Relationships of Fetal-Type Erythropoiesis versus Nitric Oxide Production and Glycated Hemoglobin Levels in Diabetics

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Abstract. This study investigated the potential contribution of nitric oxide (NOx) production to enhanced fetal hemoglobin (HbF) synthesis in patients with diabetes. Glycated hemoglobin (HbA1c), HbF, high sensitivity C-reactive protein (hsCRP), plasma glucose levels, and serum NOx concentrations were measured in 350 diabetics and 125 healthy subjects. There were no significant correlations between HbF and HbA1c levels, nor between HbF and plasma glucose levels. However, serum NOx concentrations in patients with HbF >1.0% (76.2±32.4 µmol/L) were significantly higher than those with HbF≤1.0% (47.3 ± 29.8 µmol/L, p <0.05). Inversely, patients with moderately increased serum NOx levels >98.1 µmol/L (75th percentile of patients) exhibited significantly higher HbF levels than those with decreased serum NOx levels <34.2 µmol/L (25th percentile of patients) (1.16 ± 0.41 vs. 0.62 ± 0.28%, p <0.05). After excluding the subjects with high NOx levels, elevated HbF concentrations returned to a level not significantly different from the control value. Serum NOx concentrations were significantly correlated with HbF (r = 0.32, p <0.05) and hsCRP levels (r = 0.35, p <0.05) in diabetic patients. In conclusion, long-term glycemic control does not contribute to fetal-type erythropoiesis, but increased NOx production seems to play an important role in the enhanced HbF synthesis of diabetics.

Keywords: fetal hemoglobin, nitric oxide, glycated hemoglobin, diabetes

Introduction

Fetal hemoglobin (HbF) production occurs principally during the fetal stage of human development. In healthy adults, HbF synthesis is minimal, and HbF is restricted to a specific population referred to as F cells [1]. However, the reemergence of HbF production, called fetal-type erythropoiesis, has been observed in certain inherited and acquired diseases in adulthood [2]. Because HbF co-migrates with glycated hemoglobin (HbA1c), HbF leads to overestimation of HbA1c levels during the analysis of hemoglobin fractions using either electrophoresis or ion exchange methods [3,4]. Enhanced HbF production has been reported in patients with type 1 and type 2 diabetes mellitus [5-8]. The reason for the increased HbF fraction in diabetics remains unclear. Several investigators reported that elevated HbF levels are associated with poor glycemic controls [6,7]. However, Koskinen et al [8] found that there was no significant difference in glycemic control or the duration of diabetes in patients with high and low HbF levels. Most of the studies concerning HbF production in diabetics have focused on HbA1c levels, particularly in relation to long-term glycemic control. Little is known about the possible contribution of nitric oxide (NOx) production to enhanced HbF synthesis in diabetics. This study investigated the relationships between serum NOx concentrations and fetal-type erythropoiesis in patients with diabetes mellitus.

Materials and Methods

A total of 350 patients (182 males, 168 females; age = 39-75 yr) with type 2 diabetes mellitus were investigated. Patients were evaluated in terms of serum NOx, HbF, HbA1c, high-sensitivity C-reactive protein (hsCRP), and plasma glucose levels. The control group consisted of age-matched, non-diabetic, healthy subjects (n = 125; age = 38-74 yr), who showed plasma glucose levels ≤100 mg/dl and HbA1c concentrations ≤6.0%. The subject populations comprised Koreans who were...
not pregnant, exhibited no apparent inflammation, and had no history of hemoglobinopathies, including thalassemia and sickle cell diseases. Patients with acute blood loss (n = 2), nephropathy (n = 3), and drug administration (n = 5) were excluded from the study, since these conditions may influence HbF production. Blood samples were collected prior to treatment. The Institutional Review Board of Inha University Hospital reviewed and approved the study protocol.

HbA1c and HbF levels were analyzed by high performance liquid chromatography with EDTA-anticoagulated blood using a G7 Glycohemoglobin Analyzer (Toosoh Bioscience, South San Francisco, CA), which can rapidly separate the hemoglobin components based on their electrical charge [9]. Patients with elevated HbF >1.0% were considered to have fetal-type erythropoiesis. Plasma glucose and hsCRP levels were assayed with a chemical analyzer (Hitachi 7600; Hitachi, Tokyo, Japan), with cutoff limits of HbA1c (>6.0%) and hsCRP (>0.3 mg/L), based on the manufacturer’s instruction.

