Proteomics of Red Blood Cell from Patients with Obstructive Sleep Apnea

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INTRODUCTION

Obstructive sleep apnea (OSA) is a common public health concern causing metabolic and cardiovascular consequences. Although OSA is a systemic disease, the molecular mechanisms and specific genes/proteins associated with such processes remain poorly defined.

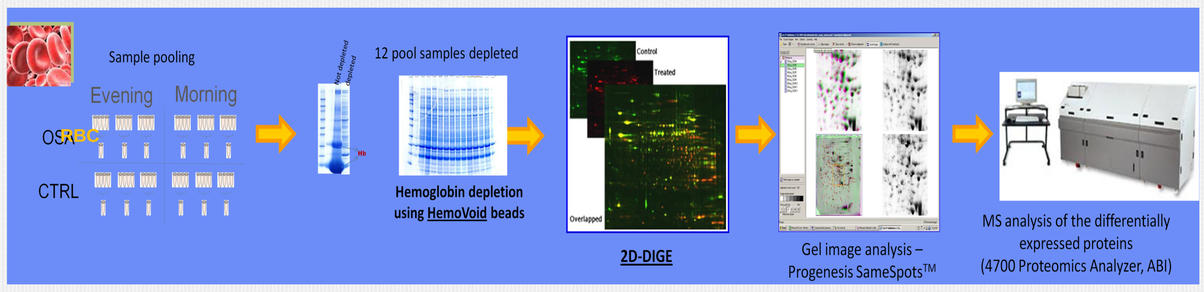
AIMS

To identify dysregulated proteins that could be useful as candidate biomarkers of diagnosis/prognosis of OSA.

METHODS

Red blood cells (RBC) were collected from peripheral blood of patients with moderate/severe OSA (n=12) and snorers (n=12) at pre-(evening) and post-(morning) sleep study so that proteome variations between these time points could be assessed. RBC samples were randomly pooled (n=4/pool) to constitute 3 biological replicates per group of patients and condition. The cytoplasm soluble fraction of RBC was prepared and depleted of hemoglobin using Hemovoid™ system and then was analyzed by 2-D Fluorescence Difference Gel Electrophoresis (2-DIGE) and mass spectrometry (MS). The images were acquired in an Imager and analyzed by Progenesis SameSpots software (NonLinear version 4.5).

Proteomic Workflow



RESULTS I

Demographic, polysomnographic and analytical characterization of study sample

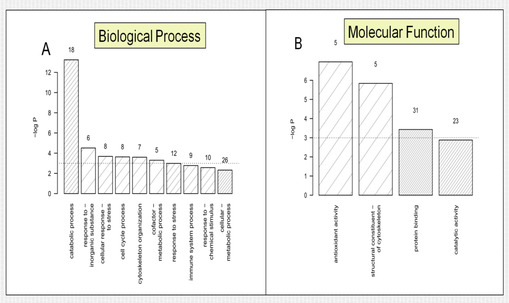
Demographic and PSG parameters	Screened Subjects		
	Control (n=12)	OSA (n=12)	p value
Age (years)	46.8 (10.0)	45.8 (7.2)	NS
Habits			
Current Smoking (%)	50%	33.3%	NS
Current Drinking (%)	58.3%	50%	NS
EPW Score	9.1 (4.3)	9.1 (4.3)	NS
Observational features			
Morning arterial pressure (mmHg)	141.3(23.1)/ 88.8 (14.6)	129.8(13.1)/78.9 (8.3)	NS
BMI (kg/m^2)	26.9 (2.5)	30.8 (1.9)	**0.003***
Abdominal perimeter (cm)	97.5 (6.9)	107.4 (8.6)	NS
Comorbidities			
Hypertension (%)	41.7%	33.3%	NS
Respiratory Diseases (%)	16.7	8.3%	NS
Dyslipidemia (%)	66.7%	83.3%	NS
Polysomnographic parameters			
RDI (events/hour)	2.7 (1.5)	41.9 (20.5)	**3.6E-5***
Arousal Index (events/hour)	12.6 (4.8)	34.6 (18.4)	**0.001***
Minimum Arterial Saturation (%)	88.2 (3.4)	80.5 (7.6)	**0.01***
T90 (%)	0.1 (0.2)	19.4 (24.6)	**0.02***
ODI (events/hour)	1.9 (1.4)	36.1 (24.7)	**0.0005***
Sleep Efficiency (%)	76.7 (14.1)	82.1 (6.8)	NS

