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

Kh.Arjmandi[1], M. Faranoush[2], Gh. Bahoush[1], P. Vossough[3], AA. Hedayatiasl[4], Sh.Ansari[1], E. Shahgholi[5]

1. Assistant professor of pediatric hematology and oncology, Iran University of Medical Sciences, Ali Asghar children's Hospital
2. Assistant professor of hematology and oncology, Semnan University of Medical Sciences, Amir al Momenin Hospital
3. Professor of pediatric hematology and oncology, Iran University of Medical Sciences, Ali Asghar children's Hospital
4. Fellowship of pediatric hematology and oncology, Iran University of Medical Sciences, Ali Asghar children's Hospital
5. Assistant professor of pediatric hematology and oncology, Tehran University of Medical Sciences, Bahrami children's Hospital

Background:
The hemoglobin disorders constitute the most prevalent group of monogenic disease A findings consistent with this is a hemoglobin/G variant indicating that the patient has HbD/G trait. Hb D/G traits are clinically benign. In this study, the epidemiology of HbD/G was reviewed in a single institution in Iran.
Methods and Materials:
We conducted the epidemiological study at Aliasghar children's hospital and all patients with HbD/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 HbD/G with serum ferritin, MCV, Hb and sex.
Conclusion:
In Iran HbD/G is relatively benign condition with mild anemia, Poikylocytosis and minimal hemolysis.
Key Words: Hemoglobin D/G, Hemoglobinopathis, 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.(3-5) 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 ($\alpha_2 \beta_2^{121\ GLu \rightarrow GLN}$). 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 A_2 & 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 described by central laboratory. As light modification of the gradient program (increasing or decreasing initial proportion of mobile phase B) or the pH of the mobile phases may be necessary to adjust the retention times of HbA, F, S, A_2, 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 11cases and Minor Thalassemia 9763 cases.

In patients with HbD/G, 7 cases were homozygous, 8 patients had HbD, G/β^+thal and 1 patient was is 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

Table2. Electrophoresis Data (101 patients)

	Mean	Median	Std.Deviation	Minimum	Maximum
Hgb A_1	52.63	60.20	23.12	0.00	89.00
Hgb A_2	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 Ferritin, MCV and hemoglobin.

In the Mann-Whitney test, there was no difference of Hb D/G, MCV and serum Ferritin levels between the sexes.

Conclusion:

Hemoglobin D/G traits are clinically benign but may be significant in offspring, who are homozygous for the variant or co-inherit it with the different variant such as Hb S or β^0 Thalassemia.

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) (Table-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. Hemoglobin electrophoresis obtained in other studies was as follows: HbF 2.5%, HbA_2 3%, HbA 38.1% and HbD/G 75%, but in our study it was HbF 0.8%, HbA_2 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	Greek	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(1.8-	1.2	2	7
HbA_2(%)	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:

1. Lukens JN: The abnormalhemoglobins: general principles In Lee GR, Foerster J, et al (eds): Wintrobes Clinical Hematology, 10 th ed.Philadelphia: Lippincott Williams& Wilkins. 1999:1329-1345
2. Sturgeon P, Itano HA: Clinical manifestation of inherited abnormal hemoglobins. The interaction of HbS with HbD. Blood1955; 10:389
3. Schneider RG, Ueda S, Alperin JB, et al: Hemoglobin Los Angeles in two Caucasian families: Hemoglobin SD disease and hemoglobin D Thalassemia. Blood 1968; 32:250-259
4. Cawein MJ, Lappat EJ, Brangle RW, et al: Hemoglobin S-D disease. Ann Intern Med1966; 64:62-70
5. Cohen PS, Israel MA: Basic molecular biology for the pediatric Hematologist/Oncologist .Am J Pediatr Hematol oncol 1989; 11:467
6. Honig GR, Adams JG: Human hemoglobin genetics. Vienna, Springer-verlag, 1986
7. Weatherall DJ, Clegg Jb, Higgs DR, et al: The Hemoglobinopathies. In: Scriver CR, Beaudet AL, Sly WS, et al: The metabolic basis of inherited disease. 6th ed. New York, McGraw-Hill, 1989
8. Beutler E: The sickle cell diseases and related disorders: Williams Hematology, 6ih ed .New York; McGraw-Hill, 2001: 581-605
9. Wang WC, Lukens JC: Sickle cell anemia and other sickling syndromes.Wintrobes Clinical Hematology, 10 th ed.Philadelphia: Lippincott Williams& Wilkins.1999:1329-1345
10. Harmening DM: Hemolytic anemias, intracorposcular defects: the Hemoglobinopathies. Clinical Hematology and Fundamentals of Hemostasis, 3rd ed. Philadelphia: FA Davis, 1997:173-192
11. Rabinovitch A: Hemoglobinopathy survey set HGB.Northfield, MN: CAP, 1992:2-3
12.Ahmed M, Stuhrmann M, Bashawri L, Kuhnau W: The beta – globin genotype in hemoglobin D. Ann-Hematol .2001 NOV; 80(11): 629-33
13. Almeida AM, Henthorn JS, and Davies SC: Neonatal screening for Hemoglobinopathies. Br –J- Hematol, 2001 Jan; 112 (1): 32-35
14. Thornburg CD, Zimmerman SA, Schultz WH, and Ware RE: An infant with homozygous Hemoglobin D Iran, J-Ped- Hematol –Oncol.2001 Jan; 23(1): 67-8
15. Angastiniotis M, Modell B: Global epidemiology of Hemoglobin disorders: Annals, 1998; 850:251-269
16. Ahmed M, Petrou M: Screening extended families for genetic hemoglobin disorders in Pakistan; NEJM, 2002, vol 347(15):1162-1168
17. Primary health care approaches for the prevention and control of congenital and genetic disorders: report of a WHO meeting, Cairo, Egypt, 6-8 December 1999. Geneva: World Health Organization, 2000. (Document no. WHO/HGN/WG/OO.l.)
18. Alwan A, Modell B. Community control of genetic and congenital disorders. EMRO technical publication series 24 Alexandria, Egypt: WHO Regional Office for the Eastern Mediterranean, 1997
19. Bittles AH. The role and significance of consanguinity as a demographic variable. Popul Dev Rev 1994; 20:561-84
20. Khattak MF, Saleem M. Prevalence of heterozygous (beta)-thalassaemia in the northern areas of Pakistan Assoc] 992; 42:32-4.
21. The laboratory diagnosis of Hemoglobinopathies. Br J Hematol 998;] 0 I: 783-92.
22. Steinberg MH, Adams JG III. Haemoglobin A (J}: origin, evolution and aftermath. Blood 1991; 78:2165-77.
23. Ahmed S, Petrou M, Saleem M. Molecular genetics of (beta)-thalassaemia in Pakistan: a basis for prenatal diagnosis. 3r J Haematol 1996; 94:476-82.
23. Mouzouras M, Camba L, Ioannou P, Modell B, Constantinides P, Gale R. Thalassaemia as a model of recessive netic disease in the community. Lancet 1980; 2:5
24.Ra.iab A, Patton MA. Analysis of the population structure in Oman. Community Genet 1999; 2:23-6.
25. Steensma DP, Hoyer JD: Hereditary Red Blood Cell disorders; Mayo Clininc Proceedings. 2001, 76(3):285-293
26. Persons DA, TisdaleJF: Gene therapy for the hemoglobin disorders; Seminar in hematology, 2004, 41:279-286