Dysfunctional lamins as mediators of oxidative stress in Emery-Dreifuss muscular dystrophy

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Abstract

Deficit of lamin A/C or emerin causes genetically transmitted Emery-Dreifuss muscular dystrophy (EDMD). As lamins are considered to be mediators of oxidative stress, the antioxidant/oxidant status was examined. The total oxidant/ antioxidant status in serum was examined in 29 cases of Emery-Dreifuss muscular dystrophy. The study included 12 autosomal-dominant laminopathies (AD-EDMD), 17 X-linked emerinopathies (X-EDMD) and 20 age-matched normal subjects. Total oxidant status (TOS) was reduced in all cases, and the total antioxidant capacity (TAC) was found to be decreased in the majority of the patients (in 82.8%). A relationship between TOS level and disease progression was noted. No correlation between TOS/TAC level and cardiological or neurological parameters was detected.
The results of the study indicate disturbances of redox balance in EDMD patients. Determination of TOS/TAC might help to assess the progress of the disease and the potential effectiveness of antioxidant therapy.

Key words: lamin deficiency, oxidative stress, Emery-Dreifuss muscular dystrophy.

Introduction

A deficit of lamins A/C or emerin in skeletal muscles and the heart causes a rare, genetically transmitted disease known as Emery-Dreifuss muscular dystrophy (EDMD). The cell defect is generalized, but skeletal muscles, heart and joints are all selectively affected, with the ultimate effects being skeletal muscle atrophy, joint contractures and dilated cardiomyopathy, which is at the onset either clinically silent or preceded by conduction block and arrhythmias [13,14]. Sudden death both in patients and in carriers is not a rare event.

Lamins serve as mediators of oxidative stress [20]. There is a relationship between lamin mutations and altered metabolism of radical oxygen species (ROS). Laminopathies are correlated with oxidative stress at the cellular level. No data have hitherto been available on antioxidant/oxidant status in EDMD.

The aim of the study described here was therefore to assess serum for total oxidant status (TOS), which represents the oxidative/antioxidative balance. The antioxidant capacity (TAC) measures the total antioxidant capacity of biomolecules. The relevant

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data obtained were compared to the clinical status of the EDMD patients.

Material and methods

A total of 29 patients with EDMD were examined. Twelve of these had autosomal-dominant EDMD associated with laminopathy (AD-EDMD), while 17 had X-linked EDMD (X-EDMD) associated with emerinopathy. The diagnoses of EDMD were confirmed clinically, by indirect immunofluorescence analysis of muscle nuclei, immunochemically by Western blotting examination of a muscle biopsy specimen, as previously described [5,15], and by molecular testing of *LMNA* and *EMD* genes, respectively (Tables I and II). Dilated cardiomyopathy was present in all the cases. However, no subjective cardiac symptoms, even when there was evident bradycardia, were present. In some patients cardiac involvement was already detected at the time of the first neurological examination. In AD-EDMD patients the left ventricle

was affected. There were also disturbances of contractility or conductivity (Table I). Dilated cardiomyopathy was a feature common to all studied cases detected already at the first examination. Cardiac symptoms appeared earlier in the AD-EDMD than in the X-EDMD group. In the latter cardiac involvement was moderate/mild overall, to severe/very severe in some cases (Table II). With disease onset, conductance and atrial involvement predominate, necessitating implantation of a pacemaker in the majority of patients. In the follow-up clinical examination dilated cardiomyopathy was found to develop later in X-EDMD than AD-EDMD sufferers.

The control group consisted of 20 healthy age-matched subjects with no history of muscle or cardiac disease.

Blood was selected for routine biochemical analyses and centrifuged at 3000 rpm for 10 minutes. Serum was separated and frozen at –30°C until used, usually up to 30 days both in patients and controls.

Table I. Clinical and laboratory data in individual autosomal-dominant Emery-Dreifuss muscular dystrophy (AD-EDMD) patients

