Multiple constitutional aetiological factors in bone marrow failure syndrome (BMFS) patients from north India


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Background & objectives: A large number of patients diagnosed with bone marrow failure syndromes (BMFS), comprising aplastic anaemia (AA) and myelodysplastic syndromes (MDS), remain aetiologically uncharacterized worldover, especially in resource constrained set up. We carried out this study to identify a few constitutional causes in BMFS patients attending a tertiary care hospital in north India.

Methods: Peripheral blood lymphocyte cultures were performed (with and without clastogens) in a cohort of 135 consecutive BMFS patients, in order to detect Fanconi anaemia (FA), Down’s syndrome (+21), trisomy 8 (+8) and monosomy 7 (-7).

Results: Constitutional factors were detected in 17 (12.6%) patients. FA defect was observed in 24.07 per cent (13/54), 16.66 per cent (1/6) and 2.85 per cent (1/35) paediatric aplastic anaemia, paediatric MDS and adult MDS patients respectively. Down’s syndrome was detected in 5.00 per cent (2/40) adult aplastic anaemia patients. None of the patients revealed trisomy 8 or monosomy 7.

Interpretation & conclusion: Presence of an underlying factor determines appropriate management, prognostication, family screening and genetic counselling of BMFS patients. Special tests required to confirm or exclude constitutional aetiological factors are not available to majority of the patients in our country. Diepoxybutane (DEB) test yielded better results than mitomycin C (MMC) test in our experience.

Key words Bone marrow failure syndromes - constitutional aetiological factors - Down’s syndrome - Fanconi anaemia

Bone marrow failure syndromes (BMFS), comprising aplastic anaemia (AA) and myelodysplastic syndromes (MDS), are a leading cause of mortality and morbidity in haematological practice. Based upon putative aetiological factors, BMFS are classified as constitutional, acquired secondary and acquired primary subtypes; the latter being diagnosed in the absence of former two types.
The definition of exact aetiology is important for proper management of patients, family screening and genetic counselling, etc. A number of patients with constitutional BMFS present with cytopaenia/s usually in association with one or more somatic abnormalities\(^1,2\). Fanconi anaemia (FA) and dyskeratosis congenita (DC) are two common constitutional bone marrow failure syndromes, presenting during childhood or at times later in adulthood as well. Chromosomal instability syndromes (FA, DC, etc.) predispose to both AA and MDS whereas constitutional chromosomal syndromes (+21, +8 and -7) are mostly known to be linked with MDS cases\(^1\).

FA and DC are so far best characterized for their clinical and genetic heterogeneity\(^1-4\). It is now well recognized that the typical phenotype may only be observed in approximately one third of these patients. Therefore exact incidence of a particular aetiological factor may remain undetermined (if based upon clinical manifestations alone) unless definitive diagnostic tests [using clastogens like diepoxybutane (DEB) and mitomycin C (MMC)] are performed in a large number of BMFS patients with or without characteristic phenotype. According to literature from the West\(^1\), the inherited factors contribute towards BMFS in nearly one third of young patients\(^1\). The acquired factors include drugs, chemicals, toxins, infections (mainly viral), etc. Such data on aetiological factors both constitutional and acquired, are not available from India, and only sporadic reports originated from Asia\(^2\). Results of MMC testing were reported recently in 29 aplastic anaemia patients from New Delhi\(^9\). Most of the constitutional BMFS require specific diagnostic tests and many (like FA) necessitate modifications in the treatment strategy. International Fanconi anaemia registry (IFAR) and dyskeratosis congenita registry have made concerted efforts to detect and characterize FA and DC defects in BMFS patients. However, there are hardly any prospective studies available describing multiple aetiological factors in both AA and MDS patients. Use of DEB test for detection of FA defect has not been reported from India.

Hence, we undertook this study to explore multiple constitutional aetiological factors in BMFS patients attending a tertiary care health facility in north India. Some of these results have been communicated briefly earlier\(^7\).

**Material & Methods**

The consecutive BMFS patients presenting between April 2002 and September 2005 to Postgraduate Institute of Medical Education & Research, Chandigarh, were included in this study. The diagnosis of aplastic anaemia and MDS was made according to the standard criteria, based upon complete blood counts, peripheral blood (PB) picture, bone marrow aspiration and trephine biopsy findings\(^8,9\). In our institute, patients up to 12 yr of age are looked after by paediatricians and rest by physicians under the respective haematology clinics.

*Conventional cytogenetic analysis of G - banded metaphases\(^10\):* PB lymphocyte culture and BM samples were processed and analyzed to identify constitutional and acquired cytogenetic abnormalities in all the patients. Monosomy 7, trisomy 8 and 21 were specially looked for. A minimum of 50 metaphases were analyzed routinely.

