The utility of hematopoietic stem cell karyotyping in the diagnosis of de novo myelodysplastic syndromes

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Abstract Myelodysplastic syndromes (MDS) are a clonal hematopoietic stem cell (HSC) disorders. Cytogenetics plays a vital role in pathogenesis, diagnosis, prognosis, and determining treatments in MDS. Cytogenetic studies on CD 34⁺ cells in Asian MDS patients are limited. The aim of conducting this study was to compare the karyotypic features of CD 34⁺ cells and their bone marrow (BM) karyotypes. BM samples of 20 primary MDS patients were collected. BM-CD 34⁺ cells were isolated by CD34 positive selection. Conventional karyotyping was performed on primary BM samples and isolated CD 34⁺ cells. Fluorescence in situ hybridization (FISH) was performed to confirm del(5q) by using XL 5q31/5q33 locus-specific probe. Chromosomal abnormalities were detected in 41 % (7/17) of BM and 40 % (8/20) of HSC karyotypes. BM and HSC karyotypes were similar (94 %) except in one (BM—normal; CD 34⁺ cells had del(5q)) where the patient showed characteristic del(5q) phenotype. The abnormalities found were del(5q)—10 % (2/20), del(11q)—5 % (1/20), del(12p)—5 % (1/20), 47,XY,+19—5 % (1/20), hypodiploidy—5 % (1/20), and random loss of chromosomes—10 % (2/20). The percentage of abnormal metaphases counted per case was higher for CD 34⁺ cell cultures than for BM cultures. Culture

failure were less for CD 34⁺ cells when compared to BM. Sample limitation for BM does not apply for CD 34⁺ cells if cultures can be maintained. Although the abnormal karyotypes counted were greater using CD 34⁺ cells than BM, there was no statistically significant difference ($p > 0.05$). Detection of karyotypic abnormalities could be greater when using CD 34⁺ cells. All the karyotypic abnormalities reported in this study had been previously known in MDS. Further large scale studies are needed to verify our findings.

Keywords Cytogenetic abnormality · Myelodysplastic syndromes · Hematopoietic stem cells · Bone marrow · FISH

Introduction

Myelodysplastic syndromes (MDS) comprise a group of hematopoietic stem cell disorders characterized by peripheral cytopenias, bone marrow dysplasia with ineffective hematopoiesis [1]. Cytogenetics have long been known to determine the pathogenesis, diagnosis, and prognosis and determine treatment in MDS.

An International Prognostic Scoring System (IPSS) was proposed in 1997 to improve the risk assessment of MDS, based on cytogenetic abnormalities, bone marrow blast percentage, cytopenias, and patient variable age and gender with MDS [2]. This system was re-evaluated in 2012 and a new scoring system for MDS was defined; the revised IPSS—R-IPSS [3]. The R-IPSS recognizes five cytogenetic sub groups [3]. Cytogenetic abnormalities are found in 40–50 % of cases of MDS by conventional karyotype [4]. The commonest recurring cytogenetic abnormalities in MDS include +8, del(5q), del(7q), del(20q), del(11q) and –Y [5]. The cytogenetic features of MDS have shown a significant difference among

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Asian populations compared to the Western populations [6–14].

MDS result from malignant transformation of a hematopoietic stem cell (HSC), which has growth advantage over normal CD 34⁺ cells resulting in clonal expansion [15]. Molecular and cytogenetic abnormalities are major determinants in transforming normal CD 34⁺ cells to malignant cells in the bone marrow (BM) [16–18]. Cytogenetic analyses on hematological malignancies have identified various clonal chromosomal abnormalities in the haematopoietic stem/progenitors as well as in the myeloid and lymphoid lineages of blood cells [19–24]. However, the cell of origin of the cytogenetic abnormalities in MDS is still debatable. The aim of this research was to conduct a pilot study to investigate the cytogenetic abnormalities of CD 34⁺ cells of primary MDS patients and compare them with BM karyotypes. In addition, this is the first cytogenetic analysis to describe chromosomal abnormalities of patients with primary MDS in Sri Lanka.

Materials and methods

Patients

Twenty BM samples of the patients diagnosed as MDS were collected during the 2 years (2013 to 2015) of patient recruitment conducted at four tertiary care hospitals in Sri Lanka (National Hospital of Sri Lanka, Colombo South Teaching Hospital, Colombo North Teaching Hospital and National Cancer Institute, Maharagama) after obtaining written informed consent. The study was conducted according to the principles of Declaration of Helsinki (2008). Ethical approvals for the study were obtained from the ethics review committees of the Faculty of Medicine, University of Colombo, Sri Lanka and the above named hospitals. Patients with secondary MDS were excluded. Patient's clinical data and investigation data were recorded in an interviewer administered questionnaire. Patient characteristics are given in Table 1. All patients were diagnosed as MDS and subtyped according to the WHO classification.

