Obstructive sleep apneia:
New insights into antioxidant activity and cellular response to stress

Fátima Vaz[1], Amélia Feliciano[1,2,3], Ana Rita Silva[1],Cristina Coelho, [1] Vukosava Millic Torres[1] Vesna Bozanic[1], Atul Malhotra[4],Paula Pinto[2,5], Cristina Bárbara[2,5], Deborah Penque[1]

[1]Laboratório de Proteómica, Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, [2]Serviço de Pneumologia, Centro Hospitalar Lisboa Norte, [3]Faculdade de Ciências Médicas da Universidade Nova de Lisboa, [4]Brigham and Women's Hospital, Harvard Medical School, Boston, USA , [5]Faculdade de Medicina da Universidade de Lisboa, Portugal

INTRODUCTION

Obstructive sleep apnea (OSA) is a sleep¬related breathing disorder characterized by recurrent episodes of apnea/and hypopnea during sleep with resultant intermittent hypoxia and sleep fragmentation, leading to cardiometabolic diseases. OSA affects 3.7% to 26% of adult population, but frequently is underdiagnosed. By 2DIGE-proteomics approach, we have investigated red blood cells (RBC) in OSA to uncover new insights into putative chronic stress-induced RBC dysfunction that lead to inflammation and metabolic syndrome associated with OSA. A number of proteins as potential candidate biomarkers for OSA, such as the cytosolic antioxidant regulators, catalase (CAT) and peroxiredoxin2 (PRX2) was identified, needing additional biochemical research validation.

AIMS

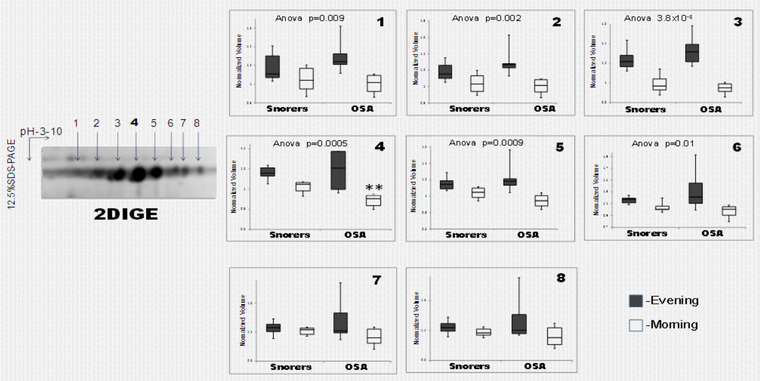
Validate potential proteomic candidate biomarkers that we have identified in OSA RBC and may be associated with OSA and OSA associated cardiometabolic disorders.

METHODS

The biochemical validation of 2DEbased proteomic results was performed by WesternBlot (WB) analysis or Enzymatic Assay for catalase (CAT) and peroxirdeoxin 2 (PRX-2) on nondepleted RBC cytoplasmic fractions prepared from blood samples collected from Snorer subjects (n=10) and OSA (n=10) patients before and after underwent six month of CPAP treatment. Samples (n=50) were individually separated in duplicates on 4-12% SDS-PAGE mini gels. A non-reduced SDS-PAGE were used to analysed PRX-2. A Kinetic Enzymatic Assay was determined for CAT activity on these RBC lysates.

RESULTS I
Catalase Validation

A



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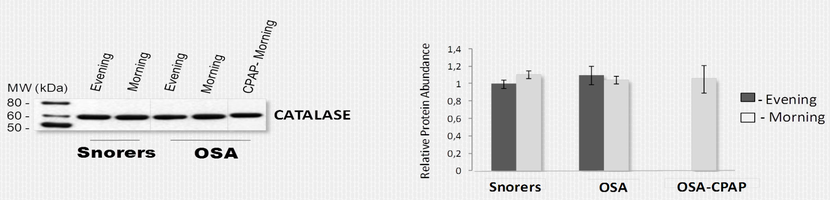

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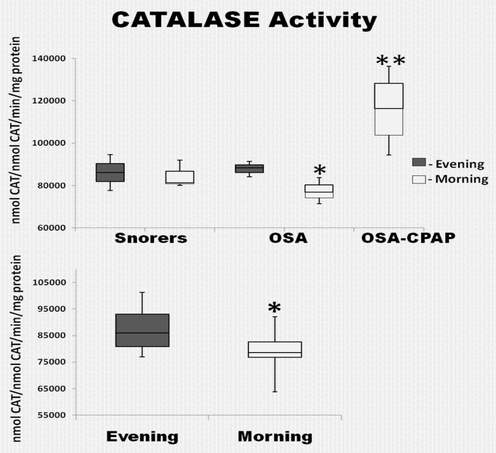


Figura 1

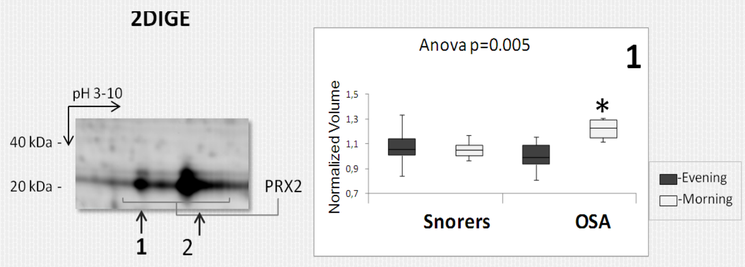
A – 2DIGE showing 8 catalase (CAT) proteoforms identified in OSA and Snorer RBC samples collected at Evening or Morning time. The 6 most acidic CAT proteoforms (1-6) were identified differentially abundant in OSA (Anova p≤ 0.05). The abundance level of the most prominent CAT proteoform (4) on the gel was significantly reduced (Anova, p≤ 0.01) in OSA Morning RBC compared with Snores ones.

