Glycosylated Hemoglobin and the Severity of Sleep Obstructive Apnea

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This research revealed a strong relation between apnea- hypopnea index (AHI), average blood oxygen saturation ($avSpO_2$) measured with the pulse oximeter, oxygen desaturation index (ODI) and glycosylated hemoglobin (HbA1c) in obstructive sleep apnea (OSA) patients, not previously diagnosed with diabetes. Data from biochemistry, fundamental biology and previous clinical monitoring reports were integrated in interpreting this relation. The analysis concluded that high levels of HbA1c limit the relevance of $avSpO_2$ in evaluating OSA severity. ODI maintains a strong association with AHI in high levels HbA1c group.

Key words: glycosylated hemoglobin, obstructive sleep apnea

Hemoglobin (Hb) is essential for blood gases transport. Quantitative or qualitative modifications of Hb (primary globin structure or posttranslational changes) have major impact on tissue metabolism. Glycosylated hemoglobin (HbA1c) is one of the possible posttranslational modifications of the adult Hb. Functional Hb is important in all circumstances, particularly in patients with chronic, severe, pulmonary diseases and hypoxemia. In these particular conditions, a simultaneous quantitative reduction of the oxygen (O_2) into the capillary blood, as the result of the respiratory failure and hypoxemia, and delivered to tissues, as the result of dysfunctional haemoglobin, occurs. When these two pathological conditions coexist, hypoxia is amplified, leading to a more pronounced metabolic dysfunction. OSA is an independent risk factor for oxidative stress and chronic inflammation, therefore any impairment of Hb transportation is relevant for patients' evolution. Obstructive sleep apnea (OSA) is a syndrome characterized by intermittent hypoxia-reoxygenation, having an impressive number of metabolic consequences, confirmed in both experimental and clinical studies. The inducer of apnea consists in the restriction of airflow, with underlying desaturation in blood O_2. The level of O_2 saturation, recorded during sleep, by pulse oximetry, and the average of O_2 desaturation, are strongly associated with an increased duration of both apneas and hypopneas, being more pronounced during apnea events [1]. Parallel recording of SpO_2 and direct O_2 measurements, in arterial blood, in diabetics, showed that HbA1c levels led to an underestimation of the decrease in O_2 recorded by pulse oximetry and expressed as SpO_2 [2]. Taking this into consideration, we conducted a study in a group of OSA patients, not previously diagnosed with diabetes, aiming to assess if the level of HbA1c influences the relationship between apnea-hypopnea index (AHI), oxygen desaturation index (ODI) and the average blood oxygen saturation ($avSpO_2$).

Experimental part

Of 275 patients screened for sleep apneea in a Sleep Laboratory of Constana Clinical Pneumophthisiology Hospital, from October 2011 to April 2015, who declared no previous diagnosis of diabetes, all OSA cases were included. The sleep study was performed with a portable cardio-respiratory sleep polygraph STARDUST II RESPIRONICS, allowing to recording nasal respiratory flow, SpO_2, cardiac frequency, snoring, body position and respiratory effort. Following respiratory polygraph exam, all patients with central sleep apnea, obesity-hypoventilation syndrome and upper airways resistance syndrome were excluded. Study procedure of blood samples collecting was approved by Local Ethical Committee. Supplementary tests were collected at a maximum 30 days interval after OSA confirmation. Invitation was launched to all OSA patients, with no further selection criteria, but only 32 patients agreed to participate. Venous blood sample was collected after 10h of fasting and HbA1c was determined by an immunoturbidimetric method in a certified Laboratory by the National Glycohemoglobin Standardization Program. Results of HbA1c were expressed as percentage of the total Hb. In order to determine the influence of HbA1c on the relation between AHI and $avSpO_2$, in the first step of the analysis, we computed the correlation between variables (AHI, $avSpO_2$, ODI and HbA1c) for the whole sample. AHI, ODI and $avSpO_2$ data were extracted from the polygraph report. ODI is defined as the number of ≥3% arterial O_2 desaturation per hour of sleep. According to the median value of HbA1c, study sample was divided in 2 groups: group 1 with lower values than median HbA1c and group 2 with higher values. We computed the regression function between AHI, as dependent variable, and $avSpO_2$, as independent variable, for the whole sample and for the 2 groups, comparing the regression coefficients among them. Results were processed with SPSS program (Statplus for Mac, 2016, v6). As variables had no normal distribution, data are presented as medians, using Mann Whitney test for comparison, chi² test for non-numerical variables and correlation between variables by Spearman rank R correlation test. Multiple regression analysis was used to compare the influences on AHI. A threshold of 95% was selected for the statistical significance.

