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# A STUDY OF BONE MARROW FAILURE SYNDROME IN CHILDREN

V. GUPTA, S. TRIPATHI, T. B. SINGH, V. TILAK<sup>1</sup>, B. D. BHATIA

## ABSTRACT

**BACKGROUND:** Bone marrow failure syndrome (BMFS), or aplastic anemia, includes peripheral blood single cytopenias, as well as pancytopenia due to inability of the marrow to effectively produce blood cells. **AIM:** To study the clinico-hematological profile and etiological factors of bone marrow failure syndrome in children. **SETTING AND DESIGN:** This prospective study was carried out in the Department of Pediatrics of a university teaching hospital over 36 months. **MATERIALS AND METHODS:** Children with pancytopenia (Hb < 10 g/dl, absolute neutrophil count <  $1.5 \times 10^9/L$ , platelet count <  $100 \times 10^9/L$ ) and bone marrow cellularity < 25% were included in the study. History of exposure to drugs, socioeconomic status, ethnicity and occupation of father were noted. Bone marrow aspiration; trephine biopsy; Ham test; viral studies for hepatitis A, B and C; and cytogenetic investigations were carried out. **STATISTICAL ANALYSIS:** Relative risk was estimated by odds ratio (OR) with 95% confidence interval (CI) in matched cases and controls. **RESULTS:** Of the 53 children studied, 6 (11.3%) were diagnosed as Fanconi anemia. Two cases had features of myelodysplastic syndrome. Forty-five children were labeled as acquired aplastic anemia, of whom one had evidence of hepatitis B infection and two patients (5.8%) had paroxysmal nocturnal hemoglobinuria. Aplastic anemia was more common in children from family with lower socioeconomic status; in Muslims; and where the father's occupation was weaving, dyeing and painting. However, the number was small to make statistically significant conclusions. No correlation could be established with exposure to drugs. **CONCLUSION:** Fanconi anemia was responsible for approximately one-tenth of the cases of bone marrow failure syndrome. Majority of the patients had acquired aplastic anemia. Hepatitis B infection was an uncommon cause of acquired aplastic anemia.

**Key words:** Aplastic anemia, bone marrow failure syndrome, Fanconi anemia, pancytopenia

## INTRODUCTION

The term 'bone marrow failure syndrome' (BMFS), or aplastic anemia, encompasses

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peripheral blood single cytopenias, as well as pancytopenia, due to inability of the bone marrow to effectively produce blood cells.<sup>[1]</sup> While acquired aplastic anemias are more common, it is possible that they occur in rare individuals who are genetically predisposed to marrow damage. At least 25% of childhood aplastic anemias may have a genetic predisposition. These patients need to be identified, since inherited and acquired disorders

differ significantly in treatment and prognosis.

There are few studies in Indian literature on bone marrow failure syndrome in children.<sup>[2,3]</sup> The present study was undertaken to evaluate the clinico-hematologic profile and etiologic factors in BMFS in children.

## MATERIALS AND METHODS

This prospective study was carried out in the Unit of Hematology-Oncology, Department of Pediatrics of a university teaching hospital over a period of 36 months, from August 2003 to July 2006. Fifty-five children ranging in age from 2-16 years (mean, 7.8 years) with a male-to-female ratio of 5:1 satisfying the inclusion criteria for aplastic anemia were included in the study. For every eligible case, two age- and sex-matched controls were selected from among the pediatric outpatients or inpatients that did not have any hematological problem. Informed consent was taken from parents/ guardians of the children. The study was approved by the ethical committee of the hospital. The inclusion criteria were (i) hemoglobin value < 10 g/dL (ii) neutrophil count of <  $1.5 \times 10^9/L$  (iii) platelet count of <  $100 \times 10^9/L$  with bone marrow cellularity of < 25%.<sup>[4]</sup> Children receiving chemotherapy or radiotherapy were excluded from the study.

Full blood count, including reticulocyte count, blood film examination, fetal hemoglobin, bone marrow aspiration and trephine biopsy, was carried out in all the cases. Liver function tests, along with viral studies for hepatitis A, B, C, were done. Ham test was done to detect the presence of paroxysmal nocturnal hemoglobinuria (PNH). Chromosomal study using Mitomycin-C (MMC) was done to

diagnose patients of Fanconi anemia.<sup>[5]</sup> Two milliliters of venous blood was collected from cases and healthy controls. Five to six drops of heparinized blood (0.1 mL of heparin/2 mL of blood) was mixed with growth media and incubated in tissue culture flask at 37°C for 96 h. Mitomycin-C was added in concentration of 40 ng/mL of media for the duration of culture. Harvesting and slide preparation were performed according to standard methods for preparation of chromosomes.<sup>[6]</sup> Slides were stained with 2% Giemsa solution. The MMC-induced chromosomal breakages were then compared with healthy controls. Chromosomal aberrations were recorded: gaps smaller than widths of the chromatids were not scored. Single chromatid breaks, isochromatid breaks and acentric fragments were all scored as 1 break each. Dicentric and ring chromosomes were scored as 2 breaks each. Radial exchange configurations were scored separately. The proportion of breaks and radial figures was expressed in percent, i.e., number of breaks or radial figures/number of mitotic figures  $\times 100$ . Occupation of father, socioeconomic status, ethnicity and exposure to drugs (chloromycetin, anticonvulsants and nonsteroidal anti-inflammatory drugs) in the last 6 months were studied in cases of acquired aplastic anemia. Socioeconomic scale was decided according to the modified Kuppuswamy scale.<sup>[7]</sup>

## Statistical analysis

Relative risk was estimated by odds ratio (OR) with 95% confidence interval (CI) in matched cases and controls.

