CORRESPONDENCE

Increased parameters of oxidative stress and its relation to transfusion iron overload in patients with myelodysplastic syndromes

INTRODUCTION

Myelodysplastic syndromes (MDS) are group of stem cell disorders characterised by peripheral cytopaenias and a variable risk of progression to acute myeloid leukaemia. Most patients have anaemia and many develop transfusion dependence and iron overload (IOL), considered to be a negative independent prognostic factor associated with a higher risk of leukemic transformation and shorter survival.[1]

Iron pool is regarded as an important regulator of the production of reactive oxygen species (ROS). The excessive production of ROS and reactive nitrogen species causes lipid peroxidation, which can suppress self-renewal, the number of haematopoietic stem cells and directly induce DNA damage and genomic instability.[2] However, the role of iron-mediated oxidative stress in the physiopathology of MDS remains uncertain.

The present study aimed to evaluate plasma nitrite (NO_2^-) and plasma malonaldehyde, secondary product of lipid peroxidation, in patients with MDS and correlate them with IOL due to transfusion dependence in MDS patients.

MATERIALS AND METHODS

Consecutive adult patients with MDS with and without IOL, followed at the University Hospital of the Federal University of Ceará, Brazil, were enrolled. The study was approved by the local ethics committee (licence 150/2009). They were classified according to the presence or absence of IOL defined as serum ferritin of 1000 ng/ml or greater. The control group consisted of healthy volunteers. Those patients with some conditions known to influence oxidative stress parameters were excluded (pregnancy, alcoholism, smoking, use of vitamins, chronic renal failure and hepatitis). Serum ferritin, serum iron and biochemical parameters, including serum ALT and AST were evaluated. The dosage of malonaldehyde, based on its reaction with thiobarbituric acid, and plasma nitrite (NO_2^-) were assessed according to the method of Green (nitrite reacts with sulfanilamide at low pH). The GraphPrism program (V.5.01) was used for statistical analysis. Significant differences between the three groups were tested by one-way analysis of variance and the t–Student–Newman–

Table–1 Clinical and laboratory characteristics of patients with (MDS), with and without iron overload and control subjects

Variable Demographic	Controls n=45	MDS without IOL n=56	MDS with IOL n=20	p value
Age (years)	74.2±11.7 (56–108)	66.8±15.6 (25–95)	68.6±17.1 (29–91)	0.042[a]
Gender (male/female)	6/39	22/34	9/11	
WHO classification				
Refractory anemia (RA)		8	1	
RA with ringed sideroblasts (RARS)		3	5	
Refractory cytopenia with multilineage dysplasia (RCMD)		38	11	
RA with excess of blasts – I		1	–	
RA with excess of blasts – II		2	2	
Secondary MDS		3	1	
Hypoplastic MDS		1	–	
Karyotype				
Favorable (normal, isolated 5q–, isolated 20q–, or deletion Y)		29	7	
Poor (any chromosome seven anomaly or>=3 aberrations)		1	–	
Intermediate (all other anomalies)		10	4	
Unknown		4	1	
No metaphase		12	8	
IPSS				
Low		14	4	
Intermediate I		20	5	
Intermediate 2		1	1	
High		–	–	
Unknown		18	10	
Secondary chemotherapy		3	–	
Biochemistry				
AST (U/L)	26.6±12.4 (7–66)	29.3±16.4 (10–98)	32.5±22.1 (8–93)	0.377
ALT (U/L)	19.0±6.3 (9–38)	25.7±19.4 (8–122)	39.5±24.5 (4–95)	<0.0001[b,c]
Serum Iron (μg/dL)	75.1±23.8 (22–120)	99.1±43.3 (22–218)	193.0±56.5 (69–296)	<0.0001[a,b,c]
Ferritin (ng/mL)	181.5±111.0 (24.0–418.1)	325.1±225.5 (19.3–850.2)	2,566±1,829 (1,003–7,108)	<0.0001[a,b,c]
Transferrin Saturation – STF *(%)*	33.6±10.0 (10.0–53.0)	38.8±19.9 (4.0–91.0)	80.0±16.1 (36.0–97.0)	<0.0001[b,c]
CLLF (mg/dL)	155.3±52.3 (40.0–283.0)	171.8±87.6 (17.0–429.0)	56.9±50.2 (5.0–168.0)	<0.0001[b,c]
CTLF (mg/dL)	230.5±56.9 (80.0–320.0)	268.4±69.7 (148.0–492.0)	233.2±44.4 (150.0–344.0)	0.0053[a,c]