No., Sex	Family history	Mutation	LAD (mm)	LVDD (mm)	EF (%)	Type of arrhythmia	Cardiac device (age)	Overall course of the disease	Muscle atrophy	C/SR	TAC (mM/l)	TOS (µM/l)
1M	S	c.1357C>T	31	42	52	SVEB, SVT, VEB, AVB1	ICD (36)	Severe	Arms, thighs	+/+	69	2.0
2M	F	c.1357C>T	44	63	36	AVB 1,2,3, atrial standstill, nsVT	Pacemaker (35) ICD (44)	Severe	Arms, thighs	+/+	55	6.2
3F	F	1357C>T	25	43	61	SVEB, SVT	ICD (21)	Moderate	Generalized	+/+	12	9.8
4F	S	c.1337A>T	26	35	61	SVEB	No	Severe	Generalized	+/+	161	3.1
5M	F	c.1355T>C	29	40	50	No arrhythmia	No	Mild	Arms, thighs	+/+	71	6.4
6M	F	c.1355T>C	43	51	45	AVB II 2:1, VEB	Pacemaker (31)	Mild	Arms	+/+	31	9.95
7F	F	c.1355T>C	32	40	64	No arrhythmia	No	Mild	No	+/+	93	3.7
8F	S	c.1072G>A	37	54	44	AVB ½/3, PAF	Pacemaker (25)	Severe	Generalized	+/+	96	3.1
9M	F	c.743T>C	30	51	55	No arrhythmia	No	Moderate	Generalized	+/–	217	12.4
10F	F	c.1072G>A	30	46	68	SVEB, VPB, AVB1	No	Moderate	Generalized	+/+	112	4.7
11F	F	c.334-336del	35	47	60	AVB1, PAF, SVEB, SVT, tachy-brady, nsVT	Pacemaker (20) ICD (28)	Severe	Generalized	+/+	32	3.95
12M	F	c.743T>C	No data			No arrhythmia	No	Mild	Arms, thighs	+/+	217	12.8

C/SR – contractures/spine rigidity, SVT – supraventricular tachycardia, SVEB – supraventricular ectopic beats, AVB – atrio-ventricular block, ICD – implanted cardioverter/defibrillator, PAF – paroxysmal atrial fibrillation, VPB – ventricular premature beats, VEB – ventricular ectopic beats, LAD (left atrium diameter) – N: 19-40 mm, LVDD (left ventricle end diastolic diameter) – N: 35-57 mm, EF (ejection fraction) – N: 60-65%

Total oxidant status was determined using a Real Assay Diagnostics kit 4. The total antioxidant capacity was determined using an OxiSelect kit (Cell Biolabs, Inc.) [8]. Routine hematoxylin-eosin staining, anti-lamin and anti-emerin staining and Western blotting muscle biopsy with anti-lamin and anti-emerin antibodies [16] were also performed.

Statistical analysis

Analyses were performed using the commercial statistical package Statistica ver. 9.0. In line with the non-normal data distribution the medians and interquartile ranges are presented. The Kruskal-Wallis analysis of variance was applied, followed by the Mann-Whitney *U* test for a two-group comparison. The relationships among variables were analyzed by Spearman's correlation coefficients. Analysis of the receiver-operating characteristic (ROC) curves was also performed. The probability value of $p < 0.05$ was regarded as statistically significant.

Results

Levels for serum TOS and total TAC reflect the redox balance between antioxidation and oxidation in EDMD. Total antioxidant capacity values in serum were significantly below those of the control in the cases of both EDMD groups, being at the level of 83.3% of patients in the case of AD-EDMD and 82.4% of patients in the case of X-EDMD (Table III). Slightly decreased values were present in both groups in 16.7 and 17.6% of the patients, respectively (appearing especially in mildly progressing cases). The values of TOS were also reduced in the EDMD patients (Table III). The ROC analysis demonstrated that the area under the curve (AUC) value of serum TAC equals 0.890, while the AUC value for TOS reached 1.000 (Fig. 1). No correlation was observed between TAC, TOS, and the cardiological or neurological parameters. A relationship between TOS level and disease progression was observed (Table IV, $p = 0.032$).

Table II. Clinical and laboratory data in individual X-linked Emery-Dreifuss muscular dystrophy (X-EDMD) patients