Nitric oxide (NOx) concentrations were measured by reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent nitrate reductase assay in the serum of patients who were on a reduced nitrate and nitrite diet. After serum nitrate was converted to nitrite by NADPH-dependent nitrate reductase, the total concentration of nitrite was determined by spectrophotometry at 540 nm [10]. Elevated NOx levels were defined as a value above the 95th percentile of serum NOx concentrations of the control group (serum NOx >67.8 µmol/L).

Subjects were categorized into one of two groups based on their HbF and HbA1c levels: patients with HbF >1.0% (n = 74) and ≤1.0% (n = 276); patients with HbA1c >6.0% (n = 101) and ≤6.0% (n = 249). Subjects were then further stratified into two groups according to their serum NOx levels: patients with serum NOx >98.1 µmol/L (n = 87) and <34.2 µmol/L (n = 87). These figures were based on the cutoff values of 75th and 25th percentiles of serum NOx levels of 350-patient populations. Data analysis was conducted using a non-parametric test (the Mann-Whitney U test). Correlation coefficients were computed by the Spearman test. All p values <0.05 were considered statistically significant.

Results and Discussion

Mean values of HbF levels were significantly higher in diabetics than in healthy subjects (0.89 ± 0.43 vs 0.56 ± 0.32%, p <0.05). The prevalence of elevated HbF levels in diabetic patients was 2.2 times higher than in the control group (21.1 vs 9.6%, p <0.05) (Table 1). These data are in general agreement with the previous results, which demonstrated that HbF synthesis was increased in patients with diabetes [5-8].

As shown in Table 2, there were no significant differences in HbF levels between patients with HbA1c >6.0% and ≤6.0%. No significant differences in HbA1c and plasma glucose levels were

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diabetic patients (n = 350)</th>
<th>Healthy subjects (n = 125)</th>
</tr>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>49.2 ± 14.3 (48): 39-75</td>
<td>47.1 ± 12.8 (47): 38-74</td>
</tr>
<tr>
<td>Serum NOx levels (µmol/L)</td>
<td>56.2 ± 34.7 (59.7):b 20.6-150.4</td>
<td>31.8 ± 17.9 (29.5): 15.2-75.6</td>
</tr>
<tr>
<td>HbF levels (%)</td>
<td>0.89 ± 0.43 (0.7):b 0.2-3.5</td>
<td>0.56 ± 0.32 (0.6): 0.3-0.9</td>
</tr>
<tr>
<td>hsCRP levels (mg/dl)</td>
<td>3.23 ± 4.31 (2.5):b 0.03-26.37</td>
<td>0.12 ± 0.35 (0.1): 0.01-1.08</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.9 ± 1.5 (5.8):b 4.6-16.5</td>
<td>5.2 ± 0.3 (5.1): 3.4-6.0</td>
</tr>
<tr>
<td>Plasma glucose levels (mg/dl)</td>
<td>167.2 ± 90.4 (161):b 127-473</td>
<td>85.9 ± 7.6 (89): 69-100</td>
</tr>
</tbody>
</table>

NOx = nitric oxide; HbF = fetal hemoglobin; hsCRP = high sensitivity C-reactive protein; HbA1c = glycated hemoglobin A1c.

a Elevated NOx levels were defined as a value above the 95th percentile of serum NOx concentration of the control group (serum NOx >67.8 µmol/L).

b p <0.05 vs. healthy subjects, computed by Mann-Whitney U test.
observed between patients with HbF >1.0% and ≤1.0%. There were also no significant correlations between HbF and HbA1c levels, nor between HbF and plasma glucose levels. These observations suggest that long-term glycemic control and recent blood glucose levels do not contribute to enhanced HbF production in diabetics. These results differed from previous reports which indicated that HbA1c concentrations were correlated with HbF levels in diabetic patients [6]. However, our data were consistent with the results of one study [8], which demonstrated that there were no detectable correlations between the amount of HbF and HbA1c in diabetic patients [6]. However, our data were consistent with the results of one study [8], which demonstrated that there were no detectable correlations between the amount of HbF and HbA1c in diabetic patients [6].

