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Original Article

Cytogenetic features in primary myelodysplastic syndrome Egyptian patients

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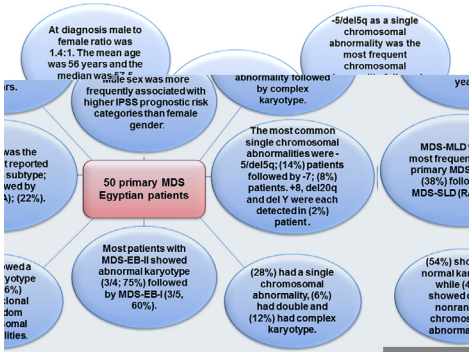
GRAPHICAL ABSTRACT



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ABSTRACT

Karyotype is the most important diagnostic and prognostic parameter in myelodysplastic syndromes (MDS) and is abnormal in approximately 50% of patients. We emphasized the importance of chromosomal analysis and reported the most frequent cytogenetic abnormalities in 50 MDS (29 males (58%) and 21 females (42%), median age: 57.5 years) Egyptian patients using conventional banding analysis (CBA). Karyotype description was conducted according to the International System for Human Cytogenetic Nomenclature (ISCN, 2013). Patients were diagnosed based on complete history, bone marrow (BM) aspirate, peripheral blood (PBL) examination, and Iron stain. MDS with multilineage dysplasia (MDS-MLD) was the most frequently encountered subtype; 19/50 (38%) followed by MDS with single lineage dysplasia (MDS-SLD); 11/50 (22%). 27/50 patients (54%) showed a normal karyotype while 23 patients (46%) showed clonal nonrandom chromosomal abnormalities. Most patients with MDS with excess blasts-II (MDS-EB-II) showed abnormal karyotype (3/4; 75%) followed by MDS-EB-I (3/5, 60%) and MDS-MLD (10/19, 53%). Among 50 primary MDS patients; 14/50 (28%) had a single chromosomal abnormality, 3/50 (6%) had double chromosomal abnormality, and 6/50 (12%) had complex karyotype. Male sex was more frequently associated with higher IPSS prognostic risk categories than female gender. The most common single chromosomal abnormalities were −5/del5q; 7/50 (14%) patients followed by −7; 4/50 (8%) patients. +8, del20q and delY were each detected in 1/50 patient (2%). Abnormalities of chromosome 5 (−5/del5q) as a single chromosomal abnormality was the most frequent chromosomal abnormality among Egyptian primary MDS patients followed by complex karyotype. Cytogenetic characteristics of MDS Egyptian patients were similar to North African and European patients. Karyotype offers useful

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information in establishing accurate diagnosis and male gender is an important predisposing factor that can predict worse prognosis in MDS patients.

Introduction

Myelodysplastic syndromes (MDS) are acquired, neoplastic disorders of hematopoietic stem cells (HSCs) characterized by ineffective and dysplastic myeloid cell differentiation and a high rate of progression to acute myeloid leukemia (AML) [1,2]. The bone marrow (BM) in MDS is hypercellular with disordered growth and maturation and clonal proliferation of abnormal cells. Peripheral blood (PBL) cytopenias are due to insufficient hematopoiesis, affecting myeloid, erythroid and megakaryocyte lineages. The disease course is highly variable, ranging from indolent to aggressive with progression to AML [3].

MDS arise *de novo*, but 10% patients may acquire MDS as a consequence of previous radio/chemo therapy for other cancers [3,4]. The median age of MDS patients at diagnosis is 65–70 years. <10% of patients are younger than 50 years with an incidence about four cases per 100,000 population. This disorder shows a slight male predominance except for isolated 5q deletion in which women predominate. The pathophysiology is a multistep process involving cytogenetic changes, gene mutations, or both. Diagnosis is based mainly on examination of peripheral blood and BM displaying cytopenias and hypercellular marrow with dysplasia, with or without excess of blasts [5].

The classification of MDS is continuously evolving and prognosis is largely dependent on the presence of chromosomal abnormalities [6]. Every new validated classification reflects better understanding of the disease, its pathogenesis and prognosis [7]. An International Prognostic Scoring System (IPSS) and a World Health Organization (WHO) classification have now been devised, which assess the type and extent of marrow cytogenetic abnormality and the cell lines affected [8]. The WHO is now the recommended classification system [9]. The original IPSS has now been replaced by a revised version [10]. The WHO 2016 classification revision of myeloid neoplasms and acute leukemia edition includes MDS with single lineage dysplasia (MDS-SLD), MDS with multilineage dysplasia (MDS-MLD), MDS with ring sideroblasts (MDS-RS), MDS with excess blasts (MDS-EB-1 and 2), MDS with isolated del (5q) and MDS unclassifiable (MDS-U) [9].