Isolation of CD34⁺ cells

From the BM samples, mononuclear cells were purified by Histopaque (Sigma-Aldrich, Gillingham, UK) density gradient centrifugation and labeled with CD34 MicroBeads (EasySep CD34 selection kit, StemCell Technologies, Canada), and were isolated using magnetic cell separation. The purity of CD34⁺ cell population was evaluated by flow cytometry (FACScan; Becton Dickinson, Heidelberg, Germany) and was 90 % or greater (See Supplemental Data Figure S1).

Cytogenetic analysis of BM

BM was cultured in marrow max media (Gibco, USA) at a concentration of 2×10^6 cells per ml and incubated for 24 h at 37 °C. Cells were arrested with colcemid (0.01 mg/ml) for 2 h before harvesting. Cells were harvested and treated with 0.075 M KCl for 20 min at 37 °C. Cells were fixed with freshly prepared fixative (methanol/acetic acid 3:1 v/v). Chromosomal spreads for analysis were prepared by dropping onto slides at 76–82 % humidity and Giemsa Trypsin Giemsa (GTG) banding was performed. At least 10–20 metaphases were counted for structural and numerical chromosomal abnormalities and 10 were analyzed. The karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN) [25] and reported according to the association for clinical cytogenetics, professional guidelines for clinical cytogenetics, acute myeloid leukemia and MDS (2012) v1.00 [26]. The number of metaphases analyzed to report a normal karyotype for BM was 10. Presence of an abnormal clone was ascertained by the observation of more than two metaphase spreads having the same structural rearrangement or chromosomal gain. Chromosomal loss had to be observed in at least three cells, to confirm an abnormal clone.

Cytogenetic analysis of CD34⁺ cells

CD 34⁺ cells were cultured at a concentration of 1.0×10^4 cells/ml in RPMI 1640 media supplemented with 20 % fetal calf serum (FCS; Invitrogen, Australia), 20 ng/mL thrombopoietin (R & D, USA), 20 ng/mL FLT3 ligand (R7D, USA), 50 ng/mL stem cell factor and 50 ng/mL IL6. Cells were incubated for 24 to 72 h at 37 °C. One hundred and fifty microliters of colcemid was added 2 h before harvesting. Cell harvesting and GTL banding were performed as described above for the BM cells and CD34⁺ cells. The karyotypes were classified according to the ISCN guidelines.

Fluorescence in situ hybridization (FISH)

BM and CD34⁺ cells of patients suspected for having del (5q) based on their BM findings were subjected to FISH analysis. XL 5q31/5q33 locus-specific probe (Metasystems, USA) was used according to the manufacturer's protocol. Briefly, 500 µL of BM was added to 10 mL of KCl and left for 20 min at room temperature. Next, 500 µL of fixative (methanol/acetic acid 3:1 v/v) was added and spun at 1500 rpm for 8 min, and supernatant was removed. Ten milliliters of the same fixative was added to the pellet, mixed and spun 1500 rpm for 8 min. A cell smear was prepared and the slide was kept for 15 min at room temperature to dry. Ten microliters of probe was added to the slide and kept for overnight hybridization. After hybridization, the slides were washed in

