INTRODUCTION

Myelodysplastic syndrome is a clonal hematopoietic stem cell neoplasm characterized by ineffective hematopoeisis and morphological dysplasia in the bone marrow, with subsequent sustained peripheral cytopenias ***(Lichtman, 2000).***

The disorder may be indolent presented by anemia or aggressive and progress to AML. Its peak age of incidence is between 50-90 years, but can appears in childhood as well, with male to female ratio 1.5/1 ***(Lichtman and Liesveld, 2006).***

The MDS develops when a clonal mutation predominates in the bone marrow, suppressing healthy stem cells. The clonal mutation may result from genetic predisposition or from hematopoietic stem cell injury ***(Lichtman and Liesveld, 2006).***

Inflammation plays a pivotal role in carcinogenesis as many solid tumors can arise on the basis of chronic inflammation. Recently, several studies have suggested an inflammatory etiology for MDS, AML and MPN ***(Takizawa et al., 2012).***

Lipocalin-2 (LCN2 or neutrophil gelatinase associated lipocalin) is an inflammatory cytokine, secreted from neutrophils and expressed in low levels in the kidney,

prostate and epithelia of the respiratory and gastrointestinal tracts**,** LCN2 plays a role in innate immunity through sequestrating iron necessary for bacterial growth ***(Yang et al., 2002).*** Moreover, it mediates the generation of reactive oxygen species (ROS) which results in DNA damage with consequent HSC aging and death ***(Lu et al., 2015).*** Thus, the protection of HSC from inflammatory stress may be of therapeutic value.

Aim of the Work

We aim to study the expression of LCN2 in the bone marrow of patient's having myelodysplastic syndrome, and to correlate it to other clinical and laboratory parameters.

MYELODYSPLASTIC SYNDROMES

Myelodysplastic syndromes (MDS) are a heterogeneous group of malignant bone marrow disorders characterized by ineffective haematopoiesis, and risk of evolution to acute myeloid leukemia (AML) ***(Damm et al., 2012).*** The hallmark of the disease is a cellular bone marrow with dysplastic changes of the hematopoietic cells in both peripheral blood and marrow associated with variable degrees of peripheral blood cytopenias ***(Germing et al., 2008).***

A- Incidence and Epidemiology

Myelodysplastic syndrome is uncommon in children and adolescents, accounting for less than 5% of all hematopoietic neoplasms in patients less than 14 years of age ***(Hasle et al., 2003).***

In USA, the surveillance, epidemiology, and end results program (SEERS) reported an incidence of MDS of more than 30/100,000 per year in people aged above 70 years (primarily in males) with a median age of 60s at diagnosis. Also in Europe the incidence is more than 30 /100,000 per year and about 20,000 patients are diagnosed with MDS per year with a median age of mid to late 60s at diagnosis ***(Germing et al., 2008).***

In Asian countries, the median age of patients with MDS are 10 years younger at diagnosis. The reason for this difference between two ethnic groups is unknown ***(Matsuda et al., 2005).***

El Husseiny et al., (2012), reported that the mean age of presentation of MDS in Egypt is lower than in developed countries. Pollution of water, use of insecticides and smoking are high risk factors for MDS among Egyptians.

B- Predisposing Factors

Several risk factors have been implicated in the etiology of MDS, including age, male gender, alcohol, cigarette smoking, ionizing radiation, immunosuppressive therapy, viral infection, benzene ***(Rund and Ben-Yehuda, 2004; Catenacci and Schiller, 2005).***

Environmental and occupational risk factors such as exposures to solvents, ammonia, exhaust gases, metals and pesticides have been suggested by few epidemiological studies ***(Nisse et al., 2001; Mufti, 2004).***

More than 80% of patients with MDS do not have an obvious history of chemical or ionizing radiation exposure. Patients without such history are described as having primary or de novo MDS. Risk factors are seen infrequently and are estimated to account for disease in

only 20% of patients, who are often designated as having secondary MDS, which has a much poorer prognosis ***(Smith et al., 2003; Steensma and Bennett, 2006).*** (Table 1)