Continued

Table–1 Continued

Variable Demographic	Controls n=45	MDS without IOL n=56	MDS with IOL n=20	p value
Hematologic features				
Hemoglobin (g/dL)	13.3±0.8 (12.0–14.9)	10.7±2.5 (4.4–16.6)	6.2±1.5 (3.6–8.2)	<0.0001[a,b,c]
Neutrophyl (mm3)	3,898±989 (1,968–5,940)	2,276±1,664 (432–8,235)	2,149±2,018 (120–7,200)	<0.0001[a,b]
Number of cytopenia				
0/1		28	7	
2/3		28	13	
Oxidative stress markers				
MDA (μM)	3.85±1.28 (1.97–7.36)	9.81±3.77 (1.46–17.11)	14.33±1.80 (11.08–18.42)	<0.0001[a,b,c]
Nitrite (nMol)	0.40±0.15 (0.21–0.86)	3.80±1.90 (1.16–8.04)	6.68±2.29 (2.65–9.93)	<0.0001[a,b,c]

[a] p-value for comparison between MDS without IOL vs control group
[b] p-value for comparison between MDS with IOL vs control group
[c] p-value for comparison between MDS with IOL vs MDS without IOL
AST (aspartate aminotransferase), ALT (alanine aminotransferase), STF (transferrin saturation), CLLT (latent capacity iron binding), CTLF (total binding capacity of iron), MDA (malonaldehyde), Nitrite (nitrite), IPSS (International Prognostic Scoring System), IOL (iron overload)

Keuls as a post test or by the Kruskal–Wallis test followed by Dunns test as appropriate. Correlation between variables was tested by Pearson or Spearman test as appropriate, and $p<0.05$ was considered to indicate statistical significance.

RESULTS

Seventy-six patients were enrolled, 20 (26.3%) with and 56 (73.7%) without IOL. The average age was 66.8 years and 68.6 years, respectively. The cntrol group consisted of 45 healthy volunteers with a mean age of 74.2 years. Demographic and laboratory data and WHO classification are shown in table 1.

The haemoglobin level in the MDS group with IOL was significantly lower ($p<0.0001$). The serum iron, transferrin saturation and ferritin levels were significantly higher in patients with IOL ($p<0.0001$) (figure 1).

Iron overloaded patients showed a significant increase in plasma nitrite and malonaldehyde when compared to other groups ($p<0.0001$ for both variables) (figure 2). The malonaldehyde level was directly correlated with the ferritin level ($r=0.3749$, $p=0.0008$). A correlation was also observed between the nitrite level and ferritin ($r=0.4853$, $p<0.0001$) (figure 3). The relationship between malonaldehyde and nitrite showed a linear association ($r=0.8260$, $p<0.0001$). Serum ALT in the MDS group with IOL was significantly higher than the two other groups ($p<0.0001$) and was directly correlated with serum ferritin ($r=0.3193$, $p=0.0049$).

DISCUSSION

MDS are a group of diseases of old age. Elderly patients are especially vulnerable to anaemia-related comorbidities and to iron-mediated toxicity secondary to transfusion dependence. Increased free iron and oxidative stress induce genotoxic effects on mitochondrial and nuclear DNA that can lead to genomic instability, clonal evolution, disease progression and an increased risk of leukaemic transformation.[3]

In the present study the evaluation of nitrite and malonaldehyde showed a significant increase in patients with MDS and IOL compared to those without IOL and the control group, and both parameters were positively correlated with the ferritin level. These findings are consistent with previous studies suggesting a relationship between IOL and the increased production of ROS in MDS.[4] Although current understanding of these effects in MDS is still waiting for more evidence, it is consensual that IOL should be monitored and managed in selected patients based on risk stratification, life expectancy, transfusion history, iron burden and ongoing transfusion requirement.

A significant decrease in mean levels of ROS and membrane lipid peroxidation was reported during therapy with deferasirox.[5 6] Effective chelation may also improve haematological parameters and transfusion requirements in a selected group of patients.[7] Therefore, it is possible that iron chelation may have additional benefits in MDS.[8] The mechanisms are unclear and are probably multifactorial, and include, at least partly, decreasing production of ROS and detoxification of redox-active species.