*Chromosomal breakage studies\(^11\):* Appropriate PB cultures with phytohaemagglutinin (PHA), with and without DEB and MMC were set up for patients as well as 130 age matched controls. PB cultures were exposed to DEB for 48 h and MMC for 72 h. The family members of positive patients were also subjected to chromosomal breakage studies. All the mitogens were handled in the hood and all the strict safety precautions were observed. Harvesting and slide making was done according to the standard techniques\(^10\). Breakage analysis was performed on unbanded metaphases, at least 100 metaphases per treatment being examined. The slides were coded for each treatment, patient and control, in order to avoid observer bias. Gaps, breaks and other rearrangements were recorded on a chart. Breaks were considered to be equal to or greater than a chromatid width apart
and gaps were less than a chromatid width apart. Each chromatid or chromosome break was scored as 1 and other rearrangements were scored as 2. Although gaps were noted, these were not given any score. Chromosomal breakage score was expressed as breaks/cell and breaks aberrant cell; breaks/cell score was used in the final analysis.

Breakage score of control, MMC-treated and DEB-treated preparations was compared using Student’s t test.

**Results**

During the study period conventional cytogenetic analysis and chromosomal breakage studies were performed in 135 BMFS patients. The study group included 94 aplastic anaemia (54 children and 40 adults; age ranged from 5 to 65 yr) and 41 MDS patients (6 children and 35 adults; age ranged from 1/4 to 86 yr) (Table). Constitutional aetiological factors were detected in 17 (12.59%) patients. Of the 94 aplastic anaemia patients, 13 were detected to have FA defect (positive for induced chromosomal breakage); all these patients being children. Two patients, aged 16 and 19, showed trisomy 21 (Down’s syndrome). Out of 41 MDS patients, 2 showed FA defect (9 and 21 yr of age). Both these patients were diagnosed to have refractory anaemia with excess of blasts. FA defect was detected in 24.07 (13/54), 16.66 (1/6) and 2.85 per cent (1/35) paediatric aplastic anaemia, paediatric MDS and adult MDS patients respectively. Down’s syndrome was detected in 5.00 per cent (2/40) adult aplastic anaemia patients. Remaining patients did not reveal any other underlying constitutional etiological factors (+8 or -7).

Breakage scores of controls (135), MMC-treated and DEB-treated preparations (from 15 FA patients) were 0.003 ± 0.006, 0.3378 ± 0.2226 \((P < 0.001)\) and 1.0314 ± 1.031 \((P < 0.001)\) respectively, and were significantly different from controls.

**Discussion**

A large number of patients seen in the haematological practice belong to the broad group of BMFS - AA and MDS. These disorders may be constitutional or acquired. The constitutional disorders are either congenital (present at birth) or inherited (genetic but not necessarily expressed at birth). Multiple aetiologies are implicated and therefore natural history of BMFS patients is very diverse. FA and DC are the best defined constitutional disorders in terms of their clinical and genetic heterogeneity. In the present study, constitutional aetiological factors were detected in 17 of 135 (12.59%) BMFS patients; FA being the underlying factor in 15 (88.23%) and Down’s syndrome was detected in 2 (11.76%) patients.

FA is the most common, but perhaps most enigmatic of the inherited disorders of bone marrow failure. It was originally described as an autosomal recessive disorder, characterized by progressive pancytopenia, various congenital abnormalities and increased predisposition to malignancy.

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**Table. Aetiological factors in bone marrow failure syndromes (BMFS)**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>BMFS subgroup</th>
<th>Age (yr) : median (range) Mean ± SD</th>
<th>Aetiological factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aplastic anaemia (AA) (n = 94)</td>
<td>Paediatric AA (n = 54)</td>
<td>8 (5 – 11) 8.14 ± 1.73</td>
<td>FA in 13</td>
</tr>
<tr>
<td></td>
<td>Adult AA (n = 40)</td>
<td>23 (13 – 65) 29.63 ± 14.45</td>
<td>Down’s syndrome in 2</td>
</tr>
<tr>
<td>Myelodysplastic syndromes (MDS) (n = 41)</td>
<td>Paediatric MDS (n = 6)</td>
<td>4 (1/4 – 9) 4.0 ± 3.27</td>
<td>FA in 1</td>
</tr>
<tr>
<td></td>
<td>Adult MDS (n = 35)</td>
<td>45 (21 – 86) 54.89 ± 34.68</td>
<td>FA in 1</td>
</tr>
</tbody>
</table>
more than 90 per cent of FA patients are expected to develop aplastic anaemia at some point in time, there seems to be a temporal continuum, whereby aplasia occurs early, leukaemia later and tumours later\textsuperscript{1}. Clinically, FA is a very heterogenous condition; the phenotype includes spectrum from extremely abnormal to normal, the haematological findings range from severe aplastic anaemia to normal and the malignant potential includes MDS, leukaemia, carcinomas, liver tumours or no malignancy\textsuperscript{1}. It is now well recognized that the diagnosis based on clinical manifestations alone can be difficult and often unreliable therefore resulting in delayed or incorrect diagnosis. Wide spectrum of genetic heterogeneity (at least 11 complementation groups and 8 defective genes identified) and the challenging task of genetic diagnosis of FA (specially FA - A subtype) is just beginning to be realized\textsuperscript{2}. However, the need to differentiate FA from non-FA aplastic anaemia cases was always felt.