Table (1): Known heritable and acquired factors associated with the development of myelodysplastic syndromes

Hereditable	Acquired
▪ Constitutional genetic disorders (trisomy 21, trisomy 8 mosaicism, familial monosomy 7) ▪ Neurofibromatosis ▪ Germ cell tumors ▪ Congenital neutropenia (Kostmann syndrome or Shwachman -Diamond syndrome) ▪ DNA repair deficiencies (e.g., Fanconi anemia) ▪ Mutagen detoxification enzyme mutation (GSTM1- null, GSTT1-null)	▪ Age ▪ Chemotherapy (alkylating agents, topoisomerase inhibitors, nucleoside analogs) and HSCT ▪ G-CSF ▪ Radiation therapy ▪ Environmental/occupational toxins ▪ Tobacco ▪ Alcohol ▪ Aplastic anemia and paroxysmal nocturnal hemoglobinuria

G-CSF= granulocyte colony–stimulating factor; HSCT =hematopoietic stem cell transplantation.

(Alter et al., 2010).

C- Pathogenesis

The clinical heterogeneity of MDS is a reflection of the various pathogenic mechanisms ***(Bejar et al., 2011).***

Older models for MDS pathogenesis proposed a multistep process initiated by a genetic insult of

hematopoietic stem cells that leads to alteration of the cellular function with the emergence and consequent evolution of AML. This clone, which has a growth advantage, is associated with morphological dysplasia, cellular dysfunction such as excess local secretion of inhibitory cytokines, ineffective haematopoiesis and defective differentiation. This model was supported by the high risk of therapy related MDS in patients who received treatment with alkylating agents such as cyclophosphamide, cholrambucil or cisplatin and the association of low dose high linear radiation with chromosome aberrations ***(Molldrem et al., 2002).***

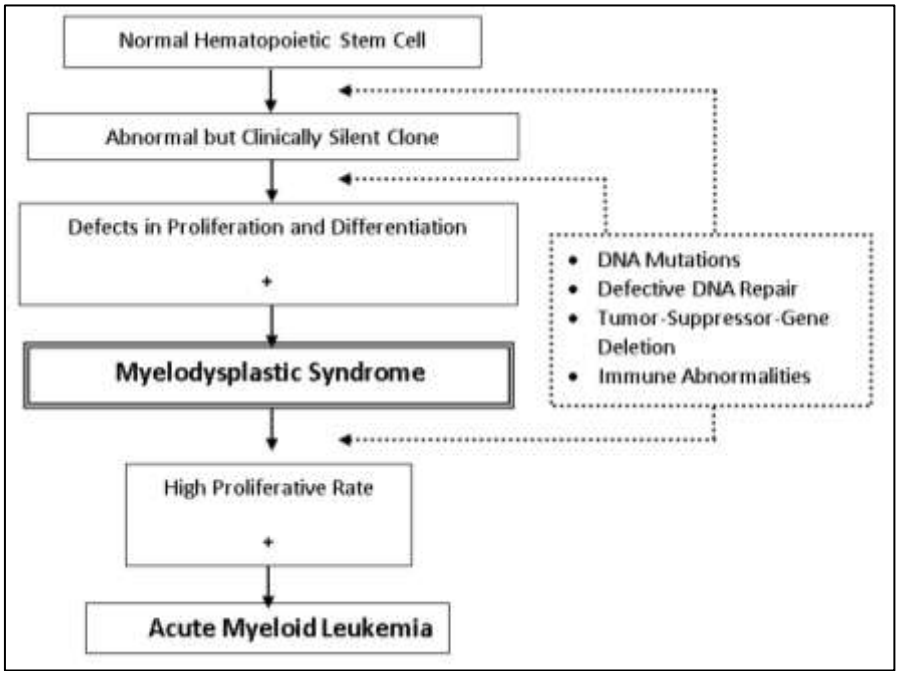

Figure (1): Hypothetical model of multistep development of MDS and AML secondary to MDS ***(Mufti, 2004).***

