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Original research article

Profile of Study of Morphological Features of Myelodysplastic Syndrome on Trephine Biopsy in Tertiary Care Hospital of Maharashtra.

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Abstract

Background: Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders and the diagnosis of MDS is mainly based on morphological findings of peripheral blood and bone marrow. The present study was undertaken to study morphological changes in bone marrow on trephine biopsy in MDS and compare with age matched controls. **Method:** All trephine biopsies done over a six year period time 2013-2018 along with complete blood count, aspirate and cytogenetic report were studied in a Tertiary Care Hospital . 40 cases diagnosed as MDS were selected and in each case, detailed morphology of cellularity and all 3 cell lines was studied including distribution of cells. Cases were compared with 20 age matched controls, in whom bone marrow biopsy was done for cytopenias which were beyond doubt not cases of MDS. **Results:** We found on peripheral smear nucleated RBC (7 cases), macrocytes (12 cases), pseudo-pelger-huet anomaly in neutrophils (10 cases) and blasts (6 cases). On bone marrow biopsy, 39 out of 40 cases showed adequate smear. 23 cases showed increased cellularity (hypercellular). Monolobated megakaryocytes (20 cases) and micromegakaryocytes were found to be statistically significant findings. Myeioid series showed left shift in all cases. Erythroid series were hyperplastic and most cases (31 cases) showed megaloblastic and normoblastic marrow. 13 cases showed dyserythropoiesis, ($p<0.01$). On bone marrow aspiration, 20 cases showed hypercellularity, 14 showed micromegakaryocytes, 22 showed monolobated megakaryocytes, 19 showed hypogranular myeloid cells. Most cases (30; 75%) showed blasts <5%. 29 cases showed hyperplastic, megaloblastic and normoblastic erythroid series. Dyserythropoiesis was seen in 23 cases, ($p<0.01$). 24 (60%) cases were positive for cytogenetics and 10 cases on follow got transformed to acute leukaemia (AL). **Conclusion:** The conclusion is drawn that bone marrow aspirate and trephine biopsies are complementary procedures and both are required for diagnosis and also the cytogenetics remains the crux of diagnosis in MDS.

Keywords: Myelodysplastic syndromes, Morphology, Bone marrow, Trephine biopsy, Peripheral smear, Aspiration

Introduction

The myelodysplastic syndromes (MDS) are a heterogeneous group of leukaemia-related disorders characterized by peripheral blood cytopenias with a hypercellular bone marrow exhibiting dyspoiesis [1-4].MDS can present with varying degrees of single or multiple

cytopenias including neutropenia, anemia and thrombocytopenia. Presentation of MDS can range from asymptomatic to life threatening. The MDS range from those with a relatively indolent course (eg. Refractory anaemia with or without ringed sideroblasts) to more aggressive disorders (eg. Refractory anaemia with excess blast [RAEB] and RAEB in transformation [RAEB-T]) which may have a clinical course indistinguishable from acute myeloid leukaemia (AML) [5]. Older patients are most often affected, with 80% of cases diagnosed in people older than 60 years. It has been estimated that the incidence of MDS in people younger than 14 years is less than 5 per 1 million people but for people older than 70 years, it as high as 22 to 45 per 100,000 [6-8]. Therefore, institutions with a higher proportion of elderly patients tent to encounter a greater number of cases.

Over the years, the MDS have been referred to by a number of terms, including oligoblastic leukaemia, refractory anaemia, smoldering acute leukaemia or preleukemia. The most commonly used classification scheme was published in 1982 by the French-American-British (FAB) group and revised in 1985. At present the WHO is the main reference classification for MDS. It was reviewed in 2008 and takes into account the cytopenias and a number of bone marrow blasts as main discriminant between MDS subtypes [9, 10].

