

Study of upper airway inflammation in patients with obstructive sleep apnea–hypopnea syndrome

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Background Pharyngeal lavage was reported as a novel technique for noninvasive assessment of inflammation of the pharynx.

Aim To study upper airway inflammation in patients with obstructive sleep apnea–hypopnea syndrome (OSAHS) and its correlation with systemic inflammation.

Patients and methods A total of 36 patients with known OSAHS, admitted to the Chest Department, Alexandria Main University Hospital (group I), and 15 healthy volunteers (group II) were enrolled into the study. Informed consent was taken from all participants. The two groups underwent complete history taking, assessment of BMI, measurement of serum C-reactive protein (CRP), overnight polysomnography, and oropharyngeal lavage (OPL) analysis.

Results In group I, the most prevalent cell type of OPL was lymphocytes followed by neutrophils. Compared with control group (group II), the most prevalent cell type in OPL was macrophages followed by epithelial cells. Regarding the OPL differential cell count which denotes local pharyngeal inflammation, there was a statistically significant difference between both groups ($P < 0.001$). Similarly, CRP, a marker of systemic inflammation, showed a significant statistical difference between both groups ($P < 0.001$). Strikingly, there was no statistically significant correlation between CRP and

apnea–hypopnea index ($P = 0.604$). Snoring, apnea–hypopnea index, and other sleep parameters correlated significantly with lymphocytic predominance in OPL ($P < 0.001$), whereas CRP correlates significantly with neutrophils and eosinophil's in OPL ($P < 0.001$ and $P = 0.015$, respectively).

Conclusion Our study had provided a novel vision into the pathophysiology of OSAHS in emphasizing the existence of upper airway lymphocytic inflammation. OPL is a simple, applicable, and easy noninvasive procedure for assessment of upper airway inflammation.

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Introduction

Obstructive sleep apnea–hypopnea syndrome (OSAHS) is a disorder characterized by frequent episodes of obstruction in the upper airway during sleep [1]. Evidence advocates that both local airway inflammation and systemic inflammation are involved in the pathophysiology of this apparently mechanical disorder [2,3]. Leukocytic infiltration is markedly increased in the mucosa of the upper airway of adults with OSAHS [4]. Accumulating evidence suggests that local and systemic inflammatory responses also exist in patients with OSAHS [5]. C-reactive protein (CRP) levels are raised in children with OSAHS [6,7]. Recently, Vicente et al reported a novel technique for noninvasive examination of pharyngeal inflammation called oropharyngeal lavage (OPL) [8].

Aim

The aim of this work was to study upper airway inflammation in patients with OSAHS and its correlation with systemic inflammation.

Patients and methods

Study population and patients

A total of 36 patients with known OSAHS, admitted to the Chest Department, Alexandria Main University

Hospital (group I), and 15 healthy volunteers (group II) were enrolled into the study. All patients and subjects were prospectively enrolled after obtaining informed consents during the period of May 2017 through August 2018. The study was approved by the Local Institutional Ethics Committee of the Faculty of Medicine, Alexandria University.

Study measurements

Exclusion criteria

Patients with severe associated diseases (e.g. liver failure, heart failure, kidney failure, or any neuromuscular disorders), acute or chronic inflammatory disease of the airways [e.g. chronic obstructive pulmonary disease (COPD)], or use of any drugs that may affect the sleep pattern (e.g. sedatives or anxiolytics) were excluded from this study.

All patients were subjected to the following:

- (1) Full history taking including age, sex, and history of other diseases.

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- (2) Assessment of BMI, which was calculated as weight/height (kg/m^2).
- (3) Full clinical examination, including general, local chest, and throat examination.
- (4) Measuring serum CRP.
- (5) Sleep study: Standard full PSG study was conducted with the SOMNOscreen System (SOMNOmedics GmbH; Am Sonnenstuhl, Randersacker, Germany). The following parameters were included: flow (cannula and/or thermistor), oxygen saturation, snore (cannula and/or microphone), plethysmogram, thoracic movement, abdominal movement, periodic leg movement, pulse rate, ECG, electrooculogram, electroencephalogram, and continuous positive airway pressure (CPAP)/BiPAP pressure. The polysomnographic evaluation was done automatically then combined with manual scoring. The analysis included the following:
 - (a) Apnea–hypopnea index (AHI): severity of OSAHS was classified according to the following: AHI 5 to less than 15/h mild, AHI 15 to less than 30/h moderate, AHI more than 30–50/h severe, and more than 50/h very severe.
 - (b) Oxygen desaturation index 4% (ODI 4%): ODI of more than 4%.
 - (c) Time of sleep with oxygen saturation below 90%.
- (6) OPL analysis: the samples were taken in the morning after waking up from sleep. All patients and the healthy controls rinsed their mouth with sterile 0.9% saline solution at room temperature. Next they gargled with 50 ml of sterile 0.9% saline solution in several portions for 5–10 s each one. For sample collection, they spat the saline into a sterile container. Cell counting was accomplished by a skilled investigator. The OPL was prepared on thin smear cytology slides and stained by H&E stain. Under $\times 10$ magnification, the slide was scanned to ensure even distribution of cells. Under $\times 40$ magnification in 10 Hpf, the differentiated cells in 100 cells were counted, and the total differentiated count for each cell and percentage of each was assessed.