Table 1 Patient details and hematological findings of MDS patients

Patient no.	Sex	Age	Subtype	BM blast	Hb, g/L	MCV fl	WBC, 10 ⁹ /L	Plt, 10 ⁹ /L	Findings in blood picture	BM findings
1	F	64	RCUD	<3 %	113	82.9	3.9	276	Erythroid dysplasia; macrocytes, microcytes and acanthocytes	Erythropoiesis suppressed Occasional monoblast and hyperlobated megakaryocytes Erythroid dysplasia
2	F	67	RCUD	<5 %	78	102	8.6	467	Erythroid dysplasia; macrocytes and acanthocytes	Aparticular marrow with occasional megakaryocytes
3	F	35	RCUD	<4 %	126	84	6.1	20	Erythroid dysplasia; macrocytes, microcytes Polychromatic cells and pencil cells	Erythroid dysplasia; irregular nuclei, megablastic changes, binuclearity
4	F	73	RCUD	<5 %	106	83.8	6.7	278	Erythroid dysplasia; macrocytes, Rouleaux formation	Leukocyte dysplasia; dysplastic neutrophils
5	F	31	RCUD	<5 %	126	84	3.6	190	Occasional hypersegmented neutrophils Leukocyte dysplasia; Hypersegmented, bilobated neutrophils	Occasional metamyelocytes Erythroid dysplasia; megablasts Hypercellular Occasional hypoblasted and monoblasted megakaryocytes
6	F	73	RCUD	<5 %	108	85.3	4.84	206	Low neutrophils and platelets	Hypocellular marrow
7	F	54	RCUD	<3 %	83	112	3.29	461	Erythroid dysplasia; oval macrocytes, pencil cells and acanthocytes	Dyserythropoiesis Suppressed myelopoiesis with dysplastic features
8	M	70	RCUD	<5 %	86	89.5	6.5	262	Few hypersegmented neutrophils No immature cells	Moderately hypocellular, large small megakaryocytes, atypical mononucleated cells
9	F	59	RCUD	<5 %	71	90	4.23	320	Erythroid dysplasia; macrocytes, Rouleaux formation	Erythroid dysplasia; megablasts Erythroid and leukocyte dysplasia; megakaryocytes with mono and hypoblasted nuclei
10	M	62	RCUD	7 %	68	67.8	2.7	153	Erythroid dysplasia; pencil cells, tear drop cells and acanthocytes	Trilineage dysplasia with erythroid hyperplasia, suppressed granulopoiesis and megakaryopoiesis
11	F	54	RCUD	<5 %	83.8	85.4	1.09	58	Low neutrophils and platelets	Trilineage dysplasia with active hemopoiesis
12	F	68	RCMD	<2 %	78	95	3.7	125	Erythroid and leukocyte dysplasia; tear drop cells, pencil cells and hypersegmented neutrophils	Erythroid and leukocyte dysplasia; megablasts Erythroid and leukocyte dysplasia; megakaryocytes with mono and hypoblasted nuclei
13	F	49	RCMD	<3 %	89	92	3.09	12	Trilineage dysplasia; macrocytes, dysplastic neutrophils	Trilineage dysplasia with erythroid hyperplasia, suppressed granulopoiesis and megakaryopoiesis
14	F	65	RCMD	<3 %	82	66.1	3.19	46	Trilineage dysplasia; macrocytes, pencil cells, tear drop cells, dysplastic neutrophils and large platelets	Erythroid and leukocyte dysplasia; megablasts, monoblasted megakaryocytes
15	M	74	RCMD	<3 %	115	108	3.33	77	Erythroid and leukocyte dysplasia; macrocytes and bilobed neutrophils	Hypoplastic marrow, bi-nucleated neutrophils
16	M	50	RCMD	<5 %	76	96.7	3.2	27	Macrocytes, acanthocytes, dysplastic hemopoiesis	Mildly hypercellular
17	F	67	del (5q)	<3 %	85	98.3	6.41	653	No abnormal cells	Monoblasted small megakaryocytes
18	M	55	RAEB-2	10 %	77	75.3	2.8	150	Erythroid dysplasia; pencil cells, tear drop cells and acanthocytes	Moderately hypocellular, small megakaryocytes, large atypical mononucleated cells
19	M	75	RAEB-2	>10 %	68	87.8	6.34	338	Erythroid and leukocyte dysplasia; acanthocytes and dysplastic neutrophils	Moderate hypocellular, dyserythropoiesis, dysplastic granulocytes, and megakaryocytes
20	M	66	RAEB-1	<10 %	70	85	2.56	30	Erythroid dysplasia; macrocytes, microcytes Polychromatic cells and pencil cells	Aparticular marrow with occasional megakaryocytes

BM morphology and blood values of patients were investigated in connection with BM aspiration for the present study

MDS myelodysplastic syndromes, BM bone marrow, Hb hemoglobin, WBC white blood cells, Plt platelet, RCUD refractory cytopenia with multilineage dysplasia, RCMD refractory cytopenia with multilineage dysplasia, RAEB-1 refractory anemia with excess blasts type 1, RAEB-2 refractory anemia with excess blasts type 2

0.4 % SSC for 2 min at 72 °C, followed by a washes in 0.05 % Tween 20 in 2 % SSC for 30 s and distilled water at room temperature and counterstaining with DAPI [(4',6-diamidino-2-phenylindole), 1 mg/mL; Sigma]. One hundred nuclei were individually analyzed by two individuals per each sample. The cutoff level for 10 + 1G signal greater than 5 % was considered as positive for del(5)(q31.2q33).