However, MDS remains challenging to clinician in terms of diagnosis and management. The diagnosis is essentially one of exclusion in first ruling out other disorders that can also cause peripheral blood/bone marrow cell dysplasia and cytopenias. The distinguishing biological characteristic of MDS is that it is a clonal disorder of the marrow with impaired differentiation. Although a MDS can be suspected from the clinical history and the peripheral blood counts, the diagnosis is usually made by morphologic inspection of the peripheral blood, bone marrow aspirate and bone marrow trephine biopsy specimen and cytogenetics confirmation [8, 11, and 12]. Morphological examination has several advantages: it is a simple, technically easy, not expensive method, which gives quick results; moreover, it has prognostic importance, and should be supplemented, but not replaced, by other tests.

The present study was carried out with objectives to study morphological changes in bone marrow on trephine biopsy in MDS and compare morphological features with age matched controls. Aspirate findings were studied to evaluate in detail the cytology, prior to biopsy. Also evaluate morphological findings in the background of cytogenetic study this forms the crux of diagnosis and estimate morphological features pointing towards transformation to acute leukaemia.

Materials and Methods

All trephine biopsies done over a six years period from 2013-2018 along with complete blood count, aspirate and cytogenetic report were studied in a Tertiary Care Centre of central india. This was a four years retrospective (jan2013-December 2016) and two years prospective study (jan2017-Dec 18) conducted in pathology department of Indira Gandhi Government Medical College , Nagpur. We studied those patients in whom biopsy and aspirate was performed and sent in Zenkers fluid to Department of Pathology after that it was processed in autotechnicon and was stained by haematoxylin and Eosin stain.

A detailed proforma was maintained including age, sex, chief complaints, investigative findings, biopsy and aspirate reports. Cases diagnosed as MDS were selected and in each case, detailed morphology of cellularity and all 3 cell lines was studied including distribution of cells. Cases were compared with age matched controls, in whom bone marrow biopsy was done for cytopenias which were beyond doubt not cases of MDS. We studied a minimum of 60 trephine biopsies. For age bracket in decades from the third decade to the seventh, at least one control was used and 40 cases of MDS were included. The control cases were selected based on hemogram findings, response to therapy and follow up, after diagnosis was made that was clearly other than MDS. For example in patients with anaemia, elevated TIBC, reduced serum iron, reduced vit B12 levels would all suggest towards a nutritional deficiency. Patient with thrombocytopenia, response to dapsone or steroid suggested towards responding cytopenia and in patients with leucopenia that was spontaneously recovering or recovery following antibiotic or supportive management (like following enteric fever or any viral infection) where trephine biopsy was done and they were selected as age matched control.

Observations and Results

Total 40 cases of myelodysplastic syndrome and 20 age matched controls were enrolled in the study. MDS was commonly seen in 51-60 years and more in males (55%). Most patients presented with weakness (100%) as shown in Table 1.

Table 1: Demographic dataand clinical Features

Age group	**No. of cases**	**Percentage**
31-40	4	10
41-50	6	15
51-60	15	37.5
61-70	10	25
71-80	5	12.5
Gender	**No. of cases**	**Percentage**
Female	18	45
Male	22	55
Clinical Features	**No. of cases**	**Percentage**
Weakness	40	100
Dyspnoea	18	45
Fever	8	20
Weight loss	3	7.5
Giddiness	2	5
Chest pain	1	2.5
Pedal oedema	1	2.5
Headache	1	2.5

Anaemia was seen in almost all the cases. Significant leucopenia in the range of 1501-4000/cumm was seen in 47.5% cases. Platelet count in most cases was below normal, in a range of 20,001 and 1 Lakh/cumm. Mean corpuscular volume (MCV) was found to be higher than normal in a majority of cases. Red cell distribution width (RDW), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not found to be important parameters in contributing to the diagnosis of MDS, (Table 2).