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Results and discussions

There were no differences between study population (n=275) and study sample (n=32) in terms of age, gender distribution, smoking status, symptoms, comorbidities and body mass index (BMI) distribution (chi² test, $p > 0.05$), excepting AHI which was significantly lower in the sample ((chi² test, p=0.002). Descriptive statistics of the sample and of the 2 groups are presented in table 1.

According to the current classification of OSA [3], 3 patients had mild OSA, 7 moderate OSA and 22 severe forms of OSA. Severe OSA cases had a significant higher median of HbA1c (p=0.004). A strong negative correlation between AHI and $avSpO_2$ for all 32 cases was found (R^2 = -0.66209, $p < 0.001$). In both group 1 and group 2, the relation between AHI and $avSpO_2$ was maintained, but it was weaker for group 2 (R^2=-0.61230, p=0.02) versus group 1 (R^2=-0.68757, p=0.002). The correlation between HbA1c and AHI was direct and significant for study sample (R^2=0.35470, p=0.046), became stronger for group 2 (R^2=0.62984, p=0.012) and lost its significance for group 1 (R^2=0.01241, p=0.962). These findings support the assumption that higher values of HbA1c have had more influence on the relation between AHI and the $avSpO_2$. Regression analysis showed a strong relation between AHI, $avSpO_2$ and HbA1c (R^2=0.39045, $p <0.001$). Data analysis inside groups revealed a different relation between AHI and $avSpO_2$ in group 1 and group 2. In both groups, the regression equation showed a significant relation for group 1 (p=0.005) and 2 (p=0.023), with higher coefficient of determination in group 1 (R^2 = 0.377280) versus group 2 (R^2 = 0.28622). This coefficient of determination indicates the contribution of the factors (in our case, $avSpO_2$ and HbA1c) to the level of AHI. High values of AHI, noticed in OSA patients of group 2, in the presence of a similar level of $avSpO_2$ in both groups (fig. 1 and fig. 2), were in concordance with regression analysis.

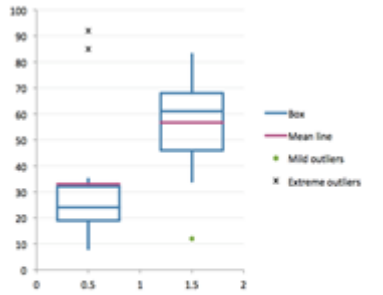
Therefore, we consider these results as a higher contribution of $avSpO_2$ to AHI in group 1 compared to group 2. So, the average SpO_2 -AHI relation seems to be more influenced by other factors, one of which is HbA1c, in group 2, as shown by the high Spearman's Rho coefficient (0.3547, p=0.046). Statistical analysis demonstrates that levels of HbA1c influence the relation between AHI and $avSpO_2$. A similar methodology applied in multiple regression analysis of AHI, as dependent variable, and ODI, and HbA1c, as independent variables, showed similar

	Group 1	Group 2	Sample	Statistical test	
Variable					p level
Number of cases	17	15	32		
Age (median)	60	57	57.5	Mann Whitney	0.673
Smoking*				Chi^2	0.037
Current	4	5	9		
Former	0	4	4		
Never smoker	13	6	19		
Symptoms				Chi^2	0.848
Snoring	17	15	32		
Night apnea	14	15	29		
Nighttime awakenings	13	15	28		
Gasping during sleeping	9	5	14		
Nicturia	10	11	21		
Morning headache	8	5	13		
OSA co-morbidities				Chi^2	0.8422
COPD	2	3	5		
Asthma	2	2	4		
HBP	13	13	26		
Ischemic heart disease	5	3	8		
Heart failure	1	2	3		
Arrhytmia	0	2	2		
Stroke	1	1	2		
ENT diseases	6	4	10		
BMI (median)	31.2	37.5	37.35	Mann Whitney	0.25
Normal weight	1	0	1		
Overweight	4	1	5		
Obesity	12	14	26		
AHI (median)	24	61	46	Mann Whitney	0.005
SpO_2 (median)	94	92	93	Mann Whitney	0.11
ODI (median)	28.2	59.9	49.1	Mann Whitney	0.006

