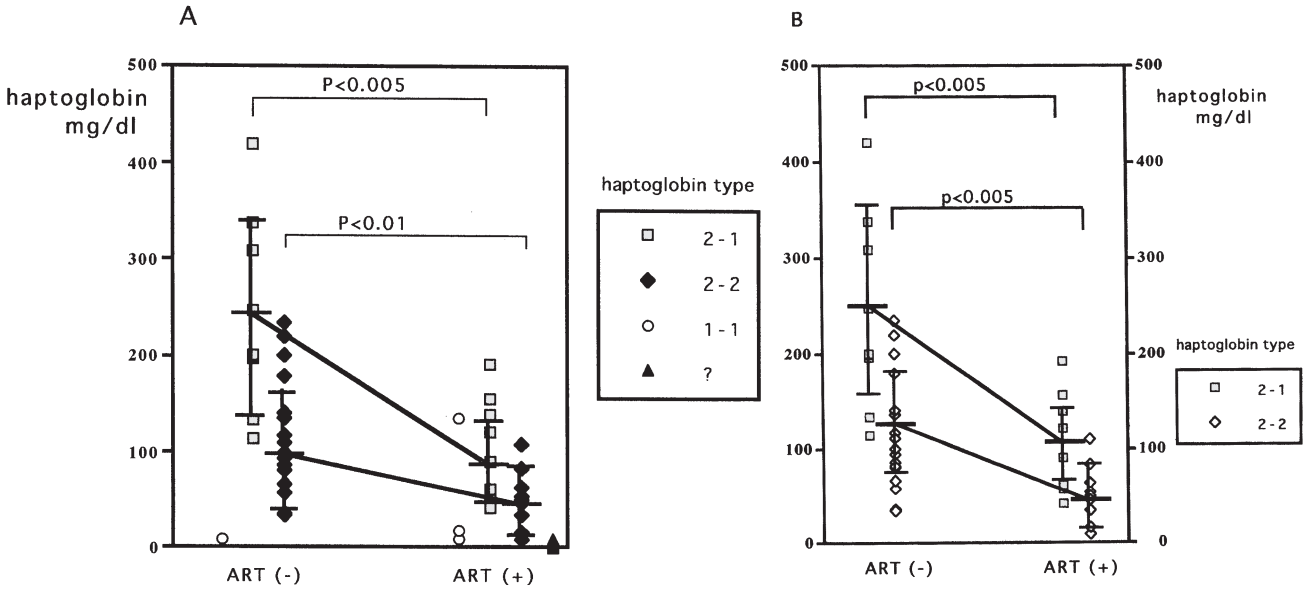

Figure 2 A : Effects of ART on serum haptoglobin in HIV-1 infected patients.
B : Effects of ART on serum haptoglobin in HIV-1 infected patients without hemophilia and HCV co-infection.

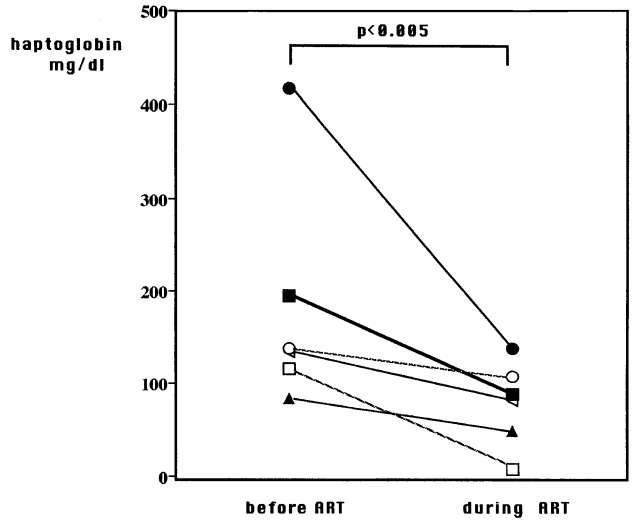

Figure 3 Change of serum haptoglobin before and during ART in the same patients.
Duration of ART were 4–60 months, and the average duration was 27.2±22.2 months.

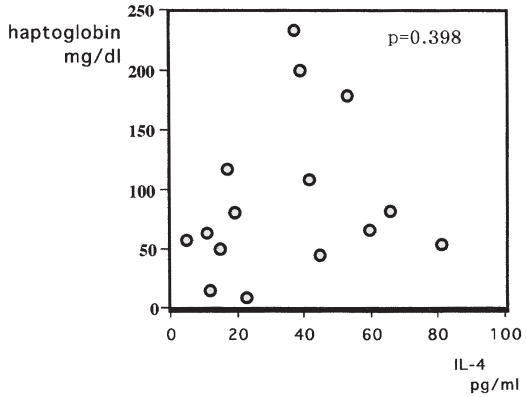

Figure 4 Corelation between serum haptoglobin and serum IL-4.
Haptoglobin type in all of them was 2–2 type.

hemophilia or hepatitis C[6–9]. Regarding our data, the Hp level in patients with either hemophilia or an HCV infection also tended to decrease compared with those without hemophilia and HCV, especially in patients with the 2–1 Hp type (Data not shown). However, we also found a significant decrease in the Hp levels during ART in HIV-1-infected patients without hemophilia or HCV, for both the 2–1 and 2–2 Hp type (Figure 2 B).