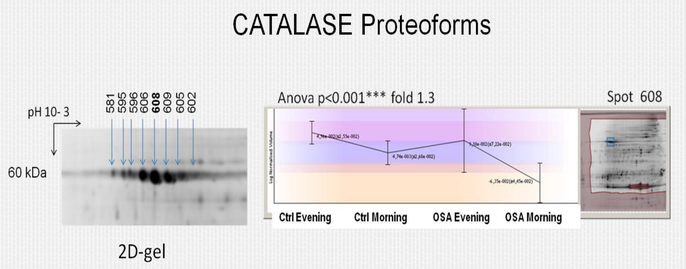
Values displayed in mean +/- SD

Analytical parameters	Screened Subjects		
	Control (n=12)	OSA (n=12)	p value
Glycemic Profile			
Glucose (70-110 mg/dl)	95.2 (8.5)	96.7 (12.0)	NS
Hb A1C (4-6%)	5.7 (0.4)	5.8 (0.4)	NS
Insulin (3-25 mU/L)	11.0 (6.2)	20.6 (12.5)	**0.04***
HOMA-IR (≥3.8)	2.6 (1.6)	5.0 (3.0)	**0.04***
Lipid Profile			
Cholesterol (< 190 mg/dl)	206.2 (44.3)	193 (30.8)	NS
Triglycerides (<150 mg/dl)	127.3 (69.7)	217.1 (127.7)	NS
Cardiovascular Marker			
Homocysteine (3.7-13.9 µmol/L)	15.6 (4.3)	15.1 (2.9)	NS
Urinary Catecholamines			
Adrenalin (1.7-22.4 µg/24 h)	14.97 (15.5)	10.8 (9.3)	NS
Nor-Adrenalin (12.1-85.5 µg/24 h)	73.5 (34.0)	74.98 (37.4)	NS
Dopamine (0-498 µg/24 h)	389.4 (218.4)	367.3 (169.5)	NS

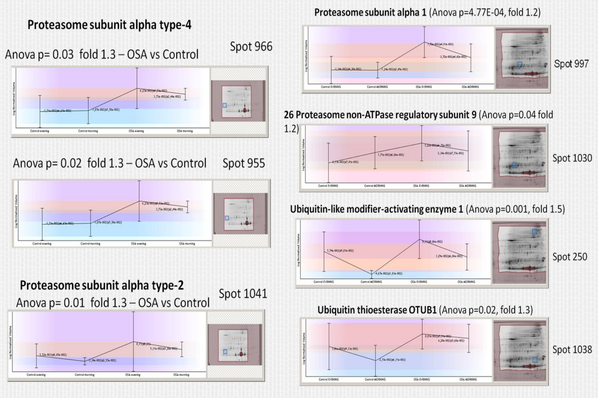
RESULTS II

One hundred sixty five protein spots were observed differentially expressed (ANOVA p<0.05) in RBC between OSA and Controls at Evening and/or Morning (pre- & post-sleep study). 70 spots of these were identified with confidence by MS (Matrix-assisted laser desorption/ionization time-of-flight - MALDI-TOF/TOF). Functional analysis by DAVID Bioinformatics Resources concerning biological process (fig A), molecular function (fig B) and cellular component indicated that these proteins were mainly cytosolic and associated with antioxidant activity and cellular response to stress. Proteoforms of catalase and peroxiredoxins-2, subunits of proteasome, ubiquitin proteins, Hsp-70, aldolase-A and nucleoside diphosphate kinases 1 (NDK) were some of these modulated proteins in OSA (see figures).