Table 2: Various blood parameters

Parameters		No. of cases	Percentage
Hb (gm %)	0-6.0	09	22.5
	6.1-10.0	26	65
	10.1-20.0	05	12.5
TLC (/cumm)	0-1500	02	5
	1501-4000	19	47.5
	4001-11000	12	30
	11001-20000	07	17.5
Platelets (/cumm)	0-20000	04	10
	20001-100000	22	55
	100001-1000000	14	35
MCV (fl)	0-70.0	02	5
	70.1-90.0	20	50
	90.1-150	18	45

On peripheral smear, presence of blasts, macrocytes, nucleated RBC and pseudo-pelger-huet anomaly was found statistically significant ($p<0.01$) finding in diagnosing MDS and distribution of patients are depicted in figure 1.

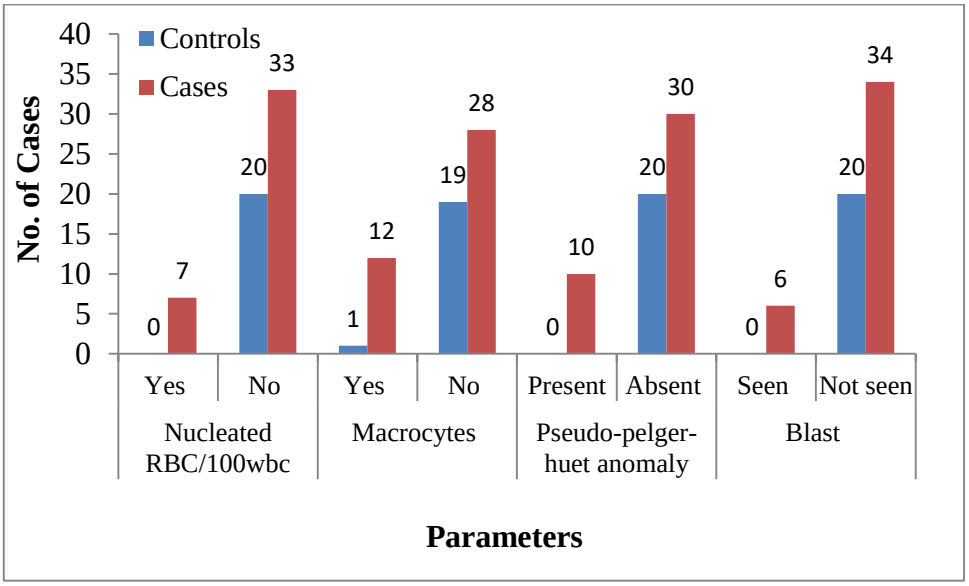

Figure 1: Interpretation of peripheral blood/ smear

On bone marrow biopsy,39 cases had adequacy adequate and 1 case had adequacy inadequate. 23 out of 40 cases of myelodysplastic syndrome shows increased cellularity. Megakaryocyte number was not found statistically significant as a marker for MDS. Monolobated megakaryocytes and micromegakaryocytes were found to be statistically significant findings. Myeioid series showed left shift in all cases. Erythroid series were hyperplastic and most cases show megaloblastic and normoblastic marrow. 13 out of 40 cases showed dyserythropoiesis which was statistically significant as shown in table 3.

Table 3: Bone marrow biopsy

Parameters			Controls (n=20)	Cases (n=40)	P value
Adequacy		Adequate	20	39	0.99
		Inadequate	00	01	
Cellularity		Decreased	03	02	0.002
		Increased	02	23	
		Normal	15	15	
Megakaryocytes		Decreased	01	07	0.254
		Increased	03	09	
		Normal	16	24	
Megakaryocytes lobation		Normal	20	20	<0.01
		Monolobated	00	20	
Myeioid series		Left shift	00	40	<0.01
		Nil	20	00	
Erythroid series	Cellularity	Hyperplastic	00	30	<0.01
		Hypoplastic	00	06	
		Normal	20	04	
	Erythroid series	Megaloblastic	01	00	0.037
		Microblastic and megaloblastic	00	03	
		Microblastic and normoblastic	00	04	
		Normoblastic	05	02	
		Normoblastic and megaloblastic	14	31	
	Dyserythropoiesis	Absent	20	27	0.004
		Present	00	13	

