ORIGINAL ARTICLE

Clinical and Cytogenetic Profile in Patients with Myelodysplastic Syndrome

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ABSTRACT

Background: The aim was to find out incidence of clinical and cytogenetic abnormality in patients with Myelodysplastic syndrome visiting our tertiary care Centre which can help in further research regarding association of cytogenetic abnormality in MDS prevalent in our region.

Material and methods: 20 patients diagnosed as case of MDS or are in follow up and 20 healthy age and sex match voluntary blood donors. Cases and controls were taken from 1st Nov 2016 to 31st June 2018. Obtained information regarding the presence of cytopenia, bone marrow biopsy, karyotyping, age group, sign and symptoms were analyzed.

Results: In our study majority of the patients were in age between 41 and 60 years. Male outnumbered females with ratio of 1.86:1.the most common symptom was weakness followed by fever and bleeding manifestation. The commonest clinical finding was pallor, splenomegaly and lymphadenopathy. There was no family history and few of them have been exposed to pesticides. Grade IV neutropenia and thrombocytopenia was present in 25% and 50% of the patient respectively. 65% of the patient showed pancytopenia and hypercellularity as well. On karyotyping 8 abnormalities were detected out of which most common was Monosomy 7 seen in 20% of cases. 15% patient progressed to acute myeloid Leukemia.

Conclusion: The pathogenesis of MDS is still poorly understood and variation in its frequency possibly due to environmental and biological factors. In our study mean age of incidence was 49 years with male preponderance. All the other modifiable and non-modifiable risk factors were evaluated and were in agreement with previous studies done in western population except for the mean age which is early for our population.

Key words: Myelodysplastic Syndrome (MDS), karyotype, cytogenetic, AML

INTRODUCTION

The Myelodysplastic Syndromes (MDS) are a very complex group of myeloid disorders characterized by Peripheral cytopenias, Dysplastic and ineffective hematopoiesis, Variable risk for leukemic transformation[1]

Peripheral cytopenias occur despite hypercellular or normocellular bone marrow. It is due to apoptosis of cells in early stages. These disorders can occur de novo or due to years after exposure to mutagenic chemotherapy or environmental exposures like radiation, benzene etc[2]. Incidence of MDS is more common in elderly people. Patient generally presents with symptoms of anemia. Less commonly they may also be asymptomatic or present with fever and bleeding manifestations. There is significant morbidity and mortality due to bone marrow failure or transformation to AML.

Several recurrent cytogenetic abnormalities have been seen by Karyotyping. Further study revealed genetic abnormality at molecular level and association with leukemogenic process[3]. Chromosomal abnormalities are detected in approximately 50% patients with primary MDS while 80% in patients with secondary MDS. Four single chromosome abnormalities commonly present in patients with MDS are 5q deletion, 7q deletion/ monosomy 7, trisomy 8 and 20q deletion.

Work up in patients with MDS includes complete blood count with differentials, peripheral blood smear study, bone marrow aspiration and biopsy. Cytogenetic analysis has proven to be a mandatory part of the diagnosis of MDS as well as a major indicator for predicting clinical course and outcome. Our present study is aimed to achieve the following objective

to find out incidence of clinical and cytogenetic abnormality in patients with Myelodysplastic syndrome.

MATERIAL & METHODS

The present study entitled "Clinical and Cytogenetic profile in patients with Myelodysplastic Syndrome" was conducted in the department of General Medicine, Division of Clinical Hematology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India from 1st November, 2016 to 31st June, 2018. The study was approved by Institutional Ethics committee. All cases were selected based on inclusion and exclusion criteria. A detailed history, clinical examination and all required investigations were done in all cases. 20 newly diagnosed or on follow up or with provisional diagnosis of MDS were included in the study. However patient who have received chemotherapy, MDS secondary to malignancy or patient not giving consent were excluded from study. 20 healthy voluntary blood donor age and gender match were taken as control. Written informed consent was obtained from each patient; for patients who were too ill to communicate, permission to enroll the patients in the study was obtained from the next of kin.

Complete blood count (CBC): 3 ml blood in EDTA vial was taken and analyzed on Beckmen Coulter Hematology Fully Automated Autoanalyzer LH750. Total leukocyte count, differential count, Red cell indices and platelet count was analyzed.

General Blood Picture (GBP): Finger Prick smear for cell morphology.

Bone marrow aspiration/ biopsy: Done from posterior superior iliac spine under aseptic care using disposable bone marrow aspiration needle, jamshedi needle for bone marrow biopsy.

Karyotyping: 2ml Bone marrow sample was collected in heparinized syringe. Bone marrow cells were cultured in RPMI 1640 supplemented with 20% fetal calf serum, 2mM L-glutamine, penicillin and streptomycin (100U/ml) for 24h. Then colcemid was added at concentration of 0.1µg/ml for 30min. Then hypotonic KCl (0.075M) for 12-15 min and it was fixed with methanol/acetic acid (3:1). Cell suspension was dropped on clean chilled and flame dried slides. Metaphase chromosomes were banded using GTG (Gimsa Trypsin Gimsa) banding technique and Karyotyping was done according to ISCN (International System for Human Cytogenetic Nomenclature). At least 20 metaphases were analyzed.

