Plasma lactoferrin level as a predictor to endothelial dysfunction in patients with obstructive sleep apnea

Abir Zakaria[a], Mostafa El Shazly[b] and Laila Rashed[c]

Departments of [a]Internal Medicine, [b]Respiratory Medicine and [c]Clinical Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence to Abir Zakaria, Department of Internal Medicine, 158 Haram Street, MIRAC Center, Building 2A, First Floor, Number 10, 12555 Giza, Egypt
Tel: +202 33834201;
e-mail: drabirzakaria@yahoo.com

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Background
Obstructive sleep apnea (OSA) syndrome is associated with cardiovascular complications attributed to endothelial dysfunction. There are contradictory reports on whether lactoferrin is protective or injurious to the blood vessels.

Objectives
To determine circulating plasma lactoferrin level in OSA patients in relation to endothelial dysfunction and to assess its relation to other criteria of OSA.

Patients and methods
In a cross-sectional study, 40 OSA patients were recruited after an established diagnosis in the sleep laboratory of the pulmonary medicine department. Doppler flow-mediated dilatation percentage (FMD%) was tested as an indicator of endothelial function. Anthropometric measurements, systolic and diastolic blood pressure, lipid profile, plasma lactoferrin level, fasting, and 2 h postprandial plasma glucose (PPG) were estimated in the patients and the control groups. Moreover, the apnea–hypopnea index, and the mean and nadir nocturnal oxygen saturation of OSA patients were determined.

Results
OSA patients were found to have significantly higher BMI, waist circumference (WC), neck circumference, fasting plasma glucose (FPG), 2 h PPG, low-density lipoprotein-cholesterol, and lower plasma lactoferrin, FMD%, and high-density lipoprotein (HDL)-cholesterol compared with the control group. There was a significant direct correlation between FMD%, as an indicator of endothelial function, and plasma lactoferrin level as well as HDL-cholesterol, and an inverse correlation between FMD% and BMI, WC, FPG, 2 h PPG, and basal brachial artery diameter. Multiple regression analysis showed that lactoferrin was the only independent predictor for FMD% among OSA patients.
However, plasma lactoferrin level was inversely correlated with BMI, WC, FPG, and 2 h PPG, and was directly correlated with HDL-cholesterol and FMD%. Multiple regression analysis selected BMI and FMD% as the independent predictors for lactoferrin level.

Conclusion
The present study showed that low circulating plasma lactoferrin levels in OSA patients independently predict endothelial dysfunction as assessed by FMD%. High BMI in OSA patients negatively influences plasma lactoferrin levels unrelated to other OSA severity predictors.

Keywords:
endothelial dysfunction, flow-mediated dilatation, lactoferrin, obstructive sleep apnea

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Introduction

Lactoferrin is an iron-binding glycoprotein of the transferrin family. It was found to be released from degranulation of neutrophils upon stimulation [1] and to inhibit upregulation of adhesion molecules on endothelial cells in addition to its known antimicrobial function. Moreover, it was known to inhibit tumor-induced blood vessels [2]. However, in a recent population-based prospective study, elevated basal lactoferrin was found to be a predictor of future fatal ischemic heart disease [1]. Therefore, there is an apparent contradiction on the effect of lactoferrin on endothelial function, whether protective or harmful.

Obstructive sleep apnea (OSA) is considered a risk factor for cardiovascular diseases, including hypertension, coronary heart disease, and strokes [3]. Endothelial dysfunction, found in previous studies to be associated with OSA [4], can be considered as a preclinical predictor of future vascular events [5]. Endothelial dysfunction among OSA patients has many proposed as a pathogenic

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mechanism including oxidative stress [6], endothelial apoptosis [3], and inflammatory injury [7]. Whether lactoferrin defends or injures the endothelium in the pathogenesis of OSA remains to be elucidated.

Objectives of the study

In the current study, we aimed to detect the level of circulating plasma lactoferrin in relation to endothelial dysfunction, as assessed by flow-mediated dilatation percentage (FMD%), in OSA patients. We attempted to sort the importance of lactoferrin level among other indicators of OSA severity, such as the apnea–hypopnea index (AHI), mean and nadir nocturnal oxygen saturation, as modulators of endothelial function. Central obesity, hypertension, glucose intolerance, or diabetes mellitus, found to be more prevalent among patients with OSA, compared with healthy individuals, were also taken into consideration as factors that might influence endothelial function.