On bone marrow aspiration, 20 cases out of 40 shows increased cellularity , 14 shows micromegakaryocytes (small), 22 showed monolobated megakaryocytes, 19 showed hypogranular myeloid cells. Most cases (30; 75%) showed blasts <5%. Hypogranular cells and giant myelocytes, metamyelocytes and band forms were found to be statistically significant findings. 29 cases showed hyperplastic, megaloblastic and normoblastic erythroid series. Dyserythropoesis was seen in 23 cases which were statistically significant, (Table 4). The increased histiocytes, increased plasma cell was not found statistically significantfinding for MDS.

Table 4: Bone marrow aspirate

Parameters		Controls (n=20)	Cases (n=40)	P value
Cellularity	Decreased	01	05	<0.01
	Increased	01	20	
	Normal	18	15	
Megakaryocytes size	Large	01	01	0.01
	Small	00	14	
	Normal	19	25	
Megakaryocytes lobation	Normal	20	18	<0.01

		Monolobated	00	22	
Myeloid cells granularity		Hypogranular	01	19	0.003
		Normal	19	21	
Blast in Myeloid series		<5	-	30	-
		5 to 20	-	10	
Giant Myeloid cells		Giant cells	00	09	0.02
		Normal cells	20	31	
Erythroid series	Cellularity	Hyperplastic	04	29	<0.01
		Hypoplastic	00	04	
		Normal	16	07	
	Erythroid series	Megaloblastic	00	02	<0.01
		Microblastic and normoblastic	00	09	
		Normoblastic	08	00	
		Normoblastic and megaloblastic	12	29	
	Dyserythropoiesis	Absent	-	17	-
		Present	-	23	

Cytogenetic testing was done in all 40 cases, among them 24 (60%) were positive and 16 (40%) were negative. Out of 24 positive cases, 5 cases showed multiple chromosomal defects, 6 cases showed trisomy 8, 4 cases showed deletion 5q, 5 cases showed deletion 20q, 2 cases showed monosomy 5. 2 cases showed deletion 9q and monosomy 7 respectively as depicted in figure 2.

In the cases that were negative for cytogenetics, they were included in the study only after absence of desired response to haematinic's, growth factors, antibiotics and after ruling out bone marrow involvement in non-haematological malignancy.