B - Western blot and the respective histogram showing that there were no significant changes in the CAT relative abundance whatever patients/conditions were analyzed.

C- Catalase Kinetic Activity (nmol Catalase/minute/mg of protein) were measured in RBC total cytosolic fraction of each patient/condition groups. The upper histogram representation shows that CAT activity was significantly lower (Anova p≤ 0.05) in OSA Morning RBC samples in comparison with both Snores Morning and OSA Evening samples. In contrast, CAT activity was significant higher (Anova p≤ 0.001) in OSA patients after underwent six month of CPAP treatment in comparison with both Snorers and non-treated OSA patients. The lowest histogram representation shows that CAT activity was significantly lower (Anova, p≤ 0.05) in Morning RBC samples regardless of patient conditions.

RESULTS II
Peroxiredoxin Validation

A



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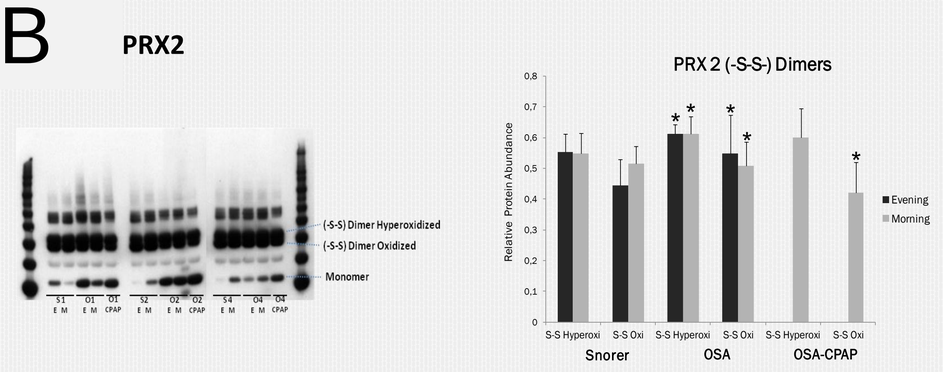

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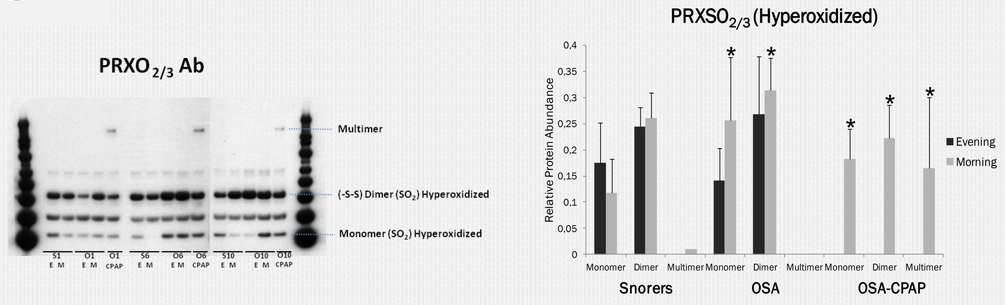

Figura 2

A – 2DIGE showing two peroxiredoxin-2 (PRX-2) proteoforms indentified on OSA and Snorer RBC samples collected at Evening or Morning time. The acidic PRX 2 proteoform (1) was identified significantly higher abundant in OSA Morning samples compared with Snorer's ones. No significant differences were observed among conditions for basic PRX-2 proteoform at 20 kDa (2).

B and **C - Western Blots** (non-reduced SDS-PAGE) showing representative immunoblots for PRX-2 and PRX-$O_{2/3}$ (Hyperoxidized forms), respectively, identified in three OSA/Snores patients as example.

HMW Dimeric (S-S) forms (hyperoxidized) and LMW (-S-S-) forms (Oxidized) of PRX 2 were highly increased in OSA RBC collected at Evening/Morning compared with Snorer's ones. After CPAP treatment at least LMW (-S-S-) oxidized dimeric forms of PRX-2 were significantly reduced (**Histogram B**).

By using a specific antibody for ($SO_{2/3}$) hyperoxidized forms of PRX, we observed that both monomeric and dimeric forms of PRX2 were highly hyperoxidized in OSA Morning samples compared with Snorer's ones (**Histogram C**). CPAP treatment decreased significantly hyperoxidized monomeric and dimeric forms of PRX2. Multimeric hyperoxidized forms were observed almost exclusively after CPAP treatment, which may correspond to CPAP-induced chaperone activity on PRX2.

CONCLUSION

Our data indicate that in OSA RBC the activity and redox/oligomeric state events of CAT and PRX2, respectively showed dysregulated maybe in consequence of OSA-induced chronic oxidative stress.

CPAP treatment was effective to partially recover OSA-induced CAT and PRX2 dysregulation by probably improving patient's antioxidant capacity.

We propose CAT and PRX2 as a promising biomarker for monitoring OSA severity and/or CPAP treatment effectiveness.

Work partially supported by Harvard Medical School-Portugal Program (HMSP-ICJ/0022/2011), FCT/Poly-Annual Funding Program and FEDER/Saúde XXI Program (Portugal).

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.