Statistical analysis

All statistical analyses were shown using IBM SPSS software package (IBM SPSS statistics for Windows, version 20.0.; IBM Corp., Armonk, New York, USA). Qualitative data were described using number and percent, whereas normally quantitative data were expressed in mean \pm SD, and abnormally distributed

data were expressed in median (interquartile range). Comparison between different groups regarding categorical variables was tested using χ^2 -test. If normal data distribution existed, parametric tests were applied. If the data were abnormally distributed, nonparametric tests were used. For normally distributed data, comparison between the two studied groups was done using independent t -test. For normally distributed data, comparison between more than two populations was analyzed by F -test (analysis of variance) and post-hoc test (least significant difference). For abnormally distributed data, comparison between two independent population was done using Mann–Whitney test, whereas Kruskal–Wallis test was used to compare between different groups, and pairwise comparison was assessed using Mann–Whitney test. Correlations between two quantitative variables were assessed using spearman coefficients regarding normality of the data. Significant results were detected at the level of P value less than 0.05.

Results

A total of 36 patients with documented OSAHS (group I), admitted to the Chest Department, Alexandria Main University Hospital, and 15 healthy volunteers (group II) were included in this study after obtaining informed consents.

Patients in both groups were matched regarding age and sex (Table 1). Regarding anthropometric measures, namely, weight, height, and BMI, there was a statistically significant difference between both groups ($P=0.036$; Table 1).

In group I, the most prevalent cell type of OPL was lymphocytes followed by neutrophils. Compared with control group (group II), the most prevalent cell types in OPL were macrophages followed by epithelial cells. Regarding the OPL differential cell count, which denotes local pharyngeal inflammation, there was a statistically significant difference between both groups ($P<0.001$). Similarly, CRP, a marker of systemic inflammation, showed a significant statistical difference between both groups ($P<0.001$; Table 1).

Regarding the sleep laboratory parameters, namely, snoring, AHI, T90, and ODI 4%, there was a significant statistical difference between both groups ($P<0.001$). Both groups were matched for comorbidities, namely, hypertension and diabetes (Table 1).

Table 2 shows the correlation between AHI, T90, ODI 4%, and CRP with different parameters in group I

Table 1 Comparison between the two studied groups according to different parameters

	Cases (n=36)	Control (n=15)	Test of Significance	P
Sex				
Female	20 (55.6)	6 (40)	$\chi^2=1.025$	0.311
Male	16 (44.4)	9 (60)		
Age	51.8±5.4	50.8±4.7	t=0.645	0.522
Weight	124.4±23	94.2±9.2		
Height	163.3±4.4	172.6±7.1	t=0.025	0.036*
BMI	46.7±8.89	31.8±5.64		
Lavage differential cell count				
Neutrophils	11 (5–19.3)	2 (1–3)	U=33.50*	<0.001*
Lymphocytes	18 (10–34.8)	9 (7–10)	U=93.50	<0.001*
Eosinophil's	0.5 (0–2)	0 (0–1)	U=221.0	0.269
Macrophages	0.5 (0–12.5)	77 (75–78)	U=0.00*	<0.001*
Epithelial cells	54 (40–70)	13 (10–14)	U=37.0	<0.001*
CRP	14 (11.3–22.5)	4 (3–6)	U=21.0*	<0.001*
AHI				
Median (25–75)	22.5 (13.0–35.0)	2.0 (1.0–4.0)	U=0.0*	<0.001*
Mean±SD	24.4±12.9	2.4±1.9		
T90				
Median (25–75)	22.5 (13.0–43.5)	1.0 (0.0–1.0)	U=0.0*	<0.001*
Mean±SD	26.7±15.3	0.5±0.5		
ODI 4%				
Median (25–75)	19 (12.0–44.8)	1.0 (0.0–1.4)	U=0.0*	<0.001*
Mean±SD	25.6±17.1	0.8±0.7		
Snoring				
Nonsnorer	10 (27.8)	12 (80)	$\chi^2=13.138^*$	0.001*
Mild snorer	13 (36.1)	3 (20)		
Severe snorer	13 (36.1)	0		
DM				
Negative	15 (41.7)	5 (33.3)	$\chi^2=0.308$	0.579
Positive	21 (58.3)	10 (66.7)		
HTN				
Negative	13 (36.1)	5 (33.3)	$\chi^2=0.036$	0.850
Positive	23 (63.9)	10 (66.7)		

