The Prognostic Significance of HbF in Childhood Haematological Malignancies

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Abstract The degree of increase in foetal haemoglobin (HbF) synthesis in haematological malignancies may be associated with the degree of malignancy. The aim of the present study was to quantify HbF levels in various childhood haematological malignancies and also, to ascertain its prognostic significance by comparing the results with the already established standard prognostic factors. Newly diagnosed cases of haematological malignancies in the paediatric age group were included in the study. HbF levels were estimated in each case of the study group along with HbF levels of control group comprising healthy children of same age group. The estimation was done by HPLC and Modified Betke's method. 50 cases of newly diagnosed haematological malignancies were studied out of which most of the cases were of acute lymphoblastic leukaemia (ALL) [$n = 30(60\%)$] followed by acute myeloid leukaemia (AML) [$n = 8(16\%)$], Hodgkin's lymphoma [$n = 7(14\%)$], non-Hodgkin's lymphoma [$n = 5(10\%)$]. Raised HbF levels were found in 43.3 % cases of ALL (13/30) and 37.5 % cases of AML (3/8). No

significant rise in HbF level was found in cases of lymphomas. There was correlation between raised HbF level and poor prognostic factors in cases of ALL but no such correlation was found in cases of AML. HbF levels are often elevated in childhood leukaemias as compared to childhood lymphomas. Thus, the concentration of HbF in acute childhood leukaemia may be considered as a prognostic factor.

Keywords Foetal haemoglobin · Paediatric haematological malignancies

Introduction

Foetal haemoglobin (HbF) is recognised as an indicator of haematological disorders, as well as of malignant diseases. Unlike most tumour markers, HbF is not a direct product of tumour cells [1]. Its production and accumulation in the blood cells is induced by conditions of malignancy [2]. Concentrations of some HbF-inducing growth factors are raised in the serum of patients with cancer, providing an explanation for increased concentrations of HbF [3]. And in some forms of leukaemia there may be a genuine reversion to foetal erythropoiesis while in others the emergence of cells containing HbF appears to be part of a rapid regeneration process occurring after a period of marrow aplasia [4].

The causes of elevated HbF production in leukaemias are most likely multifactorial. The degree of increase in HbF synthesis may be associated with the degree of malignancy: the more aggressive the disease, the more augmented the HbF synthesis and is not restricted to any particular type of leukaemia [5].

The leukaemias account for the largest number of cases of childhood cancer and are the primary cause of cancer related mortality of children. According to International

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Classification of Childhood Cancer, Third edition (ICCC-3), precursor cell leukaemias were the most frequent haematological malignancies (HM) in children and constituted 60 % of all HMs (an age-adjusted incidence rate of 42.7 per million children-years). The second most frequent childhood HM was Hodgkin lymphoma (11.2 % of all HMs), yielding an age-adjusted standardized incidence rate of 6.3 per million children-years. With regard to myeloid lineage, acute myeloid leukaemias were the most frequent with a rate of 7.9 per million children-years [6].

There are over 20 factors known to be associated with prognosis in children with acute lymphoblastic leukaemia (ALL) and the number of prognostic factors in acute myeloid leukaemia (AML) is much smaller [5]. Thus, the aim of the present study was to quantify HbF levels in various childhood haematological malignancies and also, to ascertain its prognostic significance by comparing the results with the already established standard prognostic factors.

Materials and Methods

Newly diagnosed cases of haematological malignancies in the paediatric age group were included in the study. Cases of haematological malignancies on treatment or on follow up along with patients with age more than 12 years were excluded from this study. The study was conducted in the department of Pathology in R G Kar Medical College and Hospital, Kolkata from November 2009 to December 2010.

Clinical history and physical examination was noted in each case and each one of them was subjected to complete haemogram and bone marrow aspiration. FNAC as well as biopsy of lymph node were done in cases of lymphomas.

HbF levels were estimated in each case of the study group along with HbF levels of control group comprising healthy children of same age group and compared with the standard reference values [7]. The estimation was done by HPLC (Biorad VARIANT Haemoglobin testing system lot no. L70010502 Beta thal short program) and Modified Betke's method [8].