MDS frequently has unbalanced translocations, and deletions, implicating loss of function or haplo-insufficiency of tumor suppressor genes [11]. BM chromosomal abnormalities occur in almost half of *de novo* cases [12]. Deletions are the most common cytogenetic abnormalities in *de novo* MDS. The gain can arise in both primary and therapy-related MDS. Balanced translocations are uncommon in patients with MDS and are mainly related to unfavorable prognosis [11]. The cytogenetic abnormalities recorded in the 2008 WHO classification [13] continue to exist MDS-defining in a cytopenic patient, even in the absence of diagnostic morphologic dysplasia. Del (5q) remains as the only genetic abnormality that defines a specific MDS subtype. One of the biggest challenges confronting hematologists is how to separate MDS from reactive cases of cytopenia and dysplasia. Despite the fact that cytogenetic findings are not used to outline specific subtypes of MDS, they may be strongly correlated with prognosis and may help in establishing diagnosis in unconfirmed MDS cases as well as MDS unclassifiable thus, a complete BM karyotype remains a critical test in any newly diagnosed MDS patient [9].

The present study was undertaken to discover the most frequent cytogenetic abnormalities in primary MDS Egyptian patients diagnosed by PBL and BM morphology and to correlate abnormal karyotypic findings with different MDS WHO subtypes in addition to clarify the association between gender and different IPSS prognostic risk categories.

Patient and methods

Fifty *de novo* MDS Egyptian patients were included. BM aspiration and PBL samples were collected at the time of diagnosis at National Cancer Institute (NCI), Cairo University and were sent for karyotype and analysis at the laboratory of Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City University at the period between October 2010 and December 2016. Diagnosis of MDS was based on full history taking, clinical assessment and laboratory investigations including complete blood count, bone marrow aspirate with iron stain, and conventional banding analysis (karyotype). At the time of analysis, a second revision was made and patients were classified according to the 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms [9]. *All patients gave written informed consent. The study was approved from the Institutional Research Board of GEBRI.*

Prussian blue reaction (Perls' stain for iron)

Prussian blue reaction was assessed on BM smears. Ferric iron deposits in tissue then react with soluble ferrocyanide in the stain, to form insoluble Prussian blue dye in situ, then visualized microscopically as blue or purple deposits, within cells. In brief, BM aspirate was spread on to glass slides, air dried, and fixed with methanol. Equal volume of 2% of potassium ferrocyanide and 2% hydrochloric acid solution were mixed in staining jar and slides are immersed in the solution for 15–20 min. then removed and rinsed with distilled water. Counterstaining with saffranin for 30 s. then allowed to dry and examined [14].

Conventional banding analysis (CBA)

Chromosomal banding analysis was performed by G-banding techniques [15]. Unstimulated BM cells were cultured in double (2 parallel cultures for each patient) for 16 h in Roswell Park Memorial Institute (RPMI) 1640 medium with L-glutamine and 25 mM HEPES *(Cambrex Bioproducts, Belgium)* supplemented with 15% Fetal calf serum (FCS) *(Cambrex Bioproducts, Belgium)* and penicillin/streptomycin mixture 1000 u Pen/10,000 ug strept *(Cambrex Bioproducts, Belgium)* (complete medium). On day 2, cells were pulsed with 20 μl of 10 μg/ml colcemid (GIBCO) for 25 min. and treated with hypotonic 0.075 M potassium chloride (KCL) solution for 30 min. at 37 °C. Cells were then fixed in methanol: acetic acid (3:1). At least 20 metaphase cells were analyzed using Cytovision Software *(Applied Biosystem)* for each sample after trypsinization and staining slides with Giemsa. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature, 2013 [16]. Cytogenetic abnormalities used to define MDS in cytopenic patients were according to WHO, 2016 criteria.

PBL and BM morphology

Morphological assessment for diagnosing MDS, defining cytopenias and rating of dysplasia were done and revised according to WHO 2016 criteria [9].