## RESULTS

Fifty-five children satisfying the inclusion

criteria were included in the study. Two cases were excluded in the analysis as the data was inadequate. Fifty-three children were studied. The clinical profile of all the 53 patients is presented in Table 1. The largest number of patients was in the age group of 5-10 years (81.1%) with a mean age of 7.8 years. Males outnumbered females by 5:1. Pallor was the chief presenting symptom, being present in 100% cases; followed by bleeding manifestation (92.0%) and fever (68.0%). Majority of the patients had a short history of 15-30 days before presenting to the hospital. Hematological profile of the cases is presented in Table 2. The mean hemoglobin level was 3.3 g/dL (range 2-6 g/dL). The mean total leukocyte count (TLC), absolute neutrophil count (ANC) and total platelet count were  $2.8 \times 10^9/L$ ,  $0.6 \times 10^9/L$  and  $25.3 \times 10^9/L$  respectively. Six cases showed a striking increase in both chromosome breaks and chromatid exchange radial figure and thus were diagnosed to have Fanconi

**Table 1: Clinical profile of the cases**

Characteristic	Cases	Number (%)
Age group (years)	<5	5 (9.4)
	5-10	43 (81.1)
	>10	5 (9.4)
Sex distribution	Male	44 (83.0)
	Female	9 (17.0)
Duration of symptoms (days)	<15	5 (16.7)
	15-30	14 (46.7)
	31-45	5 (16.7)
	>45	6 (20.0)
Symptoms	Fever	20 (66.7)
	Anemia	30 (100.0)
	Bleeding	28 (93.3)

**Table 2: Hematological profile of the cases**

Parameter	Range	Mean	Median	SD
Haemoglobin (g/dL)	2-6	3.3	3.0	1.4
Total leucocyte count ( $\times 10^9/L$ )	1-5	2.8	3.0	0.9
Absolute neutrophil count ( $\times 10^9/L$ )	0.2-1	0.6	0.6	0.2
Total platelet count ( $\times 10^9/L$ )	5-92	25.3	18.0	19.6

SD - Standard deviation

anemia defect [Figure 1]. Two of these cases had abnormalities of the thumb, whereas the third case had only short stature. Rest of the 3 cases had no physical abnormality. The data on remaining patients showed only moderate response to Mitomycin-C, which was similar to the response of control subjects. Two cases were diagnosed as myelodysplastic syndrome (MDS).

In the remaining 45 patients, 1 tested positive for hepatitis B surface antigen. Ham test was found to be positive in 2 patients. These patients had normoblastic hyperplasia in the bone marrow. Tables 3 and 4 show the incidence of acquired aplastic anemia in relation to the socioeconomic status, ethnicity and occupation of father. There was a higher incidence of aplastic anemia in children from

**Table 3: Socioeconomic status and ethnicity of cases and controls**

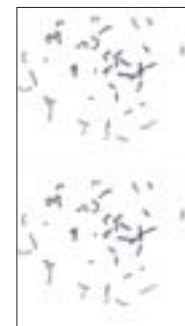
SE Group	Cases (n = 45)	Controls (n = 90)	Relative risk	95% CI
V	10 (22.2)	20 (22.2)	0.883	0.377-2.1
IV	18 (40.0)	32 (35.6)	1.21	0.58-2.535
III	14 (31.1)	30 (33.3)	0.903	0.412-1.98
II	2 (4.4)	5 (5.6)	0.791	0.191-3.28
I	1 (2.2)	3 (3.3)	0.95	0.22-1.415
Ethnicity				
Hindu	31 (68.9)	84 (93.3)	0.385	0.314-0.471
Muslim	14 (31.1)	6 (6.7)	2.596	2.118-3.181

SE - Socioeconomic; CI - Confidence interval, Figures in parentheses are in percentage

**Table 4: Occupational exposure of father in cases and controls**

Occupation	Cases (n = 45)	Controls (n = 90)	Relative risk	95% CI
Farmer	15 (33.3)	32 (35.6)	0.91	0.413-1.982
Weaver	10 (22.2)	5 (5.6)	4.86	1.55-15.234
Labour	3 (6.7)	11 (12.2)	0.513	0.135-1.94
Painter	4 (8.9)	5 (5.6)	1.66	0.422-6.567
Trader	4 (8.9)	9 (10.0)	0.88	0.081-3.034
Carpenter	5 (11.1)	13 (14.4)	0.740	0.25-2.23
Mechanic	2 (4.4)	5 (5.6)	0.385	0.314-0.471
Government job	2 (4.4)	10 (11.1)	0.372	0.077-1.783

CI - confidence interval, Figures in parentheses are in percentage



**Figure 1:** Chromosome breaks and gaps in a metaphase spread in a child with Fanconi anemia

family with lower socioeconomic status; and when the occupation of the father was weaving, dyeing and painting. The incidence was also higher in Muslim patients. However, the numbers are small to draw significant statistical conclusion. No definite association could be established with intake of drugs.