Recent studies suggest that an inflammatory process may act as a pathogenic driver for MDS through a group of inflammation-associated immature cells, termed myeloid derived suppressor cells (MDSCs). These were markedly expanded in the local bone marrow of MDS patients and induce the secretion of the suppressive cytokines IL-10 and TGF-b) as well as other intermediates generated during chronic inflammation ***(Yang et al., 2015).***

D-Classification of MDS

For more than two decades the French-American-British (FAB) classification has provided a framework for the morphologic classification and diagnostic evaluation of the Myelodysplastic syndromes. However, with widespread use of this classification, it became clear that prognostic differences existed within single categories. The FAB classification has therefore been modified by a World Health Organization (WHO) expert group to take account of these prognostic differences ***(Bain, 2004).***

French-American-British Classification (FAB) of MDS

The French-American-British classification of MDS is the oldest and most well-established scheme for the classification of MDS that was developed in 1982, it has since evolved from a five subtype MDS into an eight subtype MDS. It was based mainly on the proportion of blasts in the peripheral blood and bone marrow and the presence or absence of ringed sideroblasts or increased circulating monocyte numbers ***(Bain, 2004).***

WHO classification

The WHO classification depends mainly on the degree of dysplasia and blast percentages for disease classification and specific cytopenias have only minor impact on MDS classification (Table: 2) and (Table: 3) ***(Arber et al., 2016).***

Table (2): The revised WHO classification (2016) of MDS:

MYELODYSPLASTIC SYNDROMES
MDS with single lineage dysplasia
MDS with ring sideroblasts (MDS-RS) • MDS-RS and single lineage dysplasia • MDS-RS and multilineage dysplasia
MDS with multilineage dysplasia
MDS with excess blasts • MDS-EB-1 • MDS-EB-2
MDS with isolated del(5q)
MDS, unclassifiable
Provisional entity: Refractory cytopenia of childhood
Myeloid neoplasms with germ line predisposition

(Arber et al., 2016).

Table (3): Peripheral blood (PB) and bone marrow (BM) findings and cytogenetics of MDS

Name	Dysplastic lineages	Cytopenias	Ring sideroblasts as % of marrow erythroid elements	BM and PB blasts	Cytogenetics by conventional karyotype analysis
MDS with single lineage dysplasia (MDS-SLD)	1	1or2	<15%	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with multilineage dysplasia (MDS-MLD)	2or3	1-3	<15%	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with ring sideroblasts (MDS-RS)					
MDS-RS with single lineage dysplasia (MDS-RS-SLD)	1	1or2	≥15%	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS-RS with multilineage dysplasia (MDS-RS-MLD)	2or3	1-3	≥15%	BM <5%, PB <1%, no Auer rods	Any, unless fulfills all criteria for MDS with isolated del(5q)
MDS with isolated del(5q)	1-3	1-2	None or any	BM <5%, PB <1%, no Auer rods	del(5q) alone or with 1 additional abnormality except -7 or del (7q)
MDS with excess blasts (MDS-EB)					

Table (3): continue

Name	Dysplastic lineages	Cytopenias	Ring sideroblasts as % of marrow erythroid elements	BM and PB blasts	Cytogenetics by conventional karyotype analysis
MDS-EB-1	0-3	1-3	None or any	BM 5%-9% or PB 2%-4%, no Auer rods	Any
MDS-EB-2	0-3	1-3	None or any	BM 10%-19% or PB 5%-19% or Auer rods	Any
MDS, unclassifiable (MDS-U)					
with 1% blood blasts	1-3	1-3	None or any	BM <5% , PB 1% , no Auer rods	Any
with single lineage dysplasia and pancytopenia	1	3	None or any	BM <5%, PB <1%, no Auer rods	Any
based on defining cytogenetic abnormality	0	1-3	<15%	BM <5%, PB <1%, no Auer rods	MDS-defining abnormality
Refractory cytopenia of childhood	1-3	1-3	None	BM <5%, PB <2%	Any

(Arber et al., 2016).

E- Diagnosis of MDS

Most of MDS patients are asymptomatic and therefore are diagnosed during evaluation of other comorbidities ***(Nguyen, 2009).***

Steensma and Bennett (2006) reported that, there should be a high level of suspicion for MDS in elderly patients in the setting of isolated unexplained anemia or unexplained progressive cytopenias.