Qualitative data were described using *n* (%), whereas normally quantitative data were expressed in mean±SD, and abnormally distributed data were expressed in median (interquartile range). AHI, apnea–hypopnea index; CRP, C-reactive protein; DM, diabetes mellitus; HTN, hypertension; ODI 4%, oxygen desaturation index more than 4%; T90, time of sleep with oxygen saturation below 90%. *P*: *P* value for comparing between the two studied groups. **P*≤0.05, statistically significant.

(cases). Strikingly, there was no statistically significant correlation between CRP and AHI (*P*=0.604). Snoring, AHI, T90, and ODI 4% correlated significantly with lymphocytic predominance in OPL (*P*<0.001; Fig. 1), whereas CRP correlates significantly with neutrophils and eosinophil's in OPL (*P*<0.001 and *P*=0.015, respectively; Fig. 2). Table 3 shows the relation between snoring severity and differential cell count in OPL.

Discussion

The assessment of inflammation existing in patients with OSAHS at the main source of airway obstruction (upper airway) and its relation to systemic consequences was accomplished using a novel procedure. The OPL as a technique is simple, easy, and noninvasive; besides, it estimates the local upper

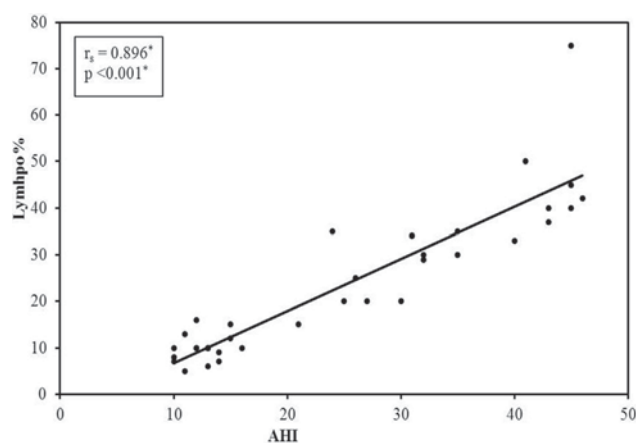
airway effects of recurrent collapse and snoring throughout repetitive cycles in patients with OSAHS. Systemic inflammation might add to weakening of the respiratory muscles, disturbed respiratory centers control, and harming the protective mechanisms that maintain airway patency in these patients [9,10].

In this study, we found that the most prevalent cell type of OPL in OSAHS group was lymphocytes followed by neutrophils, with significant difference between cases and control groups. This matched with the results of Sekosan *et al.* [3] and by Boyd *et al.* [4] who demonstrated leukocyte infiltration and lymphocytic inflammatory cells in the mucosa of upper airway in patients with OSAHS. Furthermore, Hauber *et al.* [11] evaluated the OPL as a new technique to investigate inflammation of the mucosa of the pharynx in patients

Table 2 Correlation between apnea–hypopnea index, time of sleep with oxygen saturation below 90%, oxygen desaturation index more than 4%, and C-reactive protein with different parameters in cases group

	AHI		T90		ODI 4%		CRP		Snoring	
	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P
AHI	–	–	0.860*	<0.001	0.832*	<0.001	0.089	0.604	0.574*	<0.001
Neutrophils (%)	0.148	0.390	0.121	0.482	0.103	0.548	0.805*	<0.001	0.454*	0.001
Lymphocytes (%)	0.896*	<0.001	0.894*	<0.001	0.895*	<0.001	0.038	0.826	0.657*	<0.001
Eosinophil's (%)	0.060	0.726	–0.100	0.563	–0.087	0.613	0.404*	0.015	0.158	0.269
Macrophages (%)	0.069	0.690	–0.032	0.854	–0.110	0.522	–0.116	0.501	–0.442*	0.001
Epithelial cells (%)	–0.603*	<0.001	–0.428*	0.009	–0.376*	0.024	–0.577*	<0.001	0.235	0.096