Standard poor prognostic factors were noted in each case paediatric haematological malignancies according to the standard text book and compared with the HbF levels [9–12]. Immunophenotyping could not be done as our Institution, being a State owned hospital the facility of immunophenotyping was not available. Moreover, as these patients were of low economic strata, they could not afford such expensive investigation.

All the observations were tabulated and compared. Statistical analysis of HbF levels between the study and control group were done using Chi square test and *p* value was calculated.

Results

50 cases of newly diagnosed haematological malignancies were studied out of which most of the cases were of ALL [$n = 30(60\%)$] followed by AML [$n = 8(16\%)$], HL [$n = 7(14\%)$], NHL [$n = 5(10\%)$].

Maximum no. of cases were within age group of 10–12 years comprising 20 cases of ALL, 5 cases of HL, 4 cases of AML, 3 cases of NHL. Male predominance was noted in cases of lymphomas with M:F ratio of 6:1 in HL and 4:1 in NHL while female predominance was seen in cases of leukaemia with M:F ratio of 1:1.5 and 1:1.16 in ALL and AML respectively.

Raised HbF levels were found in 43.3 % cases of ALL (13/30) and 37.5 % cases of AML (3/8). No significant rise in HbF level was found in cases of lymphomas. HbF levels of the control group was also studied which comprised of healthy children of same age group and compared with the standard reference values [7]. Then the HbF level of the control group and the study group were compared statistically. Using Pearson Chi square *p* value was calculated which was $p = 0.032$, which was statistically significant (95 % CI). The increase in HbF level in the study group was statistically significant (Table 1).

Mean HbF level in 30 cases of ALL was $1.8 \pm 0.34\%$ (range 0.7–2.9 %) whereas among the 13 cases of ALL with increased HbF level the mean HbF level was $2.4 \pm 0.25\%$ (range 2.0–2.9 %). Similarly, mean HbF level in total 8 cases of AML was $1.7 \pm 0.26\%$ (range 1.4–2.9 %) whereas in 3 cases of AML with raised HbF level, mean HbF was $2.6 \pm 0.22\%$ (range 2.1–2.9 %) (Table 2).

Standard poor prognostic factors comprising age >10 years, male sex, organomegaly/CNS manifestation, Hb > 10 g%, TLC > 50,000/cu mm, platelet count < 30,000/cu mm and morphology of blast were noted in all cases of paediatric ALL and HbF levels were compared with standard poor prognostic factors excluding cytogenetic factors. Raised HbF levels were found in all the cases of ALL where any of the poor prognostic factor(s) like organomegaly, Hb > 10 g%, TLC > 50,000/cu mm, platelet count < 30,000/cu mm were noted; giving 100 % correlation between raised HbF levels and aforementioned poor prognostic factors (Table 3).

Morphologically, French American British (FAB) L2 ALL was present in 12 cases (12/30 cases); out of which 10 cases had raised HbF levels (83.3 %). Raised HbF levels were also noted in 72 % cases of ALL presenting with age >10 years and male sex.

Mean HbF was calculated among the ALL cases with no poor prognostic factor and also in the ALL cases with one or more poor prognostic factor(s) and the value was statistically compared by Student's *t* test. *p* value was statistically significant ($p \leq 0.001$) (Table 4).

Table 1 HbF levels in the study group and control group

HbF level	Study group	Mean $\pm$ SD (%)	Range (%)	Control group	Mean $\pm$ SD (%)	Range (%)
Increased	16	2.4 $\pm$ 0.24	2.0–2.9	7	2.2 $\pm$ 0.10	2.0–2.3
Normal	34	0.8 $\pm$ 0.22	0.2–1.4	43	0.5 $\pm$ 0.10	0.2–0.9

Using Pearson Chi square test p value was calculated which came out to be: -0.032 , which was statistically significant (95 % CI). Mean HbF levels in the overall control group was 0.8 ± 0.20 % (range 0.2–2.3 %) whereas mean HbF in the overall study group was 1.8 ± 0.42 % (range 0.2–2.9 %)