Statistical analysis

t test was used to compare the mean differences of abnormal metaphases of CD34⁺ cells and BM karyotypes. The statistical significance of the results was considered at ($p < 0.05$).

Results

Patient characteristics

The age group ranged from 31 to 75 years with the median age of 67 years. The majority ($n = 13$, 65 %) were women. On peripheral blood counts and morphology and BM morphology, the following subtypes were identified prior to karyotyping (Table 1). Refractory anemia with excess blasts-1 (RAEB-1) was diagnosed in 1 patient, refractory anemia with excess blasts-2 (RAEB-2) was diagnosed in 2 patients, refractory cytopenia with multilineage dysplasia (RCMD) was diagnosed in 5 patients, MDS with isolated del(5q) was diagnosed in 2 patients and 11 patients were diagnosed with refractory cytopenia with unilineage dysplasia (RCUD).

Cytogenetic analysis of BM

Of the 20 patients analyzed, 10 (50 %) had normal karyotypes and 7 (35 %) had single chromosomal abnormalities including del (5q), del (11q), del (12p) and trisomy 19 (See Supplemental Data Figure S2). BM cultures of 3 (15 %) patients did not grow to obtain in any analyzable metaphase spreads (Table 2). The percentages of metaphase cells having chromosomal abnormalities are given in Table 4. Distribution of clonal abnormalities in the MDS subtypes other than del (5q) were; RCUD 36 % (4/11) including random chromosomal losses in two cases, hypodiploidy and del (11q) in one case each; RCMD 44 % (2/5) which included del (12p) and 47,XY,+19 in 1 case each (Table 3). Both RAEB-2 cases did not have an analyzable spreads. The patient no. 17 showed del(5)(q14q34) in 50 % of the metaphase cells in the BM karyotype. In FISH analysis of BM, 80 % of the cells showed deletion of 5q31.2 and 5q32-33 (See Supplemental Data Figure S3). The percentage of abnormal metaphase counts in each patient is given in Table 4.

Cytogenetic analysis of CD34⁺ cells

The cytogenetic abnormalities of CD34⁺ cells were similar (94 %) to those of BM except in one case. Patient no. 7 (Table 2) who did not show any chromosomal abnormalities on BM karyotype (See Supplemental Data Figure S4 A) however showed del(5q) in HSC karyotype (See Supplemental Data Figure S4 B). The abnormality was detected in 62 % of the HSC metaphases confirmed by FISH analysis where 62 % of the cells showed deletion of 5q31.2 and 5q32-33 (See Supplemental Data Figure S4 C). Detailed karyotypes of CD34⁺ cells are shown in Table 2 and percentages of metaphase cells shown the chromosomal abnormalities are given in Table 4. Patient no. 17 who also had del(5q) in BM exhibited deletion of 5q31.2 and 5q32-33 in 80 % of HSC metaphases in FISH analysis. The metaphase counts for CD 34⁺ cells were higher than that of BM but there was no statistically significant difference between the two.

Discussion

MDS is a rare disease in blood and BM with the incidence rate of 4.6 per 100,000 inhabitants of population in USA [27] and the incidence rate of MDS in Japan is 3.8 cases per 100,000 for men and 2.4 cases per 100,000 for women [28]. The incidence rate of MDS in Sri Lanka is not known and according to the latest publication of National Cancer Registry published by the Ministry of Health, Sri Lanka, 69 MDS patients have been reported in the year of 2007 [29]. Accordingly, the estimated incidence rate of MDS in Sri Lanka is nearly 4 per 1,000,000 (one million) people. We conducted the patient recruitment for 2 years (2013 to 2015) from four tertiary care hospitals in Sri Lanka (National Hospital of Sri Lanka, Colombo South Teaching Hospital, Colombo North Teaching Hospital and National Cancer Institute, Maharagama). We collected 20 BM samples for this study which we believe as a representative sample of Sri Lankan MDS population.

In this study, we describe the cytogenetic features of BM and CD 34⁺ cells of patients with primary MDS in Sri Lanka. The frequency of cytogenetic abnormalities of MDS patients in this study was 41 %. The reported frequency of chromosomal abnormalities in Caucasians using conventional cytogenetics is 40–70 % [10]. The frequencies reported in Asian populations is as follows: 39 % in China, 50 % in Taiwan, 44.1 % in Thailand, 44 % in Korea, 53 % in Japan, 47.5 and 54.48 % in India, and 42.3 % in Pakistan [6–13]. The frequency of chromosomal abnormalities in our study group of Sri Lankan MDS patients is consistent with the reported data on Asian populations.