Table 1
COMPARISON BETWEEN THE 2 GROUPS

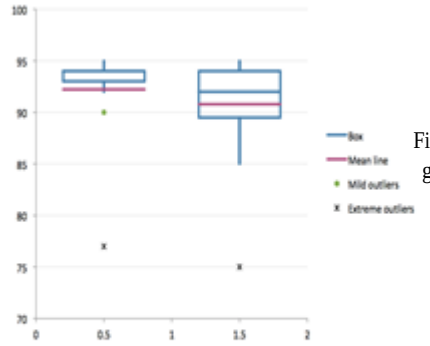
Legend: COPD = obstructive lung diseases; HBP = high blood pressure; ENT diseases = ear-nose and throat diseases; BMI = body mass index; AHI = apneea-hypopneea index; SpO_2 = peripheral blood saturation with O_2; ODI = oxygen desaturation index

determination coefficients in both groups (R^2 = 0.93083 in group 1 and R^2 = 0.93715 in group 2), reflected in a similar variance of ODI and AHI inside both groups (fig. 1 and fig. 3).



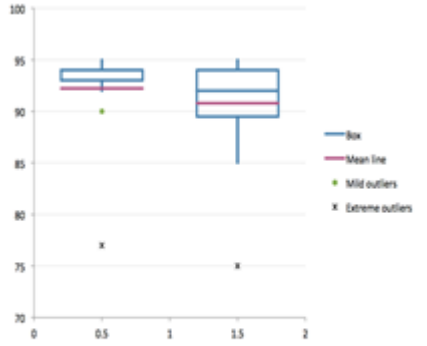
Variable	Mean	Minimum	Median	Maximum
AHI group 1	33	8	24	92
AHI group 2	56.68	12	61	83

Fig 1. AHI in group 1 and group 2
AHI = apneea-hypopneea index



Variable	Mean	Minimum	Median	Maximum
average SpO2 group 1	92.23077	77.	94.	95.
average SpO2 group 2	90.78947	75.	92.	95.

Fig 2. Average SpO_2 in group 1 and group 2
Legend: SpO_2 = peripheral blood saturation with O_2



Variable	Mean	Minimum	Median	Maximum
average SpO2 group 1	92.23077	77.	94.	95.
average SpO2 group 2	90.78947	75.	92.	95.