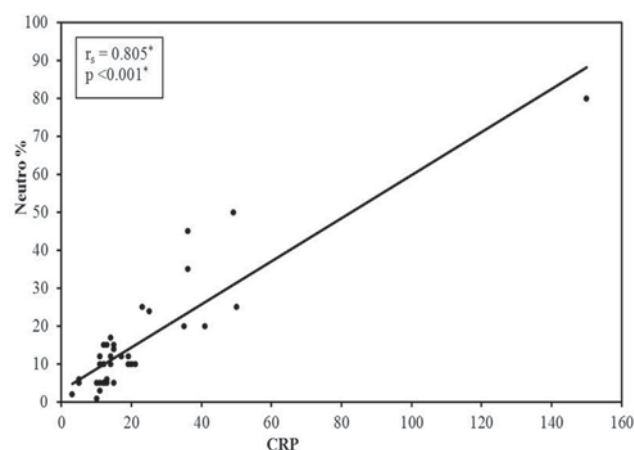
AHI, apnea–hypopnea index; CRP, C-reactive protein; ODI 4%, oxygen desaturation index more than 4%; r_s , Spearman coefficient; T90, time of sleep with oxygen saturation below 90%. * $P \leq 0.05$, statistically significant.

Figure 1

Correlation between apnea–hypopnea index (AHI) and oropharyngeal lavage (OPL) lymphocytes.

with sleep-related breathing disorders. They stated that patients with OSAHS had markedly significant numbers of lymphocytes in comparison with the control group patients. Patients having moderate or severe OSAHS had considerably higher quantities of lymphocytes compared with mild OSAHS cases, with statistical difference ($P < 0.05$). Lymphocytic count was higher in snorer than nonsnorer. It is not obvious whether local upper airway inflammation in OSAHS is the cause or the result of pharyngeal collapse. Consequently, an animal model study of frequent airway collapse established the theory that mechanical trauma occurring owing to airway collapse led to upper airway mucosal inflammation [12]. Snoring is known as a first phase in the progress of OSAHS. Our data agreed with those studies and suggest that upper airway lymphocytic mucosal inflammation could be an essential component. Additional probability is that snoring and OSAHS symbolize different categories of sleep-related breathing disorders. Extensive follow-up research studies of snorers can support and explain this issue.

Vicente *et al.* [8] revealed a high level of interleukins (ILs) mainly IL-6 and IL-8 ($P < 0.05$), although the total

Figure 2

Correlation between C-reactive protein (CRP) and oropharyngeal lavage (OPL) neutrophils.

lymphocytic count, %T lymphocytes, and %CD4+ were higher ($P < 0.05$) compared with the control group. Moreover, there were no differences between patients and control group in plasma levels of the inflammatory biomarkers as IL-6 and 8, tumour necrosis factor (TNF)- α , and CRP or lymphocytic count. No correlation for any inflammatory marker was achieved between OPL and plasma. During the follow-up of patients treated with CPAP, a reduction was found in the pharyngeal inflammatory markers with no alteration with plasma inflammatory markers. Vicente *et al.* [8] found that lymphocyte percentage was higher in severe OSAHS group compared with the control ($P < 0.05$) and the simple snorer group ($P < 0.05$). CD4+ was the main T-cell subclass and was significantly higher in severe OSAHS (55–63%) compared with both control and simple snorer groups ($P < 0.001$ and < 0.05). These alterations were predominantly caused by activated T lymphocytes. Patients on CPAP therapy revealed a reduction in the OPL inflammatory cells, which was significant for total leukocytes, percentage of lymphocytes, and percentage of CD4+ T-cells ($P = 0.034$, 0.008 , and 0.02 , respectively). Similar changes in the biomarkers were found after one-year

Table 3 Relation between snoring and different parameters in group I 'cases'