Statistical analysis: The statistical analysis was done using SPSS for Windows version 16.0 software. For categorical variable Chi-square test was used. For comparing two groups of mean Student's't' test and for more than 2 groups One-way Analysis of Variance (ANOVA) test was used. The critical value of 'p' indicating the probability of significant difference was taken as <0.05 for comparison.

RESULTS

In this study, patient in age group (0-20year), (21-40), (41-60) and (>60 years) was 10%, 25%, 35% and 30% respectively. Thus maximum patients (30%) were in age group (41-60yrs). Table 1. Out of 20 cases of MDS, 13 cases were male (65%) and 7 cases (35%) were female (table 2). Most common presentation for which patient came to us was generalized weakness in 18 cases (90%), followed by fever in 5 cases (25%) and bleeding in 4 cases (20%). Table 3

Table 1: Age wise distribution

Age group	Frequency (%)
0-20year	2 (10)
21-40year	5 (25)
41-60year	7 (35)
>60year	6 (30)
Total	20 (100)

Table 2: Sex wise distribution

Sex	Frequency (%)
Male	13 (65)
Female	7 (35)
Total	20 (100)

Table 3: Symptoms in patient with MDS

	Weakness (%)	Fever (%)	Bleeding (%)
Present	18 (90)	5 (25)	4 (20)
Absent	2 (10)	15 (75)	16 (80)
Total	20 (100)	20 (100)	20 (100)

Table 4: Risk factor in Patients

	Pesticide exposure (%)	Tobacco addiction (%)	family history (%)
Present	4 (20)	3 (15)	0 (0)
Absent	16 (80)	17 (85)	20 (100)
Total	20 (100)	20 (100)	20 (100)

Table 5: Examination finding

	Pallor (%)	Lymphadenopathy (%)	Splenomegaly (%)
Present	13 (65)	1 (5)	3 (15)
Absent	7 (35)	19 (95)	17 (85)
Total	20 (100)	20 (100)	20 (100)

Table 6: Cytopenia frequency

	Single lineage dysplasia (%)	Bicytopenia (%)	Pancytopenia (%)
Present	1 (5)	6 (30)	13 (65)
Absent	19 (95)	14 (70)	7 (35)
Present	20 (100)	20 (100)	20 (100)

Table 7: Hematological profile: Neutropenia

Count/μL	Grade*	Patients (%)
>1500	Normal	4 (20)
~1500	Grade-I	0(0)
1000-1500	Grade-II	2(10)
500 - <1000	Grade-III	9(45)
<500	Grade-IV	5(25)

* National Cancer Institute (NCI)

Table 8: Hematological profile: Thrombocytopenia

Platelets Count/mm^3	Grade*	Patients (%)
450,000 ->150,000	Normal	2(10)
75,000- <150,000	Grade-I	1(5)
50,000 - <75,000	Grade-II	2(10)
25,000 - <50,000	Grade-III	5(25)
<25,000	Grade-IV	10(50)

* National Cancer Institute (NCI)

Table 9: Bone Marrow Cellularity frequency

Bone marrow cellularity	Frequency (%)
Hypercellular	13 (65)
Hypocellular	4 (20)
Normocellular	3 (15)
Total	20 (100)

Table 10: karyotypes abnormality frequency

Karyotypes abnormality	Frequency (%)
Present	6 (30)
Absent	14 (70)
Total	20 (100)

Table 11: Cytogenetic abnormality

	Present (%)
5q deletion	2(33)
Monosomy 7	4(66)
Trisomy 8	1(16)
20q deletion	1 (16)
Patient with karyotype	6 (100)

Table 12: progression to AML

Progression to AML	Frequency (%)
Yes	3 (15)
No	17 (85)
Total	20 (100)

Patient were assessed for risk factors like pesticide exposure which was present in 4 patients (20%), addiction to tobacco in any form was present in 3 (15%) and there was no family history of MDS, other malignancies and chemotherapy in all 20 patients. Table 4. Patient were thoroughly examined and the most common finding was pallor which was present in 13 (65%) patients followed by splenomegaly in 3 (15%) and lymphadenopathy in 1 (5%) table 5.