Table 2 Percentage of cases of acute leukaemias with increased HbF levels

Haematological malignancies	No. of cases with $\uparrow$ HbF (mean $\pm$ SD, range)	Total no. of cases (mean $\pm$ SD, range)	Percentage of cases with increased HbF
ALL	13 (2.4 $\pm$ 0.25 %, 2.0–2.9 %)	30 (1.8 $\pm$ 0.34 %, 0.7–2.9 %)	43.3
AML	3 (2.6 $\pm$ 0.22 %, 2.1–2.9 %)	8 (1.7 $\pm$ 0.26 %, 1.4–2.9 %)	37.5

Table 3 Correlation of poor prognostic factors of acute lymphocytic leukaemia (ALL) & HbF levels

Poor prognostic factor	Percentage of cases of ALL	Percentage of cases of ALL with increased HbF level
Age >10 years	18 (60 %)	13 (72%)
Male	18 (60 %)	13 (72 %)
Organomegaly	12 (40 %)	12 (100 %)
Haemoglobin > 10 g%	13 (43 %)	13 (100 %)
TLC > $50 \times 10^9/L$	13 (43 %)	13 (100 %)
Platelet count < $30 \times 10^9/L$	12 (40 %)	12 (100 %)
FAB L2 morphology	12 (40 %)	10 (83.3 %)

Table 4 Comparison of the HbF values of ALL cases with no poor prognostic factor and ALL cases with one or more poor prognostic factors

	ALL cases with no poor prognostic factor	ALL cases with one or more poor prognostic factor
No. of cases	17	13
Mean HbF	1.0 %	2.4 %
Standard deviation	0.48	0.25
Range of HbF	0.7–1.6 %	2.0–2.9 %

Student's t test was calculated among the two groups and the value was found to be statistically significant ($p \leq 0.001$)

In cases of AML, HbF levels were compared with standard poor prognostic factors of AML like age <2 years, total leucocyte count > 1 lakh/cu mm, absence of Auer rods, absence of eosinophils, dysplastic megakaryocytes, FAB morphology but no correlation was found between HbF level and poor prognostic factors.

Not all the newly diagnosed patients were available for follow up, so were not included in our study. However as far as our available data, occurrence of relapse was more common in patients with raised HbF values.

Discussion

The question of HbF synthesis reactivation is an interesting topic both from the aspect of the genetic regulation of synthesis and from the aspect of the disorder of erythropoiesis in malignant diseases [12]. It is not clear how malignant transformation of granulocyte or lymphoid progenitor cells could reactivate HbF genes in erythroid cells. One of the hypothesis is that leukaemic cells excrete some mediating substance which either enhances the production of HbF or suppresses the HbF producing erythroid cells less than the cells producing adult type Hb [5]. Another hypothesis is that proteins common in foetal life often reappear in patients with malignancies when the genes operating during foetal life are reactivated like serum alpha foeto protein in hepatocellular carcinoma and carcino embryonic antigen in colonic carcinoma [5, 13]. In JCML and erythroleukaemia, Weatherall has shown a genuine reversion to foetal erythropoiesis. The fact that this reversion occurs more commonly in children may suggest that it may be affecting the basic mechanism by which the gamma chain synthesis is replaced by beta chain synthesis after birth [14, 15]. Ibarra et al. [16] stated that the findings of their study suggest that some factors intrinsic to the neoplasm might favour, in a different degree, the re-expression of HbF, which, in turn, would not necessarily mean a reversion to the foetal stages since the correlation of the gamma chains was similar to the adult pattern in 50 % of the cases studied. Pribilović et al. [17] quotes that the results of their study indicate that during the recovery of erythropoiesis, some erythroblasts synthesize haemoglobin

F. Those erythroblasts are probably the progeny of erythroid stem cells which during differentiation and proliferation escape some maturation stages.