It is expected that in the near future levels of free iron species in parallel with oxidative stress parameters will all be used to diagnose and monitor IOL in MDS.

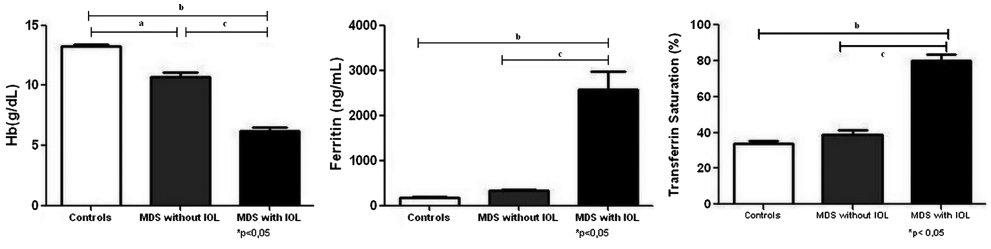

Figure 1 Haemoglobin and markers of iron status (ferritin and transferrin saturation) of patients with MDS, with and without IOL (n=20.56) and control subjects (n=45). IOL, iron overload; MDS, myelodysplastic syndrome.

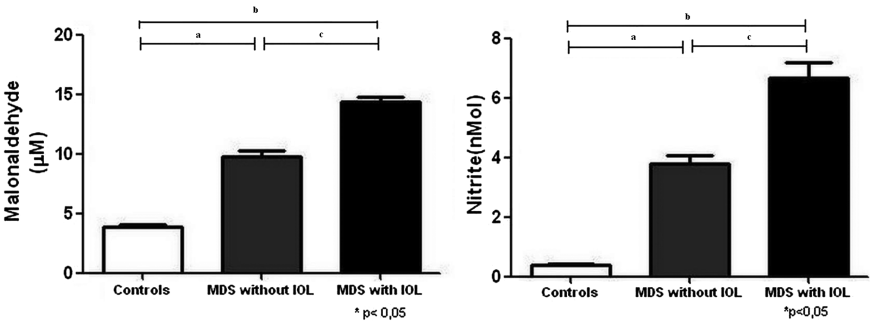

Figure 2 Oxidative parameters concentration of malonaldehyde and nitrite in individuals with MDS, with and without IOL (n=20.56) and control subjects (n=45). IOL, iron overload; MDS, myelodysplastic syndrome.

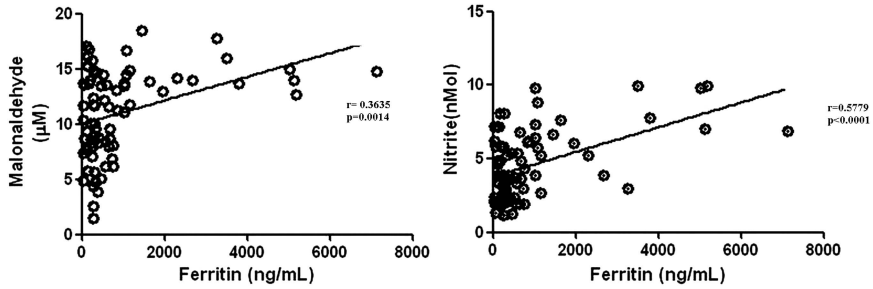

Figure 3 Dispersion graphic between ferritin and markers oxidative stress (malonaldehyde, nitrite) in patients with MDS. MDS, myelodysplastic syndrome.

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Contributors The following are employees of the laboratory of the Cancer Cytogenetics and Cytogenomics: GFdS, RFP, SMMM. The following are employees of the Department of Clinical and Toxicological Analyses: RPG, TMdJP, MCB, TEdJS. RMdF is a collaborator of the Department of Pharmacy. GFdS has a collection of biological samples from study participants after informed consent, conducted biochemical analyses and wrote the work; she is the guarantor. RFP, SMMM and MMRAM carried out the diagnosis of patients with MDS, interpreted the data and reviewed the manuscript. RPG, TMdJPC and RMdF were responsible for standardisation of techniques of oxidative stress, interpretation of data and reviewed the manuscript. MCB and TEJdS executed measurements of oxidative stress in all participants of the study and conducted statistical analysis.

Competing interests None.

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