Study of D/G Hemoglobin incidence in a sample population (single institution)

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Background:
The hemoglobin disorders constitute the most prevalent group of monogenic disease. A finding consistent with this is a hemoglobin/G variant indicating that the patient has Hb D/G trait. Hb D/G traits are clinically benign. In this study, the epidemiology of Hb D/G was reviewed in a single institution in Iran.

Methods and Materials:
We conducted the epidemiological study at Aliahsghar children’s hospital and all patients with Hb D/G were entered to this study. The hematological values, hemoglobin electrophoresis, peripheral blood smear and clinical findings were collected in the data special form.

Results:
Among 11825 hemoglobin analysis performed, we detected 101 cases of hemoglobin D/G. There were 55.4% male and 44.6% female, median age 9±13.36 yrs. Homozygous of D/G was found in seven patients with splenomegaly, jaundice, mild anemia and Reticulocytosis. Heterozygote patients were asymptomatic. There wasn’t any correlation between Hb D/G with serum ferritin, MCV, Hb and sex.

Conclusion:
In Iran Hb D/G is relatively benign condition with mild anemia, Poikylocytosis and minimal hemolysis.

Key Words: Hemoglobin D/G, Hemoglobinopathies, IRAN

Introduction:
Hereditary disorders of hemoglobin are common in many areas of the world including the Middle East.(1) In some regions of Middle East more than 10% of the population are carriers of one of these abnormal genes.(2) In Iran, abnormal hemoglobins are quite common. About 5% of the Iranian population carry Beta Thalassemia genes and 0.5% to 1 percent carry other abnormal Hemoglobin genes such as Hgb S, D, G and E. Hemoglobin D and G are a group of at least 16 beta chain variants and 6 alpha chain variants that migrate in an alkaline PH at the same electrophoretic position as Hbs. However, they do not sickle when exposed to reduced oxygen tension.³⁵ Most variants are named for the place where they were discovered. HbD Punjab and Hb D Los Angeles are identical hemoglobins, glycine being substituted for glutamic acid at the 121st position in the beta chain (α₂ β₂ 121 GLu→Gln). HbG Philadelphia is an alpha variant of D hemoglobin with a substitution of asparagine by lysine at the 68th position (6-8). Both Hb D &G are asymptomatic in the heterozygous state. Hb D disease (HbDD) is marked by mild hemolytic anemia and chronic non-progressive splenomegaly. On electrophoresis, HbD is present at over 95% with normal amounts of Hgb A₂ & F. On Alkaline electrophoresis, Hb D has the same mobility as Hb S. Hb D is separated from Hgb S on citrate agar at pH 6.0.⁹-¹⁰ These hemoglobins do not sickle and yield a negative solubility test result. No treatment is required. These hemoglobins are benign.¹¹-¹⁴

The Current survey is an epidemiological study of different types of abnormal Hb D/G at a single institution.

Methods & Materials:
We conducted the simple epidemiological study at the Ali-Asghar Children’s hospital in Tehran. All patients with diagnosis of Hgb D/G in this hospital were entered to this study. The diagnosis of disease in a child was followed by family study of Sibling and parents by CBC, retic., Hb electrophoresis, serum ferritin and review of peripheral smear. Analysis of hemoglobin variants were performed with a liquid chromatography system using a 3.5 x 0.46 cm poly CATS a column. The sample preparation, mobile phase composi-
tion and chromatographic condition are as de-
scribed by central laboratory. As light modifica-
tion of the gradient program (increasing or de-
creasing initial proportion of mobile phase B) or the pH of the mobile phases may be neces-
sary to adjust the retention times of HbA, F, S,
A2, D and G to the suggested limits. Solubility
test performed if S region was predominant.\(^{11}\)
All patients’ data sheets were completed and
analysis was done.

**Results:**
Among 11825 hemoglobin electrophoresis
analysis performed, we detected 101 cases of
hemoglobin D/G. Other Hemoglobinopathies
included: Major Thalassemia 1600 cases, Sickle
cell 141 cases, Intermediate Thalassemia 186
cases, HbH disease 23 cases, HbC disease
11 cases and Minor Thalassemia 9763 cases.