Participants and methods

In a cross-sectional study, carried out in Kasr El Ainy Hospital from May 2010 to December 2010, FMD% of the right brachial artery, as an indicator of the endothelial-derived vasodilatation, was compared between 40 consecutive patients (27 men and 13 women, age range 40–60 years) with an established diagnosis of OSA and apparently healthy age-matched 40 individuals (28 men and 12 women) as a control group. Circulating plasma lactoferrin level was compared between OSA patients and controls. The study design was approved by the medical ethical committee of the Internal Medicine Department, Faculty of Medicine, Cairo University, Egypt. An informed written consent was obtained from each participating patient as well.

In OSA patients, as well as the control group, we estimated anthropometric measurements comprising BMI, calculated as the weight in kg divided by the height in m^2, waist circumference (WC), measured by a soft tape midway between the lowest rib and the iliac crest, and neck circumference (NC), measured at the base of the neck. Systolic and diastolic blood pressure were measured in the right arm after 10 min of rest, using a standard sphygmomanometer with an appropriate cuff size, with the first and the fifth sounds considered to represent systolic and diastolic blood pressure readings, respectively. The mean of three blood pressure readings taken 5 min apart was calculated. Fasting and 2 h postprandial plasma glucose (PPG), total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein-cholesterol, and triglycerides were also estimated. Indicators of OSA severity such as AHI, mean and nadir nocturnal oxygen saturation were assessed in the patient group.

Patients with heart failure, chronic pulmonary disease, chronic renal or liver disease, smokers, or those with acute upper respiratory tract infection were excluded from the present study.

Polysomnography

In the sleep laboratory of the respiratory medicine department, overnight polysomnography was carried out. According to the AHI, defined as the total number of complete or partial cessation of breathing per one sleeping hour, caused by airway obstruction, OSA was classified into mild (AHI 5–15), moderate (AHI 15–30), and severe (AHI > 30). Moreover, nadir and mean nocturnal oxygen saturation were recorded and calculated, respectively.

Vascular reactivity assessment

Vascular endothelial-dependent reactivity assessment was performed in the vascular laboratory of the medical emergency department. Using the B-mode image of duplex ultrasound, the basal diameter of the right brachial artery was measured in the patients with OSA and in the control group after being rested in the supine position in a quiet room for 10 min. Reactive hyperemia was induced by inflating a pneumatic tourniquet distal to the brachial artery to 50 mmHg above the systolic blood pressure for 5 min. After cuff deflation and within 1 min, the diameter of the brachial artery was measured again. Arterial diameter was measured as the distance between the lumen–intima interface of the near and the far walls, as averaged from three consecutive end-diastolic measurements, guided by the R-wave of the associated electrocardiogram, whether in the basal or in the post-hyperemic phase. FMD% was defined as the percent change in brachial artery diameter between the basal and the posthyperemic phase. An ATL HDI 5000 (Washington, USA) ultrasound machine was used for the duplex ultrasound study of the brachial artery using a 7.5-MHz linear transducer.

Estimation of plasma lactoferrin level

Plasma lactoferrin levels were measured using the BIOXYTECH lactof EIA reagent set (Oxis Research, Portland, USA). Plasma samples were diluted and assayed according to the manufacturer's instructions. Intra-assay and interassay imprecision was between 5 and 10%. The lower detection limit of the assay is 1 μg/l and the degree of cross-reactivity with transferrin was less than 1%.

Statistical methods

Data were statistically described in terms of mean ± SD, median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was carried out using the Student *t*-test for independent samples when comparing two groups and the one-way analysis of variance test with post-hoc multiple two-group comparisons when comparing more than two groups. For comparison of sex, the χ^2-test was carried out. Correlation between various variables was determined using the Pearson moment correlation equation. Multivariate analysis models were used to test for the preferential effect of all studied variable(s) on FMD% values among OSA cases. A *P*-value less than 0.05 was considered statistically significant. All statistical calculations were carried out using computer programs statistical package for the social

Table 1 Comparison between obstructive sleep apnea patients and the control group