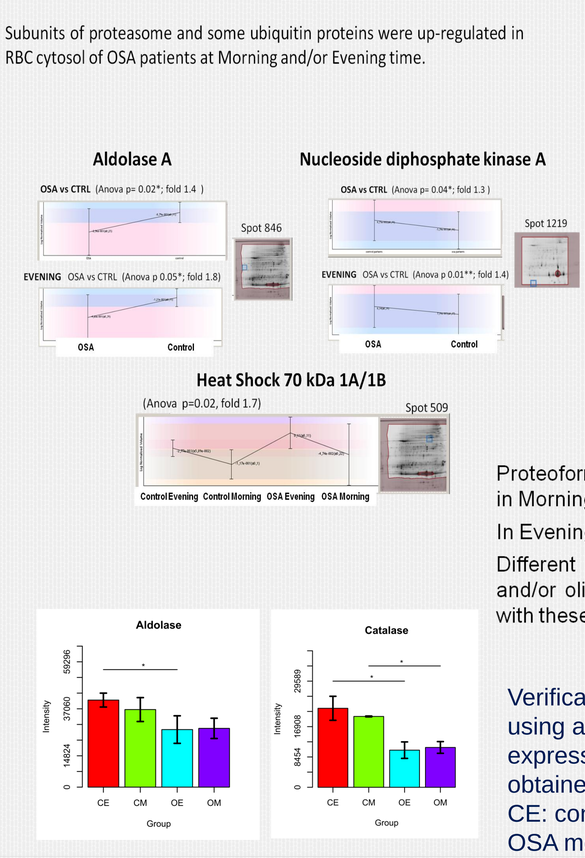




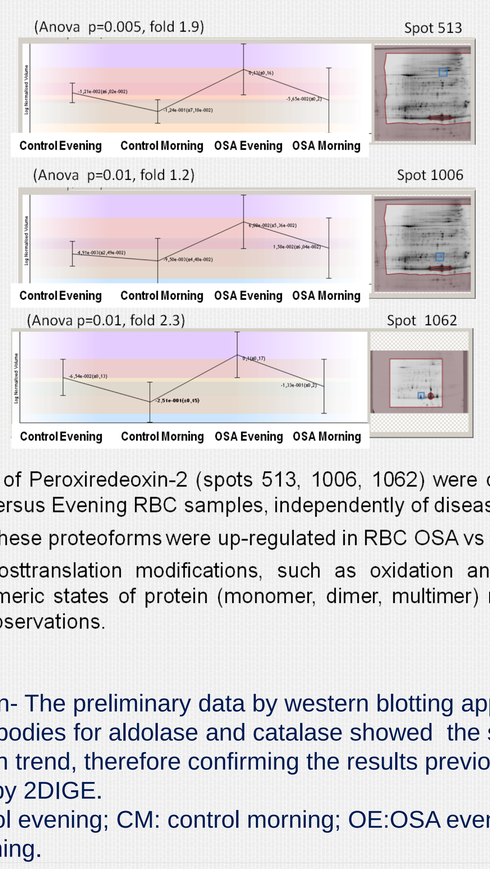
- Eight CATALASE proteoforms were identified. All of them, except spots 581 and 595 - the most basics in 2D-gel, were significantly down regulated in RBC Morning versus RBC Evening, independently of disease/health state.
- However, the proteoform 608 was significantly down regulated (ANOVA p < 0.001, fold 1.3) in OSA RBC Morning samples in comparison with Controls RBC Morning samples. Although not significant, the proteoforms 596, 606, 609, 605, 602, also were more down regulated in OSA RBC Morning in comparison with Control RBC Morning samples.



Subunits of proteasome and some ubiquitin proteins were up-regulated in RBC cytosol of OSA patients at Morning and/or Evening time.



Peroxiredoxin-2 Proteoforms



Proteoforms of Peroxiredeoxin-2 (spots 513, 1006, 1062) were down regulated in Morning versus Evening RBC samples, independently of disease/health state.

In Evening, these proteoforms were up-regulated in RBC OSA vs Controls.

Different posttranslation modifications, such as oxidation and nitrosylation, and/or oligomeric states of protein (monomer, dimer, multimer) may be related with these observations.

Verification- The preliminary data by western blotting approach using antibodies for aldolase and catalase showed the same expression trend, therefore confirming the results previously obtained by 2DIGE.
CE: control evening; CM: control morning; OE:OSA evening; OM: OSA morning.

CONCLUSION

- Antioxidant protective function of RBC, involving proteins with circadian rhythms behavior (catalase, peroxiredoxins-2), was dysregulated in OSA patients (specially at evening), probably in response to a severe oxidative stress and sleep disturbance. .
- Proteasome system seemed activated in OSA RBC, suggesting proteostasis imbalance in these cells.
- NDK, critical in erythroid development, Aldo-A, that is increased in miopathies and Alzheimer, and Hsp70 associated with stress were also found modulated in OSA RBC (evening).
- The complete identification and validation of these proteins will provide better understanding of OSA pathology that ultimately can be translated into newly effective diagnosis/prognosis tools.

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DIGE images were obtained in ITQB. This work was approved by the Ethical Committee of INSA.I.P.-Lisboa, Centro Hospitalar Lisboa-Norte., Faculdade de Ciências Médicas da Universidade Nova de Lisboa and Comissão Nacional de Proteção de Dados, Portugal.