Ibarra et al. studied the rate of HbF in 123 children with different malignancies with acute lymphoblastic leukaemia (ALL $n = 34$), acute myeloid leukaemia (AML $n = 19$), 23 cases of Hodgkin's disease (HD), 16 of non-Hodgkin lymphoma (NHL) and 31 of different solid tumours (ST). Ibarra et al. also noted that ALL and AML groups had the highest HbF rates (2.88 ± 1.93 and 2.63 ± 2.7 %, respectively), followed by HD and NHL (1.89 ± 1.09 and 1.81 ± 1.68 %, respectively). They suggested that some factors intrinsic to the neoplasm might favour, in a different degree, the re-expression of HbF [16].

The present study showed raised HbF levels in 43.3 % cases of ALL (13/30) and 37.5 % cases of AML (3/8). No significant rise in HbF level was found in cases of lymphomas. Pribilović et al. [17] found increased levels of HbF in 2/4 (50 %) ALL and 7/12 (58.3 %) AML cases and indicated that during the recovery of erythropoiesis, some erythroblasts synthesize HbF. Stojmirović [18], also found increased HbF in 59 % of the ALL patients and 72 % in acute nonlymphoblastic leukaemia.

Villabos-Arambula et al. [19] found statistically significant levels of raised HbF levels in both ALL L1 and ALL L2 by the Betke and Singer's methods (ALL-L1: 2.2 ± 1.5 %, ALL-L2: 2.0 ± 1.2 %; and ALL-L1: 2.0 ± 1.2 %, ALL-L2: 2.1 ± 1.5 % respectively) when compared with the control group (0.9 ± 0.4 %).

In cases of paediatric ALL, standard poor prognostic factors comprising age >10 years, organomegaly/CNS manifestation, Hb > 10 g%, TLC > 50,000/cu mm, platelet count < 30,000/cu mm and morphology of blast were noted and HbF levels were compared with standard poor prognostic factors excluding cytogenetic factors in the present study. Raised HbF levels were found in all the cases of ALL where any of the poor prognostic factors like organomegaly, Hb > 10 g%, TLC > 50,000/cu mm, platelet count < 30,000/cu mm were noted; giving 100 % correlation between raised HbF and aforementioned poor prognostic factors.

Morphologically FAB L2 ALL was present in 12 cases (12/30 cases); out of which 10 cases had raised HbF levels (83.3 %). Raised HbF levels were also noted in 72 % cases of ALL presenting with age >10 years.

In cases of AML, HbF levels were compared with standard poor prognostic factors of AML like age <2 years, total leucocyte count > 1 lakh/cu mm, absence of Auer rods, absence of eosinophils, dysplastic megakaryocytes, FAB morphology but no correlation was found between HbF level and the poor prognostic factors.

Rautonen and Siimes [5] showed that in patients with acute lymphoblastic leukemia (ALL), each increment of

1 g/l in initial HbF concentration resulted in a 1.13-fold rise in the risk of death or relapse (95 % CI 1.01–1.25, $p < 0.05$). In patients with myeloid leukaemias, each increment of 1 g/l in HbF was associated with a 1.20-fold (1.05 – 1.37 , $p < 0.02$) rise in the risk of death or relapse.

To conclude, HbF levels are often elevated in childhood leukaemia as compared to childhood lymphomas. The elevated levels being significantly correlated with poor prognostic factors in cases of ALL. Though simple yet appropriate and already suggested by researchers in the past, recent works have not been done in this topic as far as we have searched. This study established the correlation of increased levels of HbF in cases of ALL associated with other clinico-pathological poor prognostic factors. This study also perceived that by using simple methods like Modified Betke for estimation of HbF, prognosis of ALL patients can be ascertained at peripheral health care centres where expensive and sophisticated instruments for cytogenetics are unavailable and unaffordable.

Thus, the concentration of HbF in acute childhood leukaemia may be considered as a prognostic factor and further studies with larger series should be carried out to establish the fact.

