Influence of Erythropoietin Treatment on Hemoglobin A_{1c} Levels in Patients with Chronic Renal Failure on Hemodialysis

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We studied the influence of erythropoietin (EPO) treatment on hemoglobin A_{1c} (HbA_{1c}) levels under conditions which eliminate the effect of changes in the blood glucose concentration. HbA_{1c} levels, blood glucose, hematocrit (Hct) and reticulocyte counts were serially measured every two weeks after starting or stopping EPO administration in 15 non-diabetic hemodialysis patients. EPO treatment significantly influenced HbA_{1c} levels, and the more erythropoiesis fluctuated by changing the dose of EPO, the more HbA_{1c} levels changed, though there were no significant changes in blood glucose levels during the study period. The changes in HbA_{1c} during the 2-week period correlated inversely with both the changes in Hct during the same 2 weeks and the reticulocyte counts at that time. We concluded that the change in Hct should be kept in mind when the HbA_{1c} level is evaluated in EPO-treated patients and a formula should be proposed to correct HbA_{1c} levels based on the change in Hct or the reticulocyte count. (Internal Medicine 37: 826–830, 1998)

Key words: renal anemia, glycemic control, hematocrit, reticulocyte

Introduction

Glycated hemoglobin is formed by the binding of glucose to the N-terminal amino groups of the β chain of hemoglobin molecules. It can be separated into three distinct fractions, which are designated hemoglobin HbA_{1a}, HbA_{1b} and HbA_{1c}. Among them, HbA_{1c} is the most abundant component. The binding of glucose to hemoglobin occurs continuously during the life of red blood cells in a nonenzymatic process (1). Thus, the value of HbA_{1c} reflects the mean glycemic level of a patient over the previous 4- to 6-week period, and is widely used as a parameter of control in diabetic patients (2). Precise assessment of glycemic control in dialysis patients is particularly important because of the increasing number of diabetic patients being treated for end-stage renal failure, and HbA_{1c} is frequently used as a parameter of glycemic control.

Anemia which is caused mainly by the diminished synthesis of erythropoietin (EPO) in diseased kidneys is a universal feature among dialysis patients (3, 4). Administration of recombinant EPO typically has obtained excellent responses in the improvement of renal anemia and has brought beneficial

effects in terms of the quality of life of the patients (5). Thus EPO is recently widely used in the treatment of renal anemia and approximately 75% of the hemodialysis population are candidates for EPO (4).

Since EPO stimulates the production of red blood cells, the proportion of old and young red blood cells as well as the total number of red blood cells in peripheral blood can change according to starting, stopping or changing doses of EPO administration. The age of red blood cells has been known as a determinant of HbA_{1c} levels other than blood glucose concentration (6). However, the amount of influence of EPO treatment on the HbA_{1c} levels in dialysis patients remains unclear.

To clarify the extent of change in HbA_{1c} levels following EPO treatment under conditions which exclude the influence of blood glucose, we investigated the serial levels of HbA_{1c} after starting and stopping EPO administration in non-diabetic dialysis patients whose blood glucose concentrations were normal and stable.

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Materials and Methods

Fifteen patients (10 males and 5 females, mean age 51.8 ± 11.9 years old) with chronic renal failure on maintenance hemodialysis and receiving EPO treatment were studied. The patients were randomly selected among outpatients without diabetes mellitus. The causal diseases of renal failure were 13 chronic glomerulonephritis, 1 polycystic kidney and 1 lupus nephritis. Hemodialysis was performed for four hours three times a week using hollow fiber dialyzers.

In the pre-study period, EPO administration was stopped in all patients until their hematocrit (Hct) decreased below 25%. Subsequently EPO treatment was started; 3,000 or 1,500 units of EPO were administered intravenously at the end of each hemodialysis session. Oral or intravenous iron supplementation was simultaneously performed if their serum iron saturation values (Fe/total iron binding capacity) were below 20% or their serum ferritin levels were below 100 $\mu\text{g}/100\text{ ml}$. EPO administration was continued during the 20-week period or until their Hct increased to 35%. Thereafter EPO administrations were stopped again for 20 weeks or until their Hct decreased below 25%. Thus, study period was consisted of 2 phases; EPO administration period and no EPO administration period. HbA_{1c}, blood glucose, Hct and reticulocyte count were measured every 2 weeks at the start of the first dialysis of the week. Food intake before blood sampling was voluntary in each patient.