	Snoring			H	P
	Nonsnorer (n=10)	Mild snorer (n=13)	Severe snorer (n=13)		
Neutrophils	5.0 (5.0–12.5)	15.0 (10.0–30.0)	12.0 (5.5–15.5)	5.358	0.069
Lymphocytes	10.0 ^b (6.8–11.3)	15.0 ^{a,b} (10.0–32.0)	33.0 ^a (20.0–38.5)	12.300*	0.002*
Eosinophil's	0.0 ^b (0.0–1.0)	2.0 ^a (0.0–5.0)	0.0 ^{a,b} (0.0–1.5)	6.307*	0.043*
Macrophages	5.0 (0.0–20.0)	0.0 (0.0–3.0)	1.0 (0.0–12.5)	2.027	0.363
Epithelial cells	74.0 (44.5–86.0)	50.0 (40.0–60.0)	43.0 (39.0–39.0)	3.743	0.154

Abnormally distributed data were expressed in median (interquartile range). Means with common letters are not significant (i.e. means with different letters are significant). H: H for Kruskal–Wallis test, pairwise comparison between each two groups was done using post-hoc test (Dunn's for multiple comparisons test). P: P value for comparing between the studied groups. * $P \leq 0.05$, statistically significant.

follow-up; again the reduction in local inflammatory markers was in OPL but not in plasma. However, Li *et al.* [13] established the association between inflammation of the upper airway and severity of OSAHS in children. Children with OSAHS had significantly higher percentage of sputum neutrophil than those without OSAHS ($P=0.006$), and the percentage of sputum neutrophil was significantly associated with OSAHS ($P=0.013$).

In this study, CRP, a biomarker of systemic inflammation, showed a significant statistical difference between both groups, although we found no statistically significant correlation between CRP and AHI ($P=0.604$). Elevated measurement of systemic markers of inflammation such as CRP had been described in OSAHS [14]. As ongoing inflammatory responses play crucial roles in the progression of atherosclerosis which are definitely associated with higher cardiovascular and or cerebrovascular morbidity documented in OSAHS [15], increased serum levels of inflammatory cytokines in the atherosclerotic process, such as IL-6 and CRP, have been described in these patients. These cytokines are commonly raised in obesity, which is a confounding feature in patients with OSAHS [16]. On the contrary, some studies had not discovered an association between OSAHS and IL-6 and/or CRP levels [17,18]. Accordingly, there is disagreement about the relation between these inflammatory markers and OSAHS.

We found significant correlation between snoring, AHI, T90, and ODI 4% with lymphocytic predominance in OPL, whereas CRP correlates significantly with neutrophils and eosinophil's in OPL in OSAHS group. High lymphocyte percentage was found in severe OSAHS, and it was correlated with AHI. Subsequently, the rise in inflammatory cells mainly lymphocytes in patients with OSAHS is correlated with the severity of OSAHS [8]. Yet, Salerno *et al.* [19] demonstrated the presence inflammation of the lower airway in OSAHS using induced sputum

analysis which is a valid and commonly used procedure to assess the profile of bronchial inflammation. None of the patients were smokers nor had a history of acute or chronic pulmonary disease, and their sputum was characterized by variable grades of neutrophilic inflammation.

Inflammation associated with OSAHS was found likewise variable inflammatory biomarkers were measured mostly ILs and CRP. These markers were demonstrated and governed by obesity. There are synergistic influences of obesity and OSAHS on CRP along with reduction of CRP after treatment of OSAHS with CPAP [20]. Patients having OSAHS exhibit upper and lower inflammation of the airway. The cause of airway inflammation may be the mechanical stress on the mucosa produced by recurrent airway obstruction, nocturnal hypoxemia, and/or the associated systemic inflammation [21]. Limitations of this study are that it is a single-center experience besides the relatively limited number of included patients, but this may be expected in prospective studies. Moreover, systemic inflammatory response was assessed solely by CRP. Addition of other markers of systemic inflammation like ILs or matrix metalloproteinase [22] would rather fortify the results of this study. Moreover, follow-up of our patients after positive airway pressure therapy would rather guarantee a full view of the link between OSAHS and the oropharyngeal as well as systemic inflammation.

Conclusion

This study has provided a novel vision into the pathophysiology of OSAHS in emphasizing the existence of upper airway lymphocytic inflammation. OPL is a simple, applicable, and easy noninvasive procedure for assessment of upper airway inflammation. Additional studies are required to settle our conclusions and to better describe the cellular pathogenesis in OSAHS. Further studies are essential to evaluate the clinical significance of airway inflammation in OSAHS, and the efficiency of the

anti-inflammatory treatment on decreasing the OSAHS-induced inflammation of the airway and in revising the expected outcome of the disease. It is probable that using CPAP and reducing weight may diminish local and systemic inflammation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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