Variables	OSA patients ($n=40$) (mean ± SD)	Control group ($n=40$) (mean ± SD)	*P*-value
Age (years)	49.000 ± 4.188	48.23 ± 4.306	0.417
BMI (kg/m$^{2)}$	29.900 ± 4.911	25.668 ± 2.289	0.000
Neck circumference (cm)	38.55 ± 8.72	30.18 ± 4.782	0.000
Waist circumference (cm)	107.650 ± 16.645	101.38 ± 10.669	0.048
Flow-mediated dilatation%	4.108 ± 1.935	13.252 ± 5.788	0.000
Fasting plasma glucose (mg/dl)	99.03 ± 19.753	83.680 ± 6.708	0.000
2 h postprandial plasma glucose (mg/dl)	144.95 ± 21.125	125.88 ± 10.062	0.000
Plasma lactoferrin level (μg/l)	323.13 ± 51.605	469.70 ± 25.931	0.000
Systolic blood pressure (mmHg)	130.38 ± 9.086	129.630 ± 4.295	0.638
Diastolic blood pressure (mmHg)	81.00 ± 6.118	77.50 ± 4.668	0.005
Total cholesterol (mg/dl)	177.90 ± 21.724	177.68 ± 21.016	0.963
HDL-cholesterol (mg/dl)	43.200 ± 9.785	55.600 ± 6.172	0.000
LDL-cholesterol (mg/dl)	104.88 ± 23.365	93.27 ± 21.457	0.023
Triglycerides (mg/dl)	150.10 ± 21.137	144.05 ± 19.029	0.182

Significant *P*-value <0.05.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; OSA, obstructive sleep apnea.

Table 2 Correlation between flow-mediated dilatation percentage and other variables in obstructive sleep apnea patients

Variables	Pearson's correlation	*P*-value
Age	−0.051	0.753
BMI	−0.701	0.000
Neck circumference	0.176	0.278
Waist circumference	−0.419	0.007
Apnea–hypopnea index	0.137	0.398
Mean nocturnal oxygen saturation	−0.206	0.202
Nadir nocturnal oxygen saturation	0.259	0.107
Fasting plasma glucose	−0.415	0.008
2 h postprandial plasma glucose	−0.488	0.001
Plasma lactoferrin level	0.886	0.000
Systolic blood pressure	−0.298	0.062
Diastolic blood pressure	−0.262	0.102
Basal BA diameter	−0.394	0.012
Total cholesterol	0.142	0.381
HDL-cholesterol	0.555	0.000
LDL-cholesterol	−0.152	0.350
Triglycerides	0.289	0.070

Significant *P*-value <0.05.
BA, brachial artery; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

science (SPSS; SPSS Inc., Chicago, Illinois, USA) version 15 for Microsoft Windows.

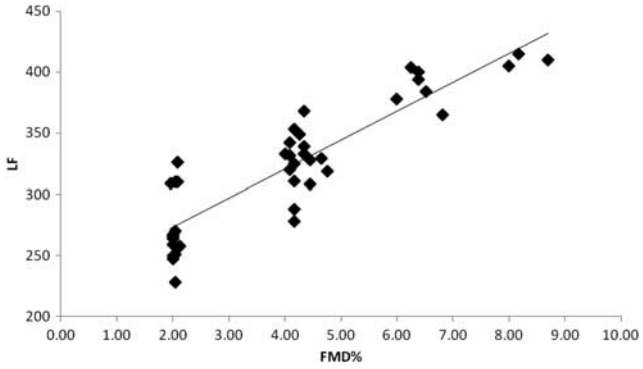
Results

OSA patients showed significantly higher BMI, WC, NC, fasting plasma glucose (FPG), 2 h PPG, low-density lipoprotein-cholesterol, and lower plasma lactoferrin, FMD%, and HDL-cholesterol compared with the control group (Table 1).

By subclassification of OSA patients according to AHI into mild, moderate, and severe types, only NC showed a statistically significant difference between subgroups ($P = 0.000$).

A statistically significant positive correlation was detected between FMD% and plasma lactoferrin level as well as HDL-cholesterol, whereas a significant negative correlation existed between FMD% on the one hand and BMI, WC, FPG, 2 h PPG, and basal brachial artery diameter on the other (Table 2).

Figure 1



Correlation between FMD% and plasma lactoferrin among obstructive sleep apnea cases. FMD%, flow-mediated dilatation percentage; LF, plasma lactoferrin.

Multiple regression analysis, including all the significantly correlated variables, showed that plasma lactoferrin level was the only independent predictor for FMD%, among OSA patients. The positive correlation between plasma lactoferrin level and FMD% is shown in Fig. 1.

Lactoferrin level was correlated inversely with BMI, WC, FPG, and 2 h PPG, and was correlated directly with HDL-cholesterol and FMD% (Table 3). Multiple regression analysis selected BMI and FMD% as the independent predictors for lactoferrin level.