The study of DEB sensitivity is extremely important for the differential diagnosis of childhood aplastic anaemia and various syndromes with congenital abnormalities. According to IFAR recommendations, patients with increased chromosomal breakage by using clastogens like DEB, are considered to have FA, whereas patients with any or every symptom or sign that is considered ‘classic’ are diagnosed as non-FA, in the absence of increased chromosomal breakage\textsuperscript{4}. DEB test was positive in all 15 FA patients whereas MMC test gave a negative result in 3 patients which would have resulted in a diagnosis of non-FA aplastic anaemia if DEB test results was not available in these patients.

Molecular studies for mutation analysis can be performed once the diagnosis of FA has been established by DEB sensitivity. Three paediatric FA patients revealed increased chromosomal breakage only once out of 3 times the DEB sensitivity was tested, twice the breakage score was just marginally higher than the MMC score and control score. It is important to remember that DEB test may give negative result at times; compound heterozygosity for FA - C mutations\textsuperscript{12} and variable response to DEB at different occasions\textsuperscript{13} being possible reasons. This underscores the need for molecular analysis in the family screening of FA\textsuperscript{10}.

Nearly 10 per cent of FA patients reported in the literature were found to be at least 16 yr of age at the time of diagnosis, although some might present much later with varied manifestations, \textit{e.g.}, acute myeloid leukaemia (AML)\textsuperscript{3}. In September 2004 we encountered a 9 yr old boy with FA defect who presented for the first time with AML - M\textsubscript{2}. The details of this patient are being communicated separately.

DC is the second most common cause for constitutional bone marrow aplasia syndromes, characterized by a combination of abnormal skin pigmentation, nail dystrophy and mucosal leucoplakia\textsuperscript{2}. Nearly 80-90 per cent patients develop bone marrow failure by the age of 30 yr\textsuperscript{2}. Three patterns of inheritance appear to be be involved: X-linked recessive, autosomal recessive and autosomal dominant\textsuperscript{1,2}. Earlier we reported two brothers with DC, one was associated with bone marrow aplasia and abnormal immune function\textsuperscript{14}. Missense mutations were subsequently reported in these DC patients\textsuperscript{15}. However, no DC patient was found in the present series.

Down’s syndrome infants present frequently with a transient myeloproliferative syndrome. Although the risk of leukaemia and MDS remains high, aplastic anaemia has also been rarely reported in association with Down’s syndrome\textsuperscript{1}. Two of our 40 (5.00\%) adult aplastic anaemia patients were found to have Down’s syndrome.

Most of the studies have focused attention on individual aetiological factors of BMFS \textit{e.g.}, FA\textsuperscript{1-4}, Down’ syndrome\textsuperscript{1,2,16}, therapy related (t - MDS / t - AML) disorders, \textit{etc}\textsuperscript{17}. Hasle \textit{et al}\textsuperscript{18} reported a population based retrospective study of 46 childhood MDS patients in Denmark, from 1980-1991. Novitzky and Prindull\textsuperscript{19} reviewed details of 340
childhood MDS patients, reported to European Society of Pediatric Haematology and Immunology between 1982 and 1996. These two studies listed more than one predisposing conditions in MDS. Down’s syndrome was the leading predisposition factor in both the studies. FA was the aetiological factor in 2 of 46 paediatric patients in the former study, whereas none of the 340 patients in the latter report was listed as FA related.

IFAR recommends that FA should be excluded in all aplastic anaemia patients by using DEB induced chromosomal breakage as the diagnostic guideline. MMC test alone can result in false negative diagnosis, as happened in our 3 patients. Same methodology with few modifications can yield diagnosis of other chromosomal instability syndromes and constitutional chromosomal disorders (+21, +8 and -7). Therefore, it is worthwhile to test for these entities in BMFS patients.

In conclusion, constitutional factors were detected in 12.6 per cent consecutive BMFS patients in a part of north India. However, the results may not be same in a vast country like India because of socio-cultural differences, such as consanguinous marriages, etc. Further studies with large sample need to be done in different parts of the country.

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References


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