Circulating Vascular Endothelial Growth Factor Levels in Patients with Obstructive Sleep Apnea

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Study Objectives: Obstructive sleep apnea (OSA) is associated with intermittent hypoxia during sleep. Vascular endothelial growth factor (VEGF) has detectable levels in the circulation and its expression is highly regulated by oxygen tension. We therefore hypothesized that serum VEGF levels will be elevated in patients with OSA.

Design: Blood samples were collected at random times during the day from 68 adults and 41 children who were clinically suspected for the presence of OSA, and who underwent overnight polysomnography.

Setting: University hospital sleep laboratory.

Participants: N/A

Interventions: N/A

Measurements and Results: For both children and adults, serum VEGF levels were significantly higher in polysomnographically confirmed OSA (AHI>15 and AI>5 in adults and children respectively) when compared to those with mild or no disease (p<0.0001). Furthermore, significant correlations were found between VEGF concentrations and respiratory disturbance index and sleep time spent at So2 <90%. In addition, VEGF levels in children were higher for any given duration of hypoxia during sleep (p<0.0001). No differences in VEGF emerged between evening and morning samples. However, temporal delays in blood sample processing were associated with spuriously increased VEGF concentrations. Exploratory analysis of the data revealed that serum VEGF concentrations of >150 pg/ml in adults and >100 pg/ml in children were predictive of OSA, when an apnea-hypopnea index >30 and an apnea index >5 were used as disease criteria in adults and children, respectively.

Conclusions: We conclude that circulating VEGF levels are frequently elevated in OSA patients, and may play a role in the regulation of tissue oxygen delivery.

Key words: Sleepiness; sleep apnea; alveolar hypoventilation; intermittent hypoxia; arousal

INTRODUCTION

SUSTAINED OR INTERMITTENT HYPOXIA IN MAMMALIAN TISSUES WILL INCREASE THE EXPRESSION OF PRO-ANGIOGENIC FACTORS SUCH AS VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF). VEGF is an endothelial cell-specific mitogen that increases oxygen delivery to peripheral tissues by stimulating angiogenesis and improving tissue capillary density.1 Increased VEGF protein expression occurs following hypoxia by enhanced transcriptional regulation of the VEGF gene through binding of the hypoxia inducible factor-1 (HIF-1) to an hypoxic responsive element in the 5' flanking region of the VEGF gene.2 VEGF expression is induced in vivo in various models of ischemia in heart, brain, lung as well as in certain tumors.3-11 However, even small changes in tissue oxygen tension within the physiologic range can lead to substantial alterations in VEGF expression.5 The unique activity of this factor in the regulation and proliferation of vascular beds allows VEGF to be measured in body fluids, such as serum, vitreous humor, and cerebrospinal fluid,6,9 where it can serve as a marker of tumor recurrence or as a prognostic factor.6-8 Furthermore, although hypobaric hypoxia exposures during high altitude climbing were not associated with significant VEGF changes in randomly collected serum samples in humans,12 circulating levels of VEGF were elevated in the serum of experimentally induced hypoxia of a rodent model.13

Obstructive sleep apnea (OSA) is a frequent condition characterized by intermittent hypoxia and alveolar hypoventilation frequently leading to arousal and sleep fragmentation. However, clinical history and physical examination are insufficiently sensitive to reach the diagnosis, and an overnight polysomnographic evaluation is therefore required. We hypothesized that circulating VEGF levels will be elevated in OSA patients, and may provide a surrogate biological marker for this condition. A brief report published well after the beginning of the present study has recently shown that both erythropoietin and VEGF serum levels are elevated in OSA patients with AHI>30.14 However, the investigators did not examine milder patients, nor did they assess potential correlations with sleep disturbances.

METHODS

Patients

Patients suspected for obstructive sleep apnea who were clinically followed at the Tulane or Kosair Children’s Hospital Comprehensive Sleep Medicine Centers were invited to participate in the study, which received institutional experimental human subject committee approval. Subjects were eligible if they...
VEGF ELISA Assay

In general, venous blood was drawn at random times of the morning, usually between 07:00 and 10:00, into glass tubes containing EDTA, citrate, or no anticoagulant. In a subset of patients, blood was drawn in the evening prior to the sleep study and the following morning. Tubes were centrifuged at 4°C for 10 minutes at 3,000 x g, and serum or plasma were frozen at -70°C until assay. In some samples, whole blood was separated into three fractions and incubated at 37°C. Centrifugation for separation of plasma was performed at 0h, 1h, and 4h. All assays for VEGF concentrations were performed by enzyme linked immunosorbent assay (ELISA) using a commercially available kit (R&D Systems, Inc., Minneapolis, MN). The lower sensitivity of the test is <3 pg/ml and linear results are obtained at a range of 7.0-1,000 pg/ml. With each assay, calibration curves were performed and showed linear sensitivity within the stipulated range as well as reproducibility agreement at r=0.99.