Discussion

OSA is a disorder characterized by recurrent attacks of breathing cessation during sleep because of upper airway obstruction, with frequent arousal attempts because of maintained efforts of breathing. The latter causes sleep deprivation and daytime tiredness in patients [8]. OSA was found to be associated with adverse cardiovascular events including hypertension, coronary heart disease, and cerebrovascular strokes, found more frequently in OSA patients in comparison with non-OSA patients [5,9]. The pathophysiologic mechanism contributing to cardiovascular

Table 3 Correlation between lactoferrin and other variables in obstructive sleep apnea patients

Variables	Pearson's correlation	*P*-value
Age	−0.070	0.667
BMI	−0.808	0.000
Neck circumference	0.149	0.360
Waist circumference	−0.427	0.006
Apnea–hypopnea index	0.145	0.371
Mean nocturnal oxygen saturation	−0.173	0.287
Nadir nocturnal oxygen saturation	0.254	0.114
Fasting plasma glucose	−0.349	0.027
2 h postprandial plasma glucose	−0.390	0.013
FMD%	0.886	0.000
Total cholesterol	0.093	0.569
HDL-cholesterol	0.550	0.000
LDL-cholesterol	−0.193	0.233
Triglycerides	0.269	0.093

Significant *P*-value <0.05.
FMD%, flow-mediated dilatation percentage; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

adverse events in OSA patients needs more elucidation. Endothelial dysfunction was suggested in previous studies to explain this phenomenon [10]. Many potential etiologies have been proposed for such a dysfunction, including repeated attacks of hypoxemia, followed by reoxygenation, with subsequent generation of reactive oxygen species [6], which exert an inflammatory effect on the endothelium. Increase in activated circulating leukocytes was also a proposed mechanism of oxidant overproduction with subsequent endothelial dysfunction in OSA patients [11,12]. Lactoferrin is an 80-kDa monomeric multifunctional nonheme iron-binding glycoprotein, comprising two lobes, each of which binds a ferric iron. It has been suggested to show anti-inflammatory, immunomodulatory, and antitumoral effects [13]. Previous studies have shown the existence of lactoferrin receptors in the monocytes, lymphocytes, adipocytes, hepatocytes, and endothelial cells [14].

In the current study, OSA patients showed significantly higher BMI. This is in agreement with previous studies [15–18]. The Wisconsin Sleep Cohort study concluded that each one standard deviation of BMI was associated with a four-fold increase in the prevalence of OSA [15]. Other studies proved that elevation of BMI above a cutoff level, suggested in one study to be $40\,kg/m^2$ [16], and in another one to be $30\,kg/m^2$ [17], increased susceptibility to OSA. Combined with a simple snoring severity score, BMI was suggested by other investigators [18] as a useful method for screening of patients, who will be referred to polysomnography.

Central rather than peripheral distribution of obesity evidenced by WC and NC were found in the Sleep Heart Health study to be independently associated with moderate-to-severe OSA, defined as AHI of 15 times/h or more [19]. This was in agreement with the present study, which showed a significant increase in NC associated with OSA deterioration from mild to severe, as assessed by AHI. This was also in agreement with previous investigators, who concluded that NC was a more useful predictor for OSA than general obesity as assessed by BMI in a prospective study and suggested using it for a confident clinical diagnosis of OSA before ordering polysomnography [20].

FMD% was found in the current study to be markedly impaired in OSA patients compared with the control group. This coincided with previous studies showing diminished FMD% in OSA patients [21,22] irrespective of obesity [21], with improvement after uvulopalatopharyngoplasty [22].

Moreover, FMD% in the present study showed a significant inverse correlation with BMI, WC, FPG, and 2 h PPG, and a significant positive correlation with plasma lactoferrin level and HDL-cholesterol. This was in agreement with previous studies that suggested a relation between impaired FMD% and obesity, especially central type, and insulin resistance [23]. Increased number and size of adipocytes was considered to induce functional abnormalities in the endoplasmic reticulum and mitochondria, with subsequent enhancement of the proinflammatory state disrupting the balance between proinflammatory and anti-inflammatory pathways, aggravating insulin resistance and enhancing excessive production of free fatty acids [24]. Central obesity is recognized as a chronic subclinical inflammatory state in which many inflammatory mediators such as interleukin-6, tumor necrosis factor-α, serum retinol binding protein-4, and plasminogen activator inhibitor-1 are operating [24]. Interleukin-6 is claimed to induce C-reactive protein production by the liver [25]. C-reactive protein inhibits nitric oxide formation by the endothelial cells promoting vasoconstriction and hence endothelial dysfunction [24]. Moreover, in obese patients, several free fatty acids, exceeding the capacity of adipose tissue, are found to accumulate in nonadipose tissues such as the liver, inducing overproduction of metabolic toxins [25], with additional impairment of insulin signaling and glucose tolerance [26].