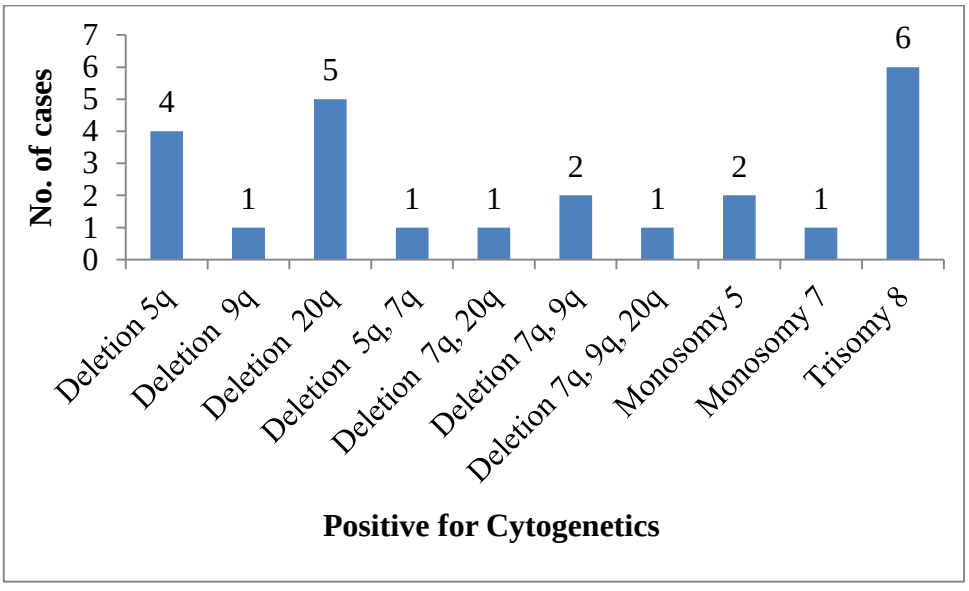

Figure 2: Distribution of Cases Positive for Cytogenetics

Out of 40, 10 cases on followup got transformed to acute leukaemia (AL) , of them 7 had blast count >5%. The other consistent finding in all cases was a prominent dyserythropoiesis, which may be a significant indicator towards transformation, (Table 5).

Table 5: Myelodysplastic syndrome cases that transformed to acute leukaemia

Sr. no.	Clinical features	Cytogenetics	Follow up period till transformation to AL	Specific finding on initial bone marrow
1	Weakness, giddiness	Deletion 20q	1 year	Dyserythropoiesis
2	Weakness, headache	Deletion 20q	3 months	Dyserythropoiesis, blasts 10% on PS
3	Weakness, breathlessness	Trisomy 8	1 year	Dyserythropoiesis
4	Weakness, fever	Negative	1 year	Dyserythropoiesis
5	Weakness, dyspnoea	Deletion 20q	6 months	Dyserythropoiesis, blasts 10% on marrow
6	Weakness, dyspnoea	Deletion 5q, 7q	1 year	Dyserythropoiesis, blasts 20% on marrow
7	Weakness, breathlessness	Negative	1 year	Dyserythropoiesis, blasts 12% on marrow
8	Weakness, dyspnoea	Trisomy 8	1.5 years	Dyserythropoiesis, blasts 8% on marrow
9	Weakness	Deletion 5q	1 years	Dyserythropoiesis, blasts 16% on marrow
10	Weakness, fever	Negative	1 years	Dyserythropoiesis,

Discussion

Myelodysplastic syndromes are hematopoietic stem cell disorders characterized by dysplastic, ineffective, clonal and neoplastic hematopoiesis. It mainly occurs in the elderly, the median age of patients with MDS exceed 65 years [8] but can affect younger individuals too. In the present study, commonest age group for both men and women was 51-60 years. No specific sex prediction was found in literature, but in current study cases were more found in males than females which is correlated with the previous studies [13, 14]. The commonest clinical presentation was weakness, which was seen in all 40 cases (100%) followed by dyspnea seen in 18 cases (45%). As per literature, clinical features of MDS are nonspecific. Few are related to cytopenias like there may be haemorrhage, susceptibility to infections and symptoms of anaemia [8].

Anaemia was seen in almost all the cases which are similar to the other studies [8, 15]. There was significant leucopenia in the range of 1500 to 4000/cumm in 19 (47.5%) cases. As per Bain leucopenia is a common feature in MDS especially neutropenia. The platelet count is usually either normal or reduced and in a minority of patients it is increased [8].Similarly present study found lower than normal platelet count in maximum (55%) cases. 25 (62.5%) cases showed a high RDW and 15 (37.5%) cases showed near high RDW values. MCV was found to be higher than normal in maximum (38) cases. We found on peripheral smear nucleated RBC (7 cases), macrocytes (12 cases), pseudo-pelger-huet anomaly in neutrophils (10 cases) and blasts (6 cases). These findings are comparable with the study done by Bain [8] and Vallespi et al [15].