Data Analysis: For all patients, circulating VEGF concentrations as sampled in the morning were plotted against corresponding AHI or AI as appropriate. For statistical comparison purposes, adults with AHI < 15/hr TST, and children with AI < 5/hr TST were considered as having mild or no OSA and were added to the control group. In addition, VEGF levels were also examined in relation to patient age and percentage of TST in which SpO2 <90%. Linear regression analysis was performed to evaluate potential relationships between above variables. Using exploratory analysis of the data, the best cutoff values for serum VEGF levels in diagnosing OSA were calculated from the receiver operator curve as sensitivity, specificity, positive and negative predictive values. Subsequent analysis on the potential incremental value of VEGF serum levels to clinical judgment was conducted by calculating the likelihood ratios. A p-value <0.05 was considered to achieve statistical significance.

RESULTS

Sixty-eight adults and 41 children completed the study.
Subject characteristics are shown in Table 1. Adults had more severe respiratory disturbances during their sleep compared to children as indicated by significantly higher apnea index and percentage time spent with SpO₂ <90% (Table 1). Of the 68 adults, 14 (21%) had AI<15/hr TST, and 21 children (51%) had AI<5/hr TST, and were therefore considered as controls.

Serum VEGF concentrations were higher in both adult and pediatric OSA patients compared to corresponding controls. Indeed, serum VEGF levels in children with OSA were 220±112 pg/ml compared to 66±23 pg/ml in controls (p<0.0001).

Table 2—Predictability of OSA at 2 severity AHI or AI criteria using a cutoff value for serum VEGF concentration of >150 pg/ml in 68 adult patients and >100 pg/ml in 41 children

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Figure 1—Scatterplot of serum VEGF levels and respiratory disturbance index in 68 adult (open circles) and in 41 pediatric (closed squares) patients. Linear regression analysis (solid line—children; dashed line—adults) revealed significant correlation coefficients (children - r: 0.81; p<0.0001; adults - r:0.83; p<0.0001). The mean slopes of the regression lines were 13.1 pg/ml/apneic event/hr TST in children and 38.7 pg/ml/apnea-hypopnea event/hr TST in adults.

Figure 2—Scatterplot of serum VEGF levels and cumulative desaturation time (SpO₂<90%) in 68 adult (open circles) and in 41 pediatric (closed squares) patients. Linear regression analysis (solid line—children; dashed line—adults) revealed significant correlation coefficients (children - r: 0.83; p<0.0001; adults - r:0.84; p<0.0001). The mean slopes of the regression lines were 52.6 pg/ml/%TST in children and 11.3 pg/ml/%TST in adults.
Similarly, serum VEGF concentrations in adult patients with OSA were 198±144 pg/ml vs. 77±76 pg/ml in controls (p<0.001). Serum VEGF levels exhibited linear correlations with both AHI and the duration of oxyhemoglobin desaturation (SpO2 <90%) during the night (Figures 1 and 2), but patient age did not contribute to the variance in the adult cohort. However, VEGF levels were higher in children than in adults for any given hypoxic duration (Figure 2; p<0.0001). Indeed, the mean slope of the regression line was 52.6 pg/ml/%TST with SpO2<90% in children compared to 38.7 pg/ml/%TST with SpO2<90% in adults (p<0.02). In addition, no significant relationship was found between the degree of alveolar hypoventilation during sleep in children and their corresponding VEGF concentrations.

The optimal OSA diagnostic cutoff for serum VEGF concentration in adult patients was calculated at 150 pg/ml. Table 2 shows the sensitivity, specificity, and positive and negative predictive values when a AHI >30 or >40 are used as criteria for diagnosis. In children, the optimal cutoff value for serum VEGF was 100 pg/ml, and AHI values of >5 and >10 were employed as diagnostic criteria (Table 2). Circulating VEGF concentrations exhibited overall satisfactory predictive values of OSA in both groups, as determined by the likelihood ratio calculations. Indeed, assuming a pre-test probability of 0.850 for adults and 0.700 for children, addition of serum VEGF testing would have improved the post-test probability to 0.988 for a AHI >30 and for AHI>40 in adult patients, and to 0.985 and 0.980 for AHI >5 and >10 in children, respectively.

Plasma levels were significantly lower than serum levels in 14 patients, but were significantly correlated (r=0.70; p=0.005; Figure 3). In 15 adult OSA patients, the morning serum VEGF levels were higher in four patients, were unchanged in six patients, and decreased in five patients (Figure 4). Incubation of whole blood for four hours was associated with time dependent increases in VEGF concentrations in both control and OSA patients (Figure 5). However, the rate at which VEGF changed over time was similar in both groups (Figure 5).