In our study, a range of chromosomal aberrations, including del (5q), del (11q), del (12p) and trisomy 19

Table 2 Patient details, hematological and cytogenetic findings of MDS patients

Patient no	Subtype	Karyotype in BM	Karyotype in HSC
1	RCUD	46,XX [12]	46,XX [7]
2	RCUD	Hypodiploidy [6]	Hypodiploidy [7]
3	RCUD	Random chromosomal losses [12]	Random chromosomal losses [14]
4	RCUD	46,XX [11]	46,XX [6]
5	RCUD	Random chromosomal losses [16]	Random chromosomal losses [8]
6	RCUD	46,XX [16]	46,XX [11]
7	RCUD	46,XX [11]	46,XX,del(5)(q14q34)[5/8] [127/200]*
8	RCUD	Failed	46,XX [7]
9	RCUD	46,XX,del(11)(q22q23) [7/16]	46,XX,del(11)(q22q23) [6/8]
10	RCUD	46,XY [15]	46,XY [8]
11	RCUD	46,XX [11]	46,XX [6]
12	RCMD	46,XX [11]	46,XX [7]
13	RCMD	46,XX,del(12)(p13) [3/10]	46,XX,del(12)(p13) [3/8]
14	RCMD	46,XX [10]	46,XX [6]
15	RCMD	46,XY [14]	46,XY [8]
16	RCMD	47,XY,+19 [6/10]	47,XY,+19 [5/8]
17	del(5q)	46,XX,del(5)(q14q34) [6/12] [98/200]*	46,XX,del(5)(q14q34) [163/200]*
18	RAEB-2	Failed	46,XY [4]
19	RAEB-2	Failed	46,XY [8]
20	RAEB-1	46,XY [14]	46,XY [9]

MDS myelodysplastic syndromes, *BM* bone marrow, *RCUD* refractory cytopenia with unilineage dysplasia, *RCMD* refractory cytopenia with multilineage dysplasia, *RAEB-1* refractory anemia with excess blasts type 1, *RAEB-2* refractory anemia with excess blasts type 2, [ ] % of metaphases with the aberration, [ ]* number of cells with the aberration/the number of cells analyzed in FISH

were found. In addition, random losses of chromosomes and hypodiploidy were also detected. FISH was performed to confirm del(5q) by using XL 5q31/5q33 locus-specific probe. This probe detects deletions in

the long arm of chromosome 5 (<https://metasystems-probes.com/en/probes/xl/d-5042-100-og/>). The orange labeled probe detects a region at 5q32 which includes *EGR1* and *CDC25C* genes (<https://metasystems-probes.com/en/probes/xl/d-5042-100-og/>).

Table 3 Type of chromosome abnormalities in MDS subtypes

WHO subgroup	Karyotype in BM			Karyotype in HSC		
	Chromosomal abnormality	No.	Total (%)	Chromosomal abnormality	No.	Total (%)
RCUD	Hypodiploidy	1		Hypodiploidy	1	
	Random chromosomal losses	2	4 (20)	Random chromosomal losses	2	5 (25)
	46,XX,del (11)(q22q23)	1		46,XX,del (11)(q22q23)	1	
RCMD	46,XX,del(12)(p13)	1	2(10)	46,XX,del(12)(p13)	1	2 (10)
	47,XY,+19	1		47,XY,+19	1	
RAEB-1	—	—	—	—	—	—
RAEB-2	—	—	—	—	—	—
del(5q)	46,XX del(5)(q14q34)	1	1 (5)	46,XX del(5)(q14q34)	1	1 (5)

MDS myelodysplastic syndromes, *BM* bone marrow, *RCUD* refractory cytopenia with unilineage dysplasia, *RCMD* refractory cytopenia with multilineage dysplasia, *RAEB-1* refractory anemia with excess blasts type 1, *RAEB-2* refractory anemia with excess blasts type 2