The patients were divided into two groups based on the extent of changes in Hct ($\pm\Delta\text{Hct}$) observed in the study period. Group I ($n=10$) consisted of patients whose ΔHct was 10% or more, and group II ($n=5$) consisted of patients whose ΔHct was less than 10%. In group I patients, differences in HbA_{1c} levels before and at 2, 4 and 6 weeks after both starting and stopping of EPO treatment were compared. Also in group I, the most prominent change in HbA_{1c} during a 2-week interval ($\Delta\text{HbA}_{1c}\text{-2W}$) in terms of both increases and decreases, were examined in each patient simultaneously with the changes in Hct during the same 2-week interval ($\Delta\text{Hct-2W}$) and the reticulocyte count at the end of the second week, and the relationships among those values were investigated.

HbA_{1c} was measured using a fully automated hemoglobin A_{1c} analyzer (Kyoto Daiichi Kagaku, Kyoto) based on the methods of a combination of reversed-phase partition and cation-exchange high performance liquid chromatography (7, 8). Blood glucose was measured by the glucose-dehydrogenase method. Hct was measured by an autoanalyzer and reticulocytes were counted by flow cytometry. Statistical analysis was performed using Student's t-test, paired t-test and Pearson's correlation coefficient.

All studies were conducted in accordance with the principles set out in the Declaration of Helsinki, and informed consent was obtained from all patients.

Results

The mean duration of the period of EPO administration was 11.5 ± 4.2 weeks in group I patients and 18.8 ± 1.8 weeks in group II patients ($p<0.01$), and mean weekly dose of EPO administration was $8,400 \pm 1,265$ units in group I patients and $5,100 \pm 823$ units in group II patients ($p<0.001$). Changes in HbA_{1c} levels during the study period were significantly greater in the patients of group I than in the patients of group II ($1.8 \pm 0.6\%$ vs $0.5 \pm 0.2\%$, $p<0.001$; Fig. 1).

After starting EPO treatment in group I patients, HbA_{1c} levels decreased with the increase of Hct over time. The HbA_{1c} values at 2, 4, and 6 weeks were significantly lower and Hct at those times were significantly higher than the pre-treatment levels (Table 1). Conversely, after stopping EPO treatment, HbA_{1c} levels increased over time and the HbA_{1c} values at 2, 4 and 6 weeks were significantly higher and Hct at 6 weeks was significantly lower than pre-treatment levels (Table 2). There were no significant changes in blood glucose and fructosamine

Figure 1. Comparison of the changes in HbA_{1c} levels ($\pm\Delta\text{HbA}_{1c}$) in patients whose Hct fluctuations with EPO treatment were over 10% (group I) and less than 10% (group II).

Table 1. Changes in HbA_{1c}, Hct, Blood Glucose and Fructosamine after the Start of EPO Administration

	pre	Weeks		
		2	4	6
HbA _{1c} (%)	6.1 ± 0.3	5.5 ± 0.4 ^{*b}	5.0 ± 0.4 ^{*a}	4.9 ± 0.7 ^{*b}
Hct (%)	23.0 ± 2.2	24.7 ± 2.3 ^{*b}	26.9 ± 4.0 ^{*b}	29.0 ± 5.1 ^{*b}
Blood glucose (mg/dl)	94.9 ± 14.5	100.2 ± 19.6	101.9 ± 17.4	93.4 ± 14.5
Fructosamine (μmol/l)	364.0 ± 24.8	362.8 ± 26.9	365.3 ± 34.5	360.5 ± 33.7

^{*a}: p<0.001, ^{*b}: p<0.01 against pre-values.

Table 2. Changes in HbA_{1c}, Hct, Blood glucose and Fructosamine after Discontinuation of EPO Administration

	pre	Weeks		
		2	4	6
HbA _{1c} (%)	5.1 ± 0.7	5.5 ± 0.6 ^{*a}	6.0 ± 0.7 ^{*b}	6.4 ± 0.7 ^{*a}
Hct (%)	34.2 ± 2.6	32.4 ± 2.3	31.1 ± 3.8	29.9 ± 4.7 ^{*c}
Blood glucose (mg/dl)	90.6 ± 17.0	99.4 ± 20.6	93.2 ± 20.8	87.6 ± 20.0
Fructosamine (μmol/l)	367.3 ± 24.5	360.3 ± 22.6	368.7 ± 29.0	364.3 ± 26.6

^{*a}: p<0.001, ^{*b}: p<0.01, ^{*c}: p<0.05 against pre-values.

levels during the study period.

The values of $\Delta\text{HbA}_{1c}\text{-2W}$ correlated inversely with both the values of $\Delta\text{Hct-2W}$ ($r=-0.730$, $y=-0.19x+0.04$, $p<0.001$; Fig. 2) and the reticulocyte count at that time ($r=-0.753$, $y=-0.09x+1.15$, $p<0.001$; Fig. 3).