All patient were thoroughly investigates as per protocol. The complete blood count reveals cytopenia in 1 (5%), Bicytopenia 6 (30%) and pancytopenia 13 (65%) out of 20 patients (Table 6). In this study, majority of patient with MDS had grade III/IV neutropenia (13/20) ranged below 1000/ μL (Table 7). In this study we found 50% patients had grade IV thrombocytopenia and 25% had grade III thrombocytopenia (Table 8). Patient bone marrow biopsy were done where the most common finding was hypercellular marrow in 13 (65%) followed by hypocellular in 4 (20%) and normocellular marrow in 3 (15%) Table 9. Karyotyping was done in all patients who come out positive for any abnormality in 6 (30%) Table 10, and the most common cytogenetic abnormality found to be Monosomy 7 which was present in 4 (66%) out of 6 positive cases which was followed by 5q deletion present in 2 (33%) of the cases. Other cytogenetic abnormality which were found in addition to the above were Trisomy 8 and 20q deletion one case of each i.e. 16% Table 11. In our study 3 patients (15%) progressed to Acute Myeloid Leukemia Table 12.

DISCUSSION

MDS is a heterogeneous disease of aging population. Incidence rate in general population is 35 to 100 per million[4]. There are various modifiable and non-modifiable risk factors. Non-modifiable risk factors include - ageing, male sex[5], inheritable genetic abnormalities, immune dysfunction, and DNA repair deficiencies.

In our study, there were 35% patients in age group (21-40 years) and (41-60 years); and 30% patients in age group (>60 years). Median age of incidence was 49 years and was in the age between 21 and 60 years. There is younger age of incidence in our study and male had higher incidence of MDS (Table: 1 & 2). In one study conducted in Mumbai revealed median age of incidence 46 year and M: F: 1.22:1[6]. In another study conducted in Chennai revealed median age at onset 55 years and M: F: 1.7:1[7].

Similar to our observations, in a study on Asian population, relatively younger population developed MDS[8]. A study revealed patient's median age in Japan was 57 years which was significantly younger than the median age of 71 years in German patients.

In this study weakness was major presenting feature (90%) followed by fever, bleeding manifestations. In this study 20% patients with MDS gave significant history of exposure to pesticide; none had family history. Lymphadenopathy was present in 5% patients and splenomegaly was present in 15% patients with MDS. The result was concordant with previous studies. (Table: 3 & 5)

Thus result was in agreement with previous studies with incidence slight earlier than western population

probably due to exposure to pesticides and other environmental factors.

Anemia is present in most cases either alone, or as a part of bi/ pancytopenia. Macrocytosis is common. Total leukocyte count is usually normal or low. Isolated neutropenia and thrombocytopenia are rare. Platelets are large and lack granules. In this study, majority of patient with MDS had grade III/IV neutropenia (13/20) ranged below 1000/ μL. We found 50% patients had grade IV thrombocytopenia and 25% had grade III thrombocytopenia. Majority (65%) of patient with MDS presented with pancytopenia followed by Bicytopenia. Single lineage dysplasia was uncommon (Table: 6-8). Majority of our patients with MDS had hypercellular (65%); 20% had hypocellular and 15% had normocellular bone marrow in the current study (Table: 9).

Cumulative genetic and environmental damage to marrow cells probably leads to MDS. Serial cytogenetic studies documented sequential changes in karyotype, and these leads to typical concomitant clinical progression[9]. In the current study we found 40% patients had karyotypes abnormality, 5q deletion was present in 10% of cases, 7q deletion/ monosomy-7 was present in 20% of cases, trisomy-8 was present in 5% cases, 20q deletion was present in 5% cases (Table: 10 & 11). In one study conducted in Mumbai incidence of cytogenetic abnormality was 52% and most common chromosomal abnormality was 7q deletion/ Monosomy 7[10]. In another study conducted in New Delhi, 47.5% patients revealed chromosomal abnormality. Monosomy 7 was most common abnormality followed by 5q deletion and others[11]. The incidence of chromosomal abnormalities varied across the different Asian populations[7].

The result of this study was in agreement with previous studies with slight lower overall cytogenetic abnormality because only four commonly occurring cytogenetic abnormality was included in study- 5q deletion, 7q deletion/ monosomy 7, trisomy 8, 20q deletion. In this study 15% patients progressed into AML having more than 60% blast with undifferentiated morphology (Table 12). In one study conducted in New Delhi 5% progressed to AML[11]. The result was concordant with previous studies.

Most published data on Myelodysplastic syndromes (MDS) are derived from Western countries, which report MDS as a disease of the elderly. However, it was observed that Asian MDS patients were younger than subjects in Western reports. Ethnogeographical differences and genetics underlying the pathogenesis of MDS among this Asian population might be responsible for occurrence of MDS in Indian population. A higher rate of transformation to acute myeloid leukemia (AML) was observed in the Chinese compared to other Asian countries.

CONCLUSION

MDS is a heterogeneous disease of aging population. Incidence of patient with MDS is increasing because of better recognition by physician, diagnostic facility. While pathogenesis of MDS is still poorly understood, there are variation in karyotypes abnormality and variation in its frequency possibly due to environmental and biological factors. There are both non-modifiable and modifiable risk factors. Further extended study will be able to give more representative data in terms of cytogenetics abnormalities and progression to AML. The limitation of the study was relatively small sample size and only four chromosomal abnormalities were included in the study.

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