No., Sex	Family history	Mutation	LAD (mm)	LVDD (mm)	EF (%)	Type of arrhythmia	Pacemaker (age)	Overall course of the disease	Muscle atrophy	C/SR	TAC (mM/l)	TOS (µM/l)
1M	F	c.1A>G	30	45	69	AVB1	No	Moderate	Arms, calves	+/+	29	0.6
2M	F	c.1A>G	32	46	54	SVEB	No	Mild	Arms, calves	+/+	20	5.95
3M	S	c.1A>G	42	57	58	FA, AVB	Yes (25)	Moderate	Arms, calves	+/+	8	1.7
4M	S	c.256C>T	40	62	40	AVB3, VEB	Yes (20)	Severe	Arms, thighs	+/+	15	2.5
5M	F	c.1A>G	30	47	55	SVEB, SVT	No	Severe	Generalized	+/+	44	7.2
6M	F	c.153delC	38	50	56	SVEB AF, SAB,	Yes (25)	Moderate	Arms	+/+	83	2.96
7M	F	c.153delC	31	51	52	AF, AVB3	Yes (31)	Moderate	Arms	+/+	62	7.4
8M	F	c.153delC	37	56	45	tachy-brady	Yes (28)	Moderate	No atrophy	−/+	302	6.2
9M	S	c.153delC	No data			PAF	Yes (19)	Moderate	Generalized	+/+	12	1.4
10M	F	c.153delC	34	52	68	AVB 2/3, AF	Yes (21)	Moderate	Generalized	+/+	19	8.8
11M	F	c.187+1G>A	27	44	No data	AVB3	Yes (15)	Moderate	Arms, calves	+/+	57	0.96
12M	F	c.187+1G>A	43	53	50	atrial standstill	Yes (35)	Mild	Arms	+/−	221	7.1
13M	F	c.266-27del18	No data	46	76	SVEB, SVT	No	Moderate	Arms, calves	+/+	334	1.4
14M	F	c.399+1G>C	35	53	65	tachy-brady	Yes (16)	Moderate	Arms, calves	+/+	18	9.4
15M	F	c.397C>T	33	49	54	AVB 1/2/3, AFL	Yes (25)	Moderate	Arms, calves	+/+	161	2.6
16M	F	c.3G>A	34	45	65	AVB 1/2/3 PAF	Yes (30)	Mild	Arms	−/+	19	2.4
17M	F	c.450-451insG	34	57	56	AVB1, VEB, SAB	Yes (18)	Severe	Generalized	+/+	71	5.5

C/SR – contractures/spine rigidity, AF – atrial fibrillation, AFL – atrial flutter, SVT – supraventricular tachycardia, SVEB – supraventricular ectopic beats, AVB – atrio-ventricular block, PAF – paroxysmal atrial fibrillation, VEB – ventricular ectopic beats, LAD (left atrium diameter) – N: 19-40 mm, LVDD (left ventricle end diastolic diameter) – N: 35-57 mm, EF (ejection fraction) – N: 60-65%.

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Table III. Total antioxidant capacity and total oxidant status in Emery-Dreifuss muscular dystrophy (EDMD) and controls

Parameters	Controls (*n* = 20)	EDMD (*n* = 29)	AD-EDMD (*n* = 12)	X-EDMD (*n* = 17)
TAC (mM/l)	232 (204-236)	62 (20-112) ([a]*p* < 0.00001)	82 (43-136) ([a]*p* = 0.00001)	44 (19-83) ([a]*p* < 0.0001, [b]*p* = 0.227)
TOS (µM/l)	57 (35-73)	4.7 (2.5-7.2) ([a]*p* < 0.00001)	5.5 (3.4-9.9) ([a]*p* < 0.00001)	3.0 (1.7-7.1) ([a]*p* < 0.00001, [b]*p* = 0.080)

Values are medians and interquartile ranges.
p values for [a]EDMD groups vs. controls, [b]AD- vs. X-EDMD

Table IV. Total antioxidant capacity, total oxidant status and progression of Emery-Dreifuss muscular dystrophy (EDMD)

Parameters	Controls (*n* = 20)	EDMD (*n* = 29)	Mild/moderate (*n* = 21)	Severe (*n* = 8)
TAC (mM/l)	232 (204-236)	62 (20-112)	62 (19-112)	63 (44-129) ([c]*p* = 0.582)
TOS (µM/l)	57 (35-73)	4.7 (2.5-7.2)	6.2 (3.0-8.8)	2.8 (1.7-3.5) ([c]*p* = 0.032)