Discussion

HbA_{1c} is the product of the chemical condensation of hemoglobin and glucose. Biosynthetic studies *in vivo* indicate that HbA_{1c} is formed slowly, continuously and irreversibly

Figure 2. Relationship between HbA_{1c} changes during a 2-week period ($\Delta\text{HbA}_{1c}\text{-2W}$) and Hct changes during the same 2-week period ($\Delta\text{Hct-2W}$). The most prominent increase and decrease in HbA_{1c} levels for each patient during both periods were recorded.

Figure 3. Relationship between HbA_{1c} changes during a 2-week period ($\Delta\text{HbA}_{1c}\text{-2W}$) and the reticulocyte count at that second week. Values were selected for each patient from both periods during which HbA_{1c} level was most prominently increased and decreased.

throughout the life span of the red blood cell (9). Thus, the levels of HbA_{1c} can reflect the glucose concentration in blood where red blood cells have been present since they were newly produced. Therefore if blood glucose concentrations are high during this period, HbA_{1c} levels are likely high.

On the other hand, the glycated rate of just produced young blood cells is reported to be lower than old cells (6). Furthermore, patients with a shortened erythrocyte life-span due to hemolysis are reported to have markedly decreased levels of HbA_{1c} (9). From those facts, the ratio of young and old erythrocytes in the peripheral blood could be a determinant for HbA_{1c} levels other than the blood glucose concentration.

In the present study, we tried to clarify the influence of EPO on HbA_{1c} levels in hemodialysis patients. We chose non-diabetic patients as subjects in attempt to eliminate the influence of the changes in blood glucose concentration. It has been well known that uremic patients, even when the underlying disease is not diabetic nephropathy, have glucose intolerance and this abnormality can be partially restored by hemodialysis (10, 11). The elevated fructosamine levels seen in the present study could be a reflection of glucose intolerance induced by uremia. However, no significant fluctuations were seen in the blood glucose or fructosamine levels throughout the study period, thus the influence of blood glucose on the fluctuations in HbA_{1c} levels can be denied in this study.

In our results, the patients whose Hct had been increasing with EPO administration showed a significant decrease of HbA_{1c} levels although the blood glucose levels remained stable. Conversely, HbA_{1c} levels significantly increased in patients whose EPO administration had been stopped. EPO accelerates the production of new erythrocytes, and the ratio of

young erythrocytes in peripheral blood must increase after initiating EPO administration. Whereas, after stopping EPO administration the ratio of old erythrocytes in peripheral blood must increase due to reduced production of new erythrocytes. Thus, fluctuations of HbA_{1c} levels after starting or stopping EPO treatment may be due to changes in the ratios of young and old erythrocytes in peripheral blood.

Diabetic nephropathy is currently one of the major causes of end-stage renal disease requiring dialysis therapy, and the population of diabetic dialysis patients has been expanding over the past two decades. It is essential that good glycemic control is maintained in patients with diabetic nephropathy, even after substitution of dialysis. Several parameters such as HbA_{1c}, fructosamine, and glycated albumin are now available to monitor long-term glycemic control. Among those parameters, HbA_{1c} has the greatest meaning in clinical practice (12) and is measured routinely in many diabetes clinics. Even when nephropathy is present, the HbA_{1c} level is demonstrated to be useful as a parameter of long-term glycemic control in diabetic patients (13).

In the use of HbA_{1c} as a parameter of glycemia in diabetic dialysis patients, however, it should be remembered that HbA_{1c} levels can be influenced by fluctuation in erythropoiesis resulting from alteration of the EPO dosage as we described in the present study. HbA_{1c} levels in dialysis diabetic patients could be roughly corrected by the value of $\Delta\text{Hct-2W}$ and more accurately by reticulocyte counts. We derived the following formula from our present results in order to be able to correct HbA_{1c} levels:

$$\text{Corrected HbA}_{1c} (\%) = \text{Actual HbA}_{1c} + 0.19H - 0.04$$

where, H is the change in Hct (%) during the previous 2 weeks.

$$\text{Corrected HbA}_{1c} (\%) = \text{Actual HbA}_{1c} + 0.09R - 1.15$$

where, R is the reticulocyte count (‰).

In conclusion, HbA_{1c} levels in hemodialysis patients can be influenced by starting or stopping EPO treatment, and HbA_{1c} levels can decrease or increase according to the increase or decrease of Hct caused by changing the EPO dosage, respectively. Correction of HbA_{1c} levels based on the values of $\Delta\text{Hct-2W}$ or the reticulocyte count might be needed to assess the HbA_{1c} level as a parameter of glycemia in diabetic dialysis patients.

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