Fig 3. ODI in group 1 and 2
Legend: ODI = oxygen desaturation index

Human adult Hb consists of a mixture of HbA (97% of the total), HbA_2 (2.5%), and HbF (0.5%). HbA is made up of four polypeptide chains: two α- and two β-chains. By chromatographic analysis, several minor human hemoglobins (HbA1A1, HbA1A2, HbA1B, and HbA1c) of HbA might be identified. These fractions are collectively referred to as HbA1 or fast hemoglobin because they migrate more rapidly than HbA in an electrical field. The majority of the glycated Hb is represented by HbA1c. HbA1c originates from the condensation of glucose with the *N*-terminal valine residue of each β-chain of HbA to form an unstable Schiff base (aldimine or pre-HbA1c). The Schiff base may dissociate or may undergo an Amadori rearrangement to form a stable ketoamine, the HbA1c. Blood cells are permeable to glucose, and persistent high glycemic values initiate glycosylation reactions [4] and made HbA1c determination an important screening, diagnostic and monitoring test for diabetes. Formation of HbA1c is influenced not only by the glycemic level but also by other factors. For example, the shorter lifespan of the erythrocytes (in anemia, hypersplenism, chronic renal failure) [5] decreases the level of HbA1c and masks an abnormal level. Among patients with anemia, the iron deficiency has a different impact on increasing HbA1c value, as a recent systematic review concluded [6], although no clear mechanism of this observation was provided. Clinical studies recommend correction of iron induced anemia before interpreting the HbA1c significance [7]. These possible biases had no influence in our results as none of the patients included in the study group had anemia, hypersplenism and/or chronic renal failure. Lower levels of HbA1c (< 5% of total Hb) are a risk factor of all causes mortality and cancer death in a large, prospective cohort [8]. The relation between HbA1c and OSA is bilateral: OSA increases HbA1c level and HbA1c has deleterious effects in patients with OSA. Our regression analysis showed strong relations between hypoxemia (estimated by the $avSpO_2$) and AHI, between AHI and HbA1c. Therefore, our results are not surprising as they are consistent with other studies [9,10]. Patients with OSA have a reduction of the insulin sensitivity [11] which is more pronounced if higher AHI is noticed during REM sleep [12]. The explanation of this process was assigned to the higher sympathetic activity and longer desaturation periods which characterize REM sleep. Glucose levels in OSA patients, during REM, are significantly raised compared to levels during non REM sleep [13]. Experimental data demonstrated an increase rate of HbA1c formation in anaerobic conditions [14], attributed to the raise of 2,3 di-phosphoglycerate in the erythrocytes, exposed to a deprived in O_2 milieu. 2,3 di-phosphoglycerate is able to initiate a labile first step of the glycation process by reversibly binding to the known glycation sites of HbA [15]. As sleep apnea is a medical condition associated with intermittent hypoxia, one could expect higher HbA1c formation. The negative effect of HbA1c in OSA patients relates to tissue hypoxia, already seriously affected. Increased values of HbA1c impair the rheological properties of blood, increase red blood cell stiffness, reduce their deformability, and affect the nitric oxide (NO) mediated vascular relaxation [16]. The increased plasma viscosity is not a direct effect of HbA1c, but recognizes the same cause: the interaction of high plasma glucose levels with other plasma proteins [17]. As a consequence, the vascular resistance increases facilitating ischemia. Smoking has been associated with higher levels of HbA1c [18] similar to our findings. Increased HbA1c was related to the general pro-oxidant status of smokers. Particularly in OSA, smoking and intermittent hypoxemia might have cumulative effects. Overestimation of SpO2, when carboxiHb exists, is well documented. Even if smoking status was different between the 2 groups of cases, current smokers were not significantly higher in group 2. So, carboxiHb was unlikely to influence these results. Another negative effect of HbA1c on tissue hypoxia is the high O_2 affinity of this form of Hb in reducing the O_2 delivery to peripheral tissues [19]. Therefore, for same values of AHI, OSA patients with high levels of HbA1c are expected to have a more pronounced hypoxia than those with normal levels of HbA1c. This assumption by the relation between AHI and HbA1c was illustrated in patients with higher levels of HbA1c and found a more pronounced influence of $avSpO_2$ on AHI in the low HbA1c group. We interpret this finding in the context of a false higher level of $avSpO_2$

measured by the pulse oximetry in patients with high HbA1c, as Pu et al described in a comparative study of arterial O_2 and SpO_2 measurement [2]. If $avSpO_2$ does not fully reflect the reality of hypoxia and desaturation in patients with high HbA1c, AHI is expected to be less influenced by the level of $avSpO_2$. $AvSpO_2$ in patients with high HbA1c should be interpreted with caution in evaluating OSA severity, similar to the presence of carboHb. As ODI is a percentage variation, it maintains the significance of an OSA severity parameter in cases with high HbA1c values ($p=0.006$). In supporting this hypothesis, we consider that the regression calculation is not a final proof. There is a need to do direct comparison of O_2 arterial pressure and SpO_2 during sleep. We are, also, aware that this study needs to be reproduced to a larger scale for conclusive remarks.

The education of the parents and of the small patients to recognize the symptoms of sleep apnoea and to apply the available effective treatments is a stringent necessity [20]. The patient's compliance is a very important key factor in the therapy of OSA [21]. Treating sleep disorders can resolve both medical and dental affections, with multiple benefits for patients [22]. After the researches of Jimbolean et al [23], training the future health promoters represent a priority, because students, physicians and nurses constitute groups in further transmission of information about OSA complications to affected population.

Conclusions

Our study underlines a strong relation between AHI, SpO_2 and HbA1c in previously non diagnosed diabetes patients and emphasizes the necessity of metabolic evaluation in all OSA patients. We integrated data from fundamental biology (HbA1c influencers of formation), clinical monitoring (comparison of pulse oximetry measurement with direct arterial O_2 measurement) in interpreting data collected from OSA patients. The relevance of $avSpO_2$ associated with high levels of HbA1c is limited and opens new themes of research on the best prognostic predictors of OSA severity in patients with high levels of HbA1c.

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