The current study showed a significant positive correlation between circulating plasma lactoferrin level and FMD%, with the former determined to be the only independent predictor for FMD% after multiple regression analysis. This finding emphasized the protective effect of lactoferrin on the endothelium in agreement with other studies, which explained this anti-inflammatory role of lactoferrin by its iron-sequestering ability, preventing the formation of reactive oxygen species by the human body and depriving the invading organism from iron, being an important nutrient for microorganisms. However, this analysis was in context of the antimicrobial role of lactoferrin [27]. Moreover, lactoferrin was proved by other investigators to prevent the release of cytokines responsible for activation and recruitment of leukocytes in inflamed tissues, with the latter suggested to exert a deleterious effect on the endothelium [28–30].

Our findings seemed apparently contradictory to the conclusions drawn by Vengen *et al.* [1], who showed that elevation of serum lactoferrin level strongly predicted the long-term risk for fatal coronary artery disease and hence endothelial dysfunction among the diabetic sub-

group of their participating cohort. However, these researchers suggested that the anti-inflammatory effect of lactoferrin may be disturbed in patients with diabetes, increasing their atherosclerotic risk [1]. Moreover, others claimed this elevation in serum lactoferrin level to be spurious because of neutrophil degranulation by clot retraction [13]. The latter was avoided in the current study by estimation of circulating plasma lactoferrin level.

Plasma lactoferrin level was shown in the present study to be inversely associated with BMI, WC, FPG, and 2 h PPG, and directly correlated with FMD% and HDL-cholesterol, with BMI being recognized as its independent predictor by multiple regression analysis.

This was in agreement with other investigators who proved that lactoferrin correlated positively with endothelial-derived vasodilatation in the obese subgroup of the participants with impaired glucose tolerance [13]. The protective effect of lactoferrin on the endothelium was explained by its inhibitory action on upregulation of adhesion molecules on endothelial cells [1]. Lactoferrin was also found to induce endothelial-dependent vasodilatation in a nitric oxide-dependent manner [31]. Moreover, previous studies showed that lactoferrin affected the peripheral opioid-mediated antinociception in animal models [32,33] through nitric oxide.

Another favorable effect of lactoferrin on lipid profile by increasing HDL-cholesterol has been recognized before in animal models [34]. This agreed with the positive correlation between plasma lactoferrin level and HDL-cholesterol in the current study. This was explained by its inhibitory effect on selective HDL-cholesterol esters' uptake by about 50% in primary human adipocytes and liposarcoma cells [35].

Lactoferrin was recognized by previous researchers to differ from other substances released from activated neutrophils by its unique anti-inflammatory properties, as it suppresses the production of inflammatory cytokines by monocytes, which may be suggested as a feedback mechanism avoiding further neutrophil recruitment and activation [36]. Lactoferrin also binds iron, which is considered to catalyze the formation of reactive oxygen species [1], known to increase in OSA patients with a deleterious effect on the endothelium. Thus, the reduced circulating lactoferrin level in OSA patients in the current study might be explained by its consumption, due to excessive reactive oxygen species production following repeated obstructive apnea or hypopnea attacks.

Moreover, lactoferrin was found in the present research to be inversely correlated with fasting and 2 h PPG levels. OSA patients were shown in previous population-based studies to be more susceptible to glucose intolerance and diabetes mellitus [37], with hyperglycemia and advanced glycation end products being suggested to inhibit lactoferrin function [38].

Unexpectedly, the present study identified no significant effect of increased AHI or lowered mean or nadir nocturnal oxygen saturation on endothelial dysfunction among OSA patients, which might be explained by the short duration of study of these variables by polysomnography over one night only. Assessment of average oxygen saturation readings of several days might have shown a significant deleterious effect of low oxygen saturation on FMD% and plasma lactoferrin levels among OSA patients, with hypoxemia always considered to be the cornerstone pathogenic factor of oxidative stress applied on the endothelium.

Conclusion

The current study showed that OSA patients were characterized by lower circulating plasma lactoferrin level, which was proved to be the only independent predictor for endothelial dysfunction evaluated by FMD%. In addition, increased BMI was found to be the independent predictor for lower plasma lactoferrin level in those patients.

Taking the small sample of the current study into consideration warranted its replication in a larger scale among OSA patients, with further verification of the mechanisms of the suggested protective effect of lactoferrin. Its potential clinical usefulness as a marker for response to treatment of OSA by nonsurgical or surgical therapeutic methods, including weight reduction and noninvasive positive pressure ventilation, might also be valuable for study in the future. Prospective studies to examine the effect of animal milk protein ingestion, which contains abundant amounts of lactoferrin [39], as an adjuvant therapy for OSA patients with evaluation of endothelial function might be considered as well.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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