Bone marrow adequacy is an important parameter. It reflects more on technique of collection and processing of biopsy specimen than disease itself. In current study 39 out of 40 cases showed adequate smear. It reflects more on technique of collection and processing of biopsy specimen than disease itself. 23 out of 40 cases showed increased cellularity (hypercellular), 2 cases show reduced cellularity which is comparable with the previous studies [8, 15-17]. The numbers of megakaryocytes were normal in 24 cases, in 7 cases they were decreased and in 9 cases the number was increased. As per Bain [8],number of megakaryocytes may be increased in MDS. 20 out of 40 cases showed monolobated megakaryocytes and remaining 20 showed normal megakaryocytes. All 40 cases of MDS showed a shift to left which is similar to the study done by Bain [8]. The erythroid series was hypercellular in 30 cases and hypocellular in 6 cases. 31 cases showed normoblastic and megaloblastic marrow. 13 cases showed dyserythropoiesis. 3 cases showed fibrosis, 3 showed necrosis, 2 showed increased in histiocytes, 2 showed increase in plasma cells and 1 case showed both increase in histiocytes and plasma cells. These are nonspecific findings and they don't have any bearing on diagnosis of MDS.

On bone marrow aspiration, 20 cases of MDS showed hypercellularity, 15 cases were of normal cellularity and 5 were hypocellular. Hypercellularity may be due to hyperplasia of erythroid or granulocytic series or both. 14 cases showed micromegakaryocytes and 25 showed normal megakaryocytes. As per literature presence of micromegakaryocytes is a very specific feature of MDS [8, 15]. Out of 40 cases 22 showed monolobated megakaryocytes and remaining showed normal multilobated megakaryocytes.19 cases showed hypogranular myeloid cells. 9 cases showed giant myelocyte, metamyelocyte and band form. All cases showed presence of blasts with 75% (30 cases) showed blasts <5%. 10 (25%) cases showed blasts ranging from 5-20%. 29 cases shows hyperplastic erythroid series. 7 cases showed normal cellularity. 29 cases shows normoblastic and megaloblastic erythroid. Dyserythropoiesis and presence of megaloblasts are almost always seen in MDS. In current study, 23 out of 40 cases showed presence of dyserythropoiesis. These findings are correlated well with the earlier studies [8, 15].8 cases showed increased histiocytes and 6 cases show increased plasma cells. The increased plasma and histiocytes are not specific features of MDS but, though they may be seen in some cases.

All 40 cases underwent cytogenetic testing among them 24 were positive for cytogenetics and 16 cases were negative. 5 cases showed multiple chromosomal defects, 6 cases showed trisomy 8, 4 cases showed deletion 5q, 5 cases showed deletion20q, 2 cases showed monosomy 5. 2 cases showed deletion 9q and monosomy 7 respectively. Patients with 5q deletion have a good prognosis with a median survival of approximately 7 years [18]. In about one-third of patients, MSD can rapidly progress to acute leukemia [7-9]. 10 out of 40 cases transformed to acute leukaemia.

Conclusion

Cytopenias of single, double or triple cell lines in an elderly age group remaining refractory to haematinics with dyserythropoiesis on peripheral smear or bone marrow is a significant pointer for MDS. Bone marrow aspirate evaluation is crucial in diagnosis of MDS. It helps to identify hypogranularity, dyserythropoiesis, dysmegakaryopoeisis and assessment of blast count. Also it helps in predicting cases which may eventually culminate in to acute leukaemia, besides being the ideal sample for cytogenetic evaluation. Specific findings on bone marrow biopsy such as increased cellularity, paratrabecular presence of megakaryocytes and erythroid cells and hypolobated or monolobated megakaryocytes should strongly incite suspicion towards MDS.The importance of biopsy in MDS cannot be underrated as only a biopsy can rule out

infective conditions and bone marrow replacement by solid organ malignancy that may present with pancytopenia and have several overlapping features on bone marrow aspirate that may mislead towards MDS.Cytogenetics remains the crux of diagnosis in MDS. Immunohistochemistry on block was not done as a consistent tool in all our cases in this study, but IHC would have been favorably contributory in diagnosis of MDS, especially in those cases that were negative for cytogenetics for MDS.

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