Table 4 Cytogenetic alterations in sorted HSCs and whole BM populations

Patient No	Subtype	BM karyotype		HSC karyotype	
		Karyotype	Abnormality in metaphase cells, %	Karyotype	Abnormality in metaphase cells, %
7	RCUD	46,XX	0	46,XX, del(5)(q14q34)	62
9	RCUD	46,XX,del(11)(q22q23)	44	46,XX,del(11)(q22q23)	75
13	RCMD	46,XX,del(12)(p13)	30	46,XX,del(12)(p13)	38
16	RCMD	47,XY,+19	60	47,XY,+19	65
17	del(5q)	46,XX,del(5)(q14q34)	50 ^a	46,XX,del(5)(q14q34)	80 ^a

MDS indicates myelodysplastic syndromes, BM bone marrow, RCUD refractory cytopenia with unilineage dysplasia, RCMD refractory cytopenia with multilineage dysplasia

^a % calculated according to FISH data

com/en/probes/x1/d-5042-100-og/). The green labeled probe specifically detects 5q32-33 region of the RPS14 gene (<https://metasystems-probes.com/en/probes/x1/d-5042-100-og/>). One green (1G) and one orange (1O) signal indicates deletions of both loci in 5q31.2 and 5q33 (<https://metasystems-probes.com/en/probes/x1/d-5042-100-og/>).

A study conducted on a large Caucasian MDS population showed that interstitial deletions of the long arm of chromosome 5 [del(5q)] with or without additional karyotypic abnormalities is the most frequent abnormality seen in MDS [10]. The common cytogenetic abnormalities found in Asian MDS population include trisomy 8, -20/del 20q, -5/del 5q, -21 and -7/del 7q and others included complex karyotypes [6–9, 11]. Of the countries in the South Asian region, a study from India reports monosomy 7 as the most frequent cytogenetic abnormality seen in MDS patients in India while another study recognizes del(5), del(7) and trisomy 8 as main abnormalities detected [11, 12]. These studies indicate that del 5q is not the most prevalent cytogenetic abnormality in Asian countries in contrast to that reported in Western MDS patients. The cytogenetic findings in our study confirm previously reported chromosomal abnormalities associated with MDS. Hypodiploidy has not been reported as a common cytogenetic aberration of MDS; however, few studies have described its association with MDS [30]. Although monosomy 7 and trisomy 8 have been reported to be common among Asian populations, our patient group did not have them. The highest number of chromosomal abnormalities was found in RAEB-2 followed by RAEB-1 patients [14].

Cytogenetic analysis of stem cells in MDS not only should help to understand the pathogenesis of MDS, but also could shed the light on therapeutic implications. Previous studies on cytogenetic analysis have shown conflicting results on the origin of cytogenetic abnormalities in the hematopoietic hierarchy [23, 31–37]. Some studies have revealed that the cytogenetically aberrant cells in MDS are constrained to myeloid

and erythroid lineages and, rarely, megakaryocytic lineages [31–36] while others report an association of a multipotential stem cell [37]. The underlying basis for these contradictory results is not yet clear but may be due to different methods used to evaluate clonality and detect cytogenetic abnormalities. The variability between patients can also affect the cytogenetic analysis. If cytogenetic abnormalities in MDS arise at the HSC level, the CD 34⁺ cells should share the same cytogenetic abnormalities as in the BM. Our findings confirm this theory as we observed similar (94 %) cytogenetic abnormalities in both BM and CD 34⁺ cells.

An important finding of our study was the detection of del (5q) in CD 34⁺ cells which was undetected by BM karyotype. This patient had del(5q) phenotype; 54-year-old female with high MCV and (BM) megakaryocytes moderately increased, showing hypolobated nuclei. Reason for the normal karyotype in this patient can be due to either failure of the abnormal clone to expand in cell culture or the number of cells expanded was not sufficient enough to detect the abnormality. We can infer the cytogenetic abnormalities present with low cell numbers may go undetected by BM karyotyping, but can be detected by karyotyping the CD 34⁺ cells.

The percentages of cytogenetically abnormal cells in BM (46 %) were less than those of CD 34⁺ cells (62 %) in all patients showed chromosomal abnormalities ($p > 0.05$). A previous study on MDS patients with -7/7q reports the presence of chromosomal abnormality in a greater proportion in CD 34⁺ cells, (CD 34⁺ cells 92 % and BM cells 60 %) [38].

In conclusion, this is the first study to report the cytogenetic abnormalities of MDS patients in Sri Lanka. Also the detection of karyotypic abnormalities could be greater when using CD 34⁺ cells. This suggests the possibility of incorporation of HSC karyotyping to the diagnostic work up of primary MDS patients with normal karyotypes. This could be validated in further studies with higher patient numbers.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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