Several experimental and clinical studies have shown that serum NOx concentrations were increased in patients with diabetes [14], although the exact mechanism of enhanced NOx production is still unclear. In our study, diabetics with elevated HbA1c levels >6.0% had significantly higher concentrations of serum NOx than those with decreased HbA1c ≤6.0% (73.4 ± 31.9 vs 50.1 ± 27.5 µmol/L, p <0.05).

As shown in Table 3, serum NOx concentrations were more strongly correlated with HbA1c levels than with plasma glucose levels. These results imply that increased NOx production in diabetics is more closely associated with long-term hyperglycemia than with their latest blood sugar levels. Our data supported the results of

**Table 2. Serum NOx and hsCRP concentrations in relation to HbF production and glycated hemoglobin levels in diabetic patients.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HbF production</th>
<th>Glycated hemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HbF &gt;1.0% (n = 74)</td>
<td>HbF ≤1.0% (n = 276)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46.3 ± 11.6 (46): 47.8 ±10.5 (47): 47.0 ± 11.4 (46): 47.6 ± 12.1 (47):</td>
<td>40.75 39.73</td>
</tr>
<tr>
<td>Serum NOx levels (µmol/L)</td>
<td>76.2 ± 32.4 (73.9): 20.6-150.4 15.2-103.6</td>
<td>73.4 ± 31.9 50.1 ± 27.5 (43.6): 20.6-103.6</td>
</tr>
<tr>
<td>HbF levels (%)</td>
<td>1.87 ± 0.54 (1.6): 0.59 ± 0.21 (0.6): 0.92 ± 0.46 0.87 ± 0.41</td>
<td>(73.9):a 20.6-150.4 15.2-103.6 (70.8):b 15.2-150.4</td>
</tr>
<tr>
<td>hsCRP (mg/dl)</td>
<td>3.98 ± 2.85 (2.7): 0.07-26.37 0.02-13.84</td>
<td>4.50 ± 2.92 2.72 ± 2.01 (3.2):b 0.04-26.37 (1.9): 0.02-12.53</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.1 ± 1.7 (5.9): 4.6-14.3 4.9-16.5</td>
<td>7.4 ± 1.9 (6.9):b 5.4 ± 0.4 (5.5): 6.1-16.5</td>
</tr>
<tr>
<td>Plasma glucose levels (mg/dl)</td>
<td>189.3 ± 78.1 (171): 130-326</td>
<td>213.9 ± 86.8 148.2 ± 51.3 (190)b 127-473 (143): 129-308</td>
</tr>
</tbody>
</table>

a p <0.05 vs diabetics with HbF ≤1.0%; b p <0.05 vs diabetics with HbA1c ≤6.0%; computed by Mann-Whitney U test.
Cosentino et al [15], which demonstrated that the prolonged exposure of endothelial cells to high glucose levels can increase NOx production.

NOx production was investigated in relation to HbF and HbA1c levels. Serum NOx concentrations in patients with HbF >1.0% were 76.2 ± 32.4 µmol/L, which was significantly higher than those values in patients with HbF ≤1.0% (47.3 ± 29.8 µmol/L, p <0.05). Serum NOx concentrations were significantly correlated with HbF levels (r = 0.32, p <0.05). HbF levels between the groups based on serum NOx concentrations were also evaluated, but there were no significant differences in HbF levels between subjects with serum NOx >67.8 µmol/L and those with serum NOx ≤67.8 µmol/L (data not shown). However, patients with moderately increased serum NOx levels >98.1 µmol/L (75th percentile of patients) exhibited significantly higher HbF levels than those with decreased serum NOx levels <34.2 µmol/L (25th percentile of patients) (1.16 ± 0.41 vs 0.62 ± 0.28%, p <0.05).

Interestingly, the mean values of HbF levels in the diabetics with decreased serum NOx levels <34.2 µmol/L were 0.62 ± 0.28%, which was not statistically different from those of the healthy controls (0.56 ± 0.32%, p = 0.17). After excluding the subjects with high NOx levels, elevated HbF concentrations returned to a level that was not significantly different from the control value, suggesting that increased serum NOx levels play a crucial role in the enhanced HbF production in diabetics.

In conclusion, increased HbF synthesis in diabetics was more closely associated with serum NOx concentrations than with HbA1c levels, suggesting that fetal-type erythropoiesis in diabetics has more important implications for enhanced NOx production than for long-term glycemic control.
Acknowledgement
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References