In addition, we reviewed the Hp levels before and during the ART therapy in the same 6 cases, and thus we recognized a significant fall in the Hp level during therapy (Figure 3). Furthermore, we found the lower the Hp levels were, the longer the duration of therapy, especially in 2–1 type (Data not shown).

Interleukin-4 (IL-4) has been reported to induce a reduction in the Hp level ; however[10], Figure 4 showed no correlation between the serum Hp and serum IL-4 levels.

Discussion

There have been many reports on the relationship between Hp and HIV infection. For example, one of them reported that HIV-infected patients carrying the Hp 2–2 phenotype tended to show a worse prognosis, which is reflected by a more rapid rate of viral replication in the absence of ART[11]. We herein report that many of the HIV-1-infected patients showed a reduction in the serum Hp (Figure 1). The serum Hp level in HIV-1-infected patients without ART has been reported to be within the normal range or to increase according to the progression of the HIV-infection[12–14]. Inflammation due to opportunistic infections or the HIV infection itself seems to be responsible for the increase in the Hp level in HIV-infected patients, because Hp is one type of acute phase protein of inflammation. In fact, C-reactive protein had been shown to correlate with Hp (Data not shown). However, we could not conclude that the main reason for the observed decrease in the Hp level by ART was that the Hp level increased by the inflammation may return to normal range during ART, because almost half of the patients showed decreased Hp levels which were lower than the normal range.

There have been several reports on the incidence of autoimmune hemolytic anemia in HIV-infected patients[2–4]. According to our data, a few patients also showed a weakly positive finding for the direct Coombs test using only the IgG method. Although some HIV-infected patients may show a positive finding for the Coombs test, a hemolytic state in such patients is very rare[12, 13]. Hypergammaglobulinemia associated with an HIV infection seems to be responsible for frequent positive finding on the Coombs test, because of the non-specific binding of immunoglobulin to red blood cells. We therefore concluded that the decrease in the Hp levels in HIV-infected patients was not related to autoimmune hemolytic anemia. Furthermore, the decrease in the Hp level in HIV-infected patients was also not related to the other types of hemolysis either, because we recognized neither an overt decrease of hemoglobin nor an overt increase in the number of reticulocytes even in patients whose Hp values were less than normal.

We found a significant decrease in the Hp levels during ART. The cause for this is still unclear at present, because the reduction in the Hp level occurs in many conditions, such as hemolysis, hemophilia, hepatitis C, and hormonal abnormality. However, hemophilia, and hepatitis C do not seem to be responsible for the decrease in the Hp levels, because HIV-1 infected patients without hemophilia and HCV showed significantly lower Hp levels during ART.

Immune reconstitution by ART and HIV infection itself induces disorders of the immune network and changes in the lymphocyte population and cytokine production, such as Interleulin-2 (IL-2), IL-4 and gamma-interferon[15–19]. In addition, it has been reported that IL-4 also inhibits the production of some acute-phase proteins, such as HP[19]. It is possible that the change in the IL-4 production induced by ART may reduce the Hp production. However, the serum IL-4 level did not correlate with the Hp reduction. Our findings indicate that the reduction in the Hp levels during ART is not induced by the changes in the IL-4 production.

Anti-HIV drugs, especially protease inhibitors, may induce many adverse effects. One of them is so-called lipodystrophy. Lipodystrophy is characterized by peripheral lipoatrophy, abdominal distension, a buffalo hump, hyperlipidemia, and insulin resistant diabetes[20, 21]. It mimics hormonal disorders, such as Cushing's syndrome[20], and the administration of cortisol can thereby induce a reduction in the Hp levels. However, another study reported that cortisol in HIV patients during ART showed no remarkable changes[20]. ART also induces hypertriglyceridemia and lactic acidosis[21, 22]. The serum triglyceride and lactate levels also showed a slight tendency to correlate with the Hp reduction, but the difference was not statistically significant (Data not shown). It is possible that the decrease in the Hp levels by ART might be one symptom of an adverse effect due to the use of anti-HIV drugs, such as lipodystrophy.

Lipodystrophy may induce arteriosclerosis since it is a metabolic disorder, and many clinicians are afraid of an increased incidence of cardiovascular disease by ART in HIV infected patients. The reduction of the Hp levels might also promote arteriosclerosis due to lipodystrophy because of a breakdown in the defense mechanism of Hp for the vascular disorders. Based on the above findings, ART may thus induce arteriosclerosis and subsequently cardiovascular diseases to a greater degree than has been previously estimated up until now.

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