Secondary MDS may be highly suspicious in those with prior exposure to any chemotherapy, radiotherapy, or environmental toxins such as benzene, pesticides, or herbicides ***(Malcovati et al., 2013).***

The diagnostic criteria aim to distinguish MDS from reactive causes of cytopenia and dysplasia (MDS-like BM dysplasia) including vitamin B12, folate deficiencies, viral infections, and exposure to certain toxic agents. It also aim to distinguish MDS from other clonal stem cell disorders ***(Schnatter et al., 2012).***

The assessment of dysplasia on peripheral blood and hypercellular bone marrow smears is the mainstay for the diagnosis of MDS. For evaluation of morphology and dysplasia in blood and bone marrow, the WHO classification of myeloid neoplasms is recommended ***(Huang et al., 2008).***

1. Clinical Features

Physical findings in patients with MDS reflect the underlying hematologic disturbance. Systemic symptoms of fever and weight loss are uncommon but generally represent late manifestations of the disease or its attendant complications. 80% of patients complain of fatigue, weakness, pallor, exercise intolerance, exertional dyspnea angina, dizziness due to anaemia, while 20% present with infections, bleeding, or bruising. Bacterial pneumonias and skin abscesses are the most common infections, occurring particularly in patients with a neutrophil count $<1x\ 10^9$ /L ***(List et al., 2004; Catenacci et al., 2005).***

2. Peripheral blood smears

It is important to review the appropriately prepared and stained peripheral blood smear in all cases with MDS and to report morphological features of the cells and the differential count in all cases ***(Valent et al., 2007).***

The main morphological abnormalities that could be found in blood smear of an MDS case are summarized in (Table 4).

Table (4): Morpholigical abnormalities seen in blood smear in MDS

Lineage	Morphological abnormality
Erythroid	Oval microcytosis Aniso-poikilocytosis Dimorphic picture Polychromasia Basophilic stippling Nucleated erythrocytes Reticulocytopenia
Myeloid	Hypogranular neutrophils Hypolobation of neutrophil nuclei (Pseudo-Pelgar-Huet cells) Coarse nuclear chromatin clumping Monocytosis (often with multiple elongated nuclear lobes) Promonocytes (with fine azurophil granules) Desgranulated eosinophils
Megakaryocytes	Giant platelets

(Hamblin and Killick, 2004).

3. Bone marrow biopsy

A trephine biopsy should be performed in all cases of suspected MDS in which bone marrow examination is indicated. Bone marrow biopsy helps in the exclusion of other clinical conditions presenting with cytopenia and provide information on marrow cellularity, megakaryocyte component, blast compartment, bone marrow fibrosis, and the presence of non hematologic cells, such as metastases. The bone marrow in MDS is usually hyper- or normocellular, but in a minority of patients (approximately 10%), the bone marrow is hypocellular (hypoplastic MDS).

The separation between these entities can be problematic because morphologic differences may be subtle. An increase in the percentage of bone marrow CD34 + cells, the presence of any ring sideroblasts, and dysplasia of either granulocytes or megakaryocytes have been shown to be useful in distinguishing hypoplastic MDS from cases of aplastic anemia ***(Bennett and Orazi, 2009).*** (Table 5)

Table (5): The main morphological abnormalities in bone marrow trephine of MDS cases

Lineage	Bone Marrow
Erythroid	Erythroid hyperplasia Multinuclearity Dyskaryorrehexis Cytoplasmic vaculation Ringed sideroblasts Howell-Jolly bodies
Myeloid	Hypogranularity of myeloid precursors Maturation arrest at myelocyte stage Increased eosinophils and/or basophils Abnormal localization of immature precursors Auer rodes (fusion of granulocytic azurophilic granules)
Megakaryocytes	Micromegakaryocytes Large mono- or binuclear megakoryocytes Megakaryocytes with widely dispersed nuclei Megakaryoblasts

(Hamblin and Killick, 2004).