## DISCUSSION

Bone marrow failure is characterized by a reduction in effective production of mature erythrocytes, granulocytes and platelets that leads to peripheral blood pancytopenia. The disorder may be acquired, inherited (genetic but not necessarily present at birth) or congenital (present at birth).<sup>[8]</sup> Fanconi anemia is the best recognized constitutional pancytopenia, which may present late with hematologic manifestations.<sup>[9]</sup> In the present study, 6 cases (11.3%) had evidence of chromosomal breaks and were diagnosed to have Fanconi anemia defect. In another study, Fanconi anemia defect was detected in 24.1% of pediatric aplastic anemia cases.<sup>[2]</sup> Two out of our 53 (3.7%) patients had features of myelodysplastic syndrome (MDS); whereas in a previous study comprising 135 BMFS

patients, 6 (4.4%) had MDS.<sup>[2]</sup>

The remaining 45 cases in our study were labeled as acquired aplastic anemia, which is characterized by the presence of a hypocellular marrow in association with peripheral pancytopenia and the absence of predictable or known myelotoxic exposure.<sup>[10]</sup> A specific cause cannot be identified in most of the cases, and these are termed as 'idiopathic.' It occurs in the western population with an annual incidence of 2-6 cases per million population.<sup>[11]</sup> It has been felt that the disease is more common in the orient.<sup>[12]</sup> However, accurate estimates for many countries are unknown, including India. Occasional studies have reported a high incidence of aplastic anemia based on hospital attendance.<sup>[13]</sup> In the present study, aplastic anemia contributed 1.1% of approximately 5,000 pediatric admissions.

Of the various etiological factors implicated in the etiology of aplastic anemia in Asia, hepatitis infection is thought to be an important cause.<sup>[12,14]</sup> However, in the present study, only one (2%) patient had evidence of hepatitis B infection. We did not come across any other Indian studies to compare the results. The patients were also analyzed for the presence of PNH by doing a Ham test. Two patients (3.8%) had evidence of PNH. There are many international studies which have shown variable incidence of PNH with aplastic anemia.<sup>[15,16]</sup> There are only occasional case reports from our country;<sup>[17,18]</sup> and therefore, it was not possible to compare the incidence with Indian studies.

The causes of aplastic anemia are for most part unknown, but several large population-

based case-control studies have studied the role of occupational and environmental factors in the etiology of aplastic anemia.<sup>[19-21]</sup> In the Thai aplastic anemia study group, a strong inverse association was found between incidence of the disease and socioeconomic status. There was also significant association with occupational exposure to solvents and agricultural pesticides.<sup>[19]</sup> In the United Kingdom Aplastic Anemia Study (UKAAS),<sup>[22]</sup> there was significant risk associated with self-reported exposure to radiation and pesticides in the workplace; and also to solvent exposure, especially benzene.

In the present study, there was a higher incidence in children from family with low socioeconomic status; and also in those children whose fathers were weavers, dyers and painters, which is similar to that found in a Thai study and in another study from Brazil. The disease was also more common in Muslim children, probably due to the fact that most of the weavers and dyers belong to the Muslim community. Most of the families either work from home or live very near their workplaces. It is possible that certain chemicals like nitrobenzene compounds used in the painting and dyeing industry may have a role in the causation of acquired aplastic anemia by having a direct toxic effect. However, the exact mechanism is unclear and needs further studies. The association with drugs could not be established.

The mainstay of treatment for patients with Fanconi anemia is androgens and steroids, alone or in combination.<sup>[8]</sup> Although 50-75% patients show some evidence of improvement, relapse is common and

complications occur. Hence the treatment is only palliative. The only curative therapy is bone marrow transplantation. In the present series, all the children with Fanconi anemia were started on androgens. Of these, three showed partial response whereas the remaining did not show any improvement. In children with acquired aplastic anemia, bone marrow transplantation from human leucocyte antigen identical sibling is the treatment of choice, offering a 75-90% chance of long-term cure.<sup>[7]</sup> In case of non-availability of a donor, immunosuppressive treatment with anti-thymocyte globulin and cyclosporine is given. Response to this therapy is 60-80%, but a few responders may relapse. There is also a higher risk of developing clonal bone marrow diseases such as leukemia or myelodysplasia after immunosuppression. None of the patients in the present series could afford bone marrow transplantation, and only one-fourth could afford immunosuppressive therapy.

## CONCLUSION

Fanconi anemia was responsible for approximately one-tenth of the cases of bone marrow failure syndrome. Majority of the patients had acquired aplastic anemia. Hepatitis B infection was an uncommon cause of acquired aplastic anemia. The disease was more common in children belonging to low socioeconomic status, Muslims and those belonging to families working in dyeing and painting; but no significant statistical conclusions could be drawn.

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