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 undiagnosed. By 2DGE-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 peroxiredoxin 2 (PRX2) was identified, needing additional biochemical research validation.

RESULTS I

Catalase Validation

A - 2DGE 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 proteofrm (4) on the gel was significantly reduced (Anova, p ≤ 0.05) in OSA Morning RBC compared with Snorers 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/measuring 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 Snorers Morning and OSA Evening samples. In contrast, CAT activity was significant higher (Anova, p ≤ 0.01) 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 - 2DGE showing two peroxiredoxin-2 (PRX-2) proteoforms identified on OSA and Snorer RBC samples collected at Evening or Morning time. The acidic PRX-2 proteofrm (1) was identified significantly higher abundant in OSA Morning samples compared with Snorers ones. No significant differences were observed among conditions for basic PRX-2 proteofrm at 20 kDa (2).

B and C - Western Blots (non-reduced SDS-PAGE) showing representative immunoblot for PRX-2 and PRX-2H (Hyperoxidized forms), respectively, identified in three OSA/Snorers patients as example.

METHODS

The biochemical validation of 2D-based proteomic results was performed by Western Blot (WB) analysis or Enzymatic Assay for catalase (CAT) and peroxiredoxin 2 (PRX-2) on nondepleted RBC cytoplasmic fractions prepared from blood samples collected from Snorers 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.

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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.