Values are medians and interquartile ranges.
p values compared progress of [c]severe vs. mild/moderate
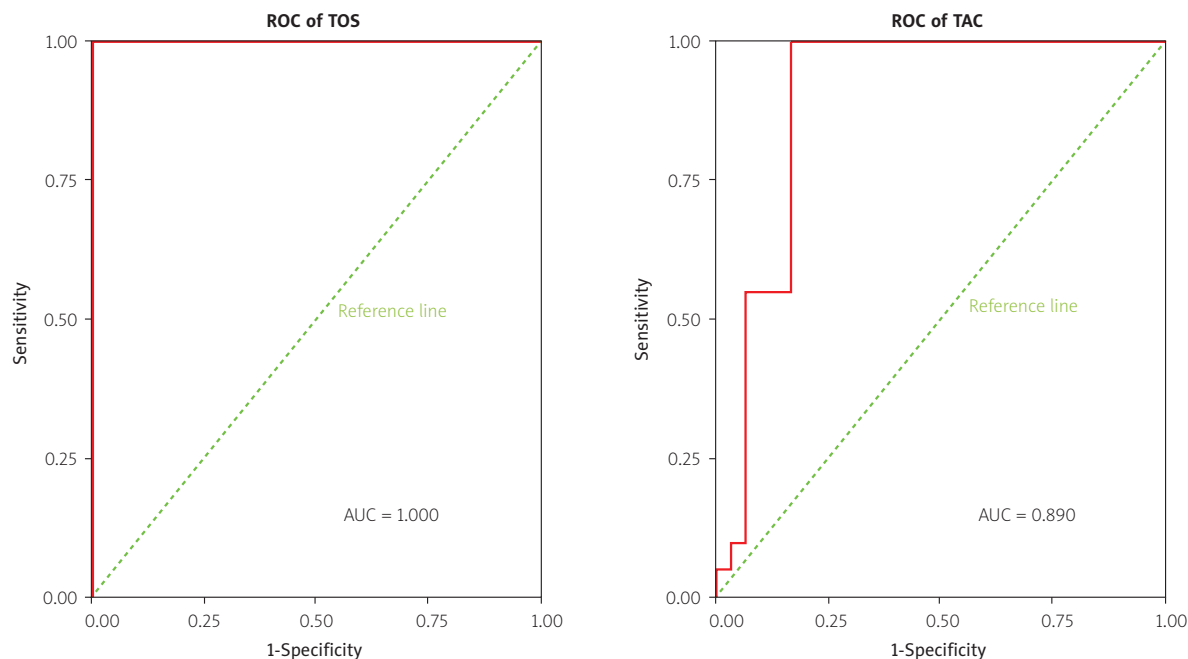
Fig. 1. Receiver-operating characteristic (ROC) curves for total oxidant status (TOS) and total antioxidant capacity (TAC) in Emery-Dreifuss muscular dystrophy patients.

Discussion

Free radicals are responsible for chemical modifications of and molecular changes in proteins, lipids, carbohydrates and nucleotides, as well as for modulation of gene expression. In biological membranes they induce changes in enzymes, and other proteins, as well as in the oxidation of –SH groups, the peroxidation of lipids, and the oxidation of polyunsaturated fatty acids. They also affect membrane transport systems. Accumulation of ROS can lead to DNA damage and the production of oxidized proteins [16,18]. Free radicals are also produced in different physiological processes under normal conditions. ROS action is typically limited by physiologically active antioxidant defense mechanisms [22]. Where the production of oxidative damaged molecules is enhanced,

this is followed by an impairment of defense that leads to the accumulation of toxic products. Several chemical compounds are able to act as antioxidants; the impact is greater when the antioxidants are acting in a set [8,21,22] and when glutathione, the most essential key agent in cell defense, is included [1,24].

Lamina provides mechanical support for the nucleus [9], modulates gene expression [2,11,17], modulates the oxidative stress response [12], acts in intracellular redox homeostasis [20], and participates in transcription and chromatin organization [2] and DNA repair [4,6,7]. Laminopathies share features including defects in the DNA damage response [4]. In emerinopathies, apart from the deficit of emerin, a decrease of lamins is also noted [16].

A relationship between *LMNA* mutation and altered ROS metabolism has already been proposed [19]. ROS have a direct damaging effect, affecting the lamin structure [16], and giving rise to persistent DNA damage [7] and telomere shortening [6]. In laminopathic cells during prelamin A accumulation ROS levels are elevated [3,10,15]. Persistent prelamin accumulation and lamin A/C depletion are likewise associated with elevated ROS levels [20].

Details of redox biology in EDMD are not known, yet. When established it is likely to be helpful as regards both the introduction of early stage pharmacological treatment and decision-making in respect of cardiac device implantations and consideration even of heart transplantation. Thus far, the only TAC member assessed in EDMD has been blood glutathione, which is present in reduced amounts in EDMD with *LMNA* mutation, and is associated with early cardiac involvement [4]. More details of redox biology in EDMD may serve as a basis for prognoses regarding progression of the disease, as well as in monitoring for the effectiveness of an antioxidant therapy. Such therapy would be useful in restoring the balance between the production of free radicals and the antioxidant capacity, with resultant reduced severity of the disease.

The results presented here for TOS and TAC levels support the opinion that oxidative stress is involved in the pathogenesis of EDMD. This finding could lead to new directions of future therapeutic intervention.

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Disclosure

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