References

1. Bertles JF (1974) Human fetal hemoglobin: significance in diseases. *Ann N Y Acad Sci* 241:638–652
2. Lavelle DE (2004) The molecular mechanism of fetal hemoglobin reactivation. *Semin Hematol* 41:3–10
3. Mroczko B, Szmitkowski M, Okulczyk B (2003) Hematopoietic growth factors in colorectal cancer patients. *Clin Chem Lab Med* 41:646–651
4. Sheridan BL, Weatherall DJ, Clegg JB, Pritchard J, Wood WG, Callender ST, Durrant IJ, McWhirter WR, Ali M, Partridge JW, Thompson EN (1976) The patterns of fetal haemoglobin production in leukaemia. *Br J Haematol* 32:487–506
5. Rautonen J, Siimes MA (1990) Initial blood fetal hemoglobin concentration is elevated and is associated with prognosis in children with acute lymphoid or myeloid leukemia. *Blut* 61:17–20
6. Marcos-Gragera R, Cervantes-Amat M, Vicente ML, de Sanjosé S, Guallar E, Godoy C, Calvo C et al (2010) Population-based incidence of childhood leukaemias and lymphomas in Spain (1993–2002). *Eur J Cancer Prev* 19:247–255
7. Glader B (2003) Anemia: general consideration. In: Greer JP, Foerster J, Lukens JN (eds) *Wintrobe's clinical hematology*, vol 1, 11th edn. Williams & Wilkins, Baltimore, p 1890
8. Wild BJ, Bain BJ (2001) Investigation of abnormal haemoglobins and thalassaemia. In: Lewis SM, Bain BJ, Bates I (eds) *Dacie and Lewis practical haematology*, 9th edn. Elsevier, Philadelphia, pp 261–262
9. Whitlock JA, Gayon PS (1999) Acute lymphocytic leukaemia. In: Pine JW Jr (ed) *Wintrobe's clinical haematology*, vol 2, 10th edn. William & Wilkins, Baltimore
10. Greer JP, Baer MR, Kinney MC (1999) Acute myelogenous leukaemia. In: Pine JW Jr (ed) *Wintrobe's clinical haematology*, vol 2, 10th edn. William & Wilkins, Baltimore

11. Longo DL (2008) Malignancies of lymphoid cells. In: Fauci AS, Kasper DL, Jameson JL, Loscalzo J (eds) *Harrison's principles of internal medicine*, vol 1, 7th edn. McGraw Hill Inc., New York, pp 691–692
12. Glader B (2004) Anaemia: general considerations. In: Pine J, Forbes A, Laser T, Mc Laughlin AM (eds) *Wintrobe's clinical haematology*, vol 1, 11th edn. Lippincott Williams & Wilkins, Philadelphia, p 948
13. Fishman WH (1976) Activation of developmental genes in neoplastic transformation. *Cancer Res* 36:3423–3428
14. Agarwal MB, Mehta BC, Rao SS (1983) Raised foetal haemoglobin in haematological malignancies. (A study of 97 cases). *J Postgrad Med* 29:15–19
15. Weatherall DJ, Edwards JA, Donohoe WTA (1968) Haemoglobin and red cell enzyme changes in juvenile myeloid leukemia. *Br Med J* 1:679–681
16. Ibarra B, Montes J, Becerra C, Prea FJ, Barras-Nunez P, Aguilar-Luna JC, Arroyo J (1991) Fetal hemoglobin in children with different neoplasms. *Sangre (Barc)* 36:383–386
17. Pribilović D, Pavlović-Kentera V (1980) Fetal hemoglobin in the blood in leukemia. *Bilt Hematol Transfuz* 8:65–70
18. Stojmirović E (1977) Fetal hemoglobin in acute leukemias. *Bilt Hematol Transfuz* 5:23–27
19. Villabos-Arambula AR, Aguilar-Luna JC, Esparza A, Perea FJ, de Loza R, Hernandez-Cordova A, Ibarra B (1993) Fetal hemoglobin and the gamma G/gamma A chain ratio in children with acute lymphoblastic leukemia L1 and L2. *Sangre (Barc)* 38:31–35