**DISCUSSION**

In the present study we found that serum VEGF concentrations are elevated in patients with OSA. Moreover as a group, children have higher serum VEGF increases than adults, when the degree of respiratory disturbance or hypoxemia are taken into account. Circulating VEGF concentrations are positively correlated with the severity of OSA when the latter is expressed as the respiratory disturbance index or as the percentage of total sleep duration in which SpO2<90%. In addition, we show that evening to morning changes in VEGF levels are not sensitive to respiratory events occurring during sleep, and that plasma levels are lower than those measured in serum. Finally, in vitro incubation of whole blood samples of OSA patients and controls is associated with increasing VEGF concentrations over time but the rate of increase is similar in the two groups.

Elevated serum VEGF concentrations have now been reported in several conditions that are not inherently associated with the typical intermittent hypoxemia seen in OSA. For example, elevated VEGF levels were found in a cohort of patients with cystic fibrosis, and were decreased after antibiotic therapy associated with clinical improvement in inflammatory markers. Similarly, increased VEGF levels occur in several oncological conditions including brain, breast, and colorectal cancers, and have been proposed as clinically applicable prognostic markers. Interestingly, patients undergoing a surgical procedure will also temporarily increase circulating VEGF levels, suggesting that multiple pathophysiological pathways such as inflammatory processes and stress may underlie the up-regulation of VEGF expression.

Our study confirms the lower concentrations of VEGF found in plasma when compared to serum. Such consistently found dif-
ferences between these often indistinguishably used blood samples could clearly affect the overall interpretation of the laboratory assay in clinical practice. Indeed, in conditions associated with either changes in platelet density\(^{29-32}\) or in conditions associated with altered leukocyte counts\(^{33}\) substantial differences may occur between plasma and serum samples. However, a robust linear relationship between plasma and serum concentrations was apparent in the subset of OSA patients studied herein, suggesting that serum samples can be potentially used in the setting of sleep-disordered breathing provided no concomitant medical conditions that affect platelet or leukocyte counts are present. Notwithstanding the potential limitations of VEGF sample processing, emphasis on achieving minimal delays between sample collection and separation cannot be overemphasized. Indeed, we also show in this study that incubation of whole blood collected in tubes containing an anticoagulant such as EDTA or citrate will lead to significant increases in VEGF concentrations over time. Therefore in the absence of strict criteria for the processing of blood samples, spurious VEGF elevations in otherwise healthy individuals may jeopardize the potential value of VEGF as an adjunct laboratory test in the diagnosis of more severe OSA, and this consideration needs to be included in future studies.

Parenthetically, it should be stressed that we did not explore the effect of anti-hypertensive or other medications, a frequent

Figure 4—Individual serum VEGF concentrations in the evening (PM) and following morning (AM) in 15 OSA patients undergoing overnight polysomnography.

Figure 5—Individual VEGF concentration changes from baseline (0) following 1 and 4 hours of incubation at 37°C in 6 controls and 9 OSA patients. In all subjects, increases in VEGF levels occurred over time but no differences emerged between OSA patients and controls.
occurrence in the adult population requiring screening for OSA, on serum VEGF concentrations. In addition, serum VEGF may not provide adequate predictive values in patients with frequent short apneic episodes without significant desaturation or conversely may become partially false positive, if a particular patient presented low AHI but significant hypoxemia. However, the dynamic responses of serum VEGF concentrations in patients with OSA could underlie the individual subject’s ability to compensate for changes induced by tissue hypoxia. More specifically and as recently shown by Schultz and colleagues, the interindividual heterogeneity in VEGF responses was closely correlated with the degree of new collateral formation in patients with coronary artery disease. It remains to be shown whether increased brain susceptibility to the intermittent hypoxia of OSA or acceleration of atherosclerotic processes in the microcirculatory network of patients with OSA reflects an overall insufficient VEGF response in these more vulnerable patients.

The increased susceptibility of children to raise VEGF circulating levels at comparable hypoxic exposures during sleep could be due to recently uncovered age-related changes in the binding and activity of HIF-1. Such differences are of sufficient magnitude such as to require a modification of the proposed optimal VEGF cutoff levels in children and adults. These age-group-defined cutoff levels appear to perform satisfactorily in our sample populations when prediction of more severe OSA is assessed using random VEGF levels. In fact, despite such encouraging findings, large scale prospective studies will obviously be necessary to confirm whether randomly obtained circulating VEGF concentrations can accurately identify the more severe adult and pediatric patients with OSA.

In summary, we have shown that elevated VEGF levels occur in OSA patients and that such increases are uniquely sensitive to sampling and processing methods of the blood samples. Furthermore, VEGF concentrations exhibit age-dependency and display significant positive correlations with OSA severity.

ACKNOWLEDGMENTS

We are grateful to the patients for their patience and cooperation and to the sleep technologists for their dedicated work.

REFERENCES