In patients with HbD/G, 7 cases were homozy-
gous, 8 patients had HbD, G/β\(^{-}\)thal and 1 pa-
tient was S/D, G. There were 55.4% male
(56), and 44.6% female (45), median age was
9±13.36yr (0.9 -56), splenomegaly noted in
6.9% (7pts) 5 cases were homozygous and 2
cases of HbD/β\(^{-}\)thal/, jaundice noted in 11.9 %
(12) and pallor in 33.7 % (34). Patients origin
were from different provinces of the country as
follows: Gilan 36 pts, Tehran 36pts(mixed
population of whole country), Khozestan 9 pts,
Mazandaran 6, Kerman 5 pts, Hamadan 3 pts .
The major Finding in peripheral blood smears
was tear drop19.8% (20), target14.9% (15),
polychromasia20.8% (21) and NRBC 0.88 ±
1.41(0-6).
Other laboratory data was shown in table 1 and
2.

| Table 1. Laboratory findings of study group (101 patients) |
|----------------|----------------|----------------|----------------|
|                | Mean | Median | Std. Deviation | Minimum | Maximum |
| RBC            | 5.12 | 5.00   | 0.76           | 2.12    | 8.00    |
| Hemoglobin     | 12.48| 12.5   | 2.29           | 3.10    | 16.80   |
| Hematocrit     | 38.22| 39.00  | 6.15           | 19.20   | 56.00   |
| MCV            | 77.57| 79.70  | 9.41           | 47.00   | 95.00   |
| MCH            | 28.33| 26.90  | 2.99           | 14.6    | 28.50   |
| MCHC           | 32.06| 32.00  | 3.37           | 30.00   | 35.00   |
| Platelets      | 332775.2| 267000 | 37252.4      | 123000  | 767000  |
| Reticulocytes  | 1.55 | 1.35   | 1.36           | 0.50    | 8.00    |
| NRBC           | 1.20 | 0.88   | 1.41           | 0.00    | 6.00    |

| Table 2. Electrophoresis Data (101 patients) |
|----------------|----------------|----------------|----------------|
|                | Mean | Median | Std.Deviation | Minimum | Maximum |
| Hgb A1         | 52.63| 60.20  | 23.12         | 0.00    | 89.00   |
| Hgb A2         | 2.00 | 1.95   | 1.40          | 0.00    | 5.70    |
| Hgb F          | 1.49 | 0.80   | 2.64          | 0.00    | 22.60   |
| Hgb D/G        | 43.21| 38.00  | 21.33         | 8.00    | 99.40   |

In nonparametric correlation analysis, we found
no correlation between HbD/G with serum Fer-
ritin, MCV and hemoglobin.
In the Mann-Whitney test, there was no differ-
ence of Hb D/G, MCV and serum Ferritin lev-
els between the sexes.

**Conclusion:**
Hemoglobin D/G traits are clinically benign but
may be significant in offspring, who are homo-
zygous for the variant or co-inherit it with the
different variant such as Hb S or β\(^{-}\) Thalas-
semia.
Most homozygous patients are asymptomatic or
have mild symptoms such as pallor, jaundice
and splenomegaly. In other study mean Hb
level, MCH, MCV, Retic Index were 10.5 gr/dl,
25.6 pg, 76 fl, 3.5%, respectively (16-20) (Ta-
ble-3). But in our study, above indexes were
12.5, 26.9, 79.7 (P=0.0008, CI =99%) and
1.35%, respectively.
Reticulocytosis in our study, was relatively
lower than other studies, perhaps due to milder
gene expression and minimal hemolysis. He-
moglobin electrophoresis obtained in other
studies was as follows: HbF 2.5%, HbA2 3%,
HbA 38.1% and HbD/G 75%, but in our study
it was HbF 0.8%, HbA2 1.95%, HbA 60.2%
(P=0.000001, CI=99%) and HbD/G 38%
(P=0.000001, CI=99%). Because of the large
number of heterozygous cases in our study,
HbA and HbD/G were, respectively, higher and lower than other studies.\(^{(20-23)}\) Identifying hemoglobin gene mutation was not performed, but variant beta 22 Glu→Gln in the beta chain was detected in Iran\(^{(24-26)}\). Overall, HbD/G in Iran is a relatively benign condition and most patients are asymptomatic.

| Table 3. Finding in some reported cases of Hb D/G with and without Thalassemia |
|-----------------------------|-----------------|-----------------|-----------------|
| Population                  | Indian          | Indian          | Italian         |
| Hb(g/dl)                    | 9.1 (8.3-14)    | 12              | 13              | 8.3              |
| MCV(fl)                     | 60 (50-750)     | 70              | 78              | 52               |
| MCH(pg)                     | 20 (12.5-24)    | 23              | 24.3            | 16.9             |
| Retics (%)                  | 4.1 (1.5-8)     | 2               | 4.2             | 4.9              |
| HbF (%)                     | 5.1 (1.8)       | 1.2             | 2               | 7                |
| HbA2 (%)                    | 5.3             | 3               | 6.4             | 5                |
| HbA (%)                     | 7               | 0               | 0               | 0                |
| HbD/G (%)                   | 82.7            | 95              | 93.6            | 82               |
| Clinical Finding            | Anemia, splenomegaly | Normal        | Splenomegaly    | Splenomegaly    |

Reference: