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ORIGINAL ARTICLE

Hypoxia induced factor-1α levels in patients undergoing adenoidectomy

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ABSTRACT

Among the most common causes of nasal congestion in childhood is adenoid hypertrophy (AH) which leads to hypoxia. In this study, we studied plasma concentrations of hypoxia induced factor-1α (HIF-1α) in children undergoing adenoidectomy. The study included a total of 86 participants: 39 patients with AH and 47 healthy individuals. Serum HIF-1α levels (ng/mL) were measured by ELISA. HIF-1α concentrations were compared to the adenoid-nasopharyngeal ratio (ANR) of patients with AH, as recorded in the medical records. We found significantly higher concentrations of HIF-1α (0.30 ± 0.47 ng/mL) in patients with AH as compared to healthy controls (0.24 ± 0.07 ng/mL, $p = .011$). HIF-1α levels were not significantly different regarding gender between patients with AH ($p = .77$) and in the control group ($p = .97$). In patients with AH, there was a moderately significant positive correlation between HIF-1α levels and Hb ($p = .000$), (correlation coefficient $r = 0.542$). There was a positive correlation between HIF-1α and ANR in patients with AH ($p = .005$, $r = 0.439$). This study indicates that AH increases HIF-1α levels. We also observed a moderately significant positive correlation between HIF-1α and ANR in patients with AH. HIF-1α levels are a potential biomarker for hypoxia in patients with AH.

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Adenoids; adenoidectomy; hemoglobin; hypertrophy; hypoxia; hypoxia-inducible factor-1 alpha; receiver operating characteristics

Introduction

The adenoids are a mass of nasopharyngeal lymphoid tissue forming part of Waldeyer's ring which acts as a defense barrier against nasal microorganisms. The adenoid tissue reaches its maximum size between the ages of 3 and 7, and then gradually regresses [1–3]. While this lymphoid tissue is a very small at birth, it may block the airway due to hypertrophy during immune system development because of frequent bacterial infections [2,4]. In these cases, hypertrophy of the adenoids can cause symptoms such as mouth breathing, nasal discharge, snoring, sleep apnea, and hyponasal speech [5–7].

Hypertrophy of adenoid tissue is common in children. Radiological imaging of adenoid tissue is an objective, non-invasive, and easy method. Determining the adenoid-nasopharyngeal ratio (ANR) from these images assists physicians with the diagnosis. However, the gold standard for diagnosis is a physical examination with flexible endoscopy [8–11].

Although the pathological mechanisms underlying the development of adenoid hypertrophy (AH) have not been fully elucidated, it is thought that elevated levels of inflammation and infection lead to production of free oxygen radicals. This results in increased lipid peroxidation and cell damage. AH cause upper airway obstruction. Elevated hypoxia due to the upper air obstruction is also thought to contribute to pathology [10,12,13]. Increased blood cell production is a classic physiological response to systemic hypoxia. One way in which cells respond to reduced oxygen levels is through the hypoxia-inducible factor HIF pathway [14,15].

Hypoxia inducible factors (HIFs) regulate oxygen sensitivity mechanisms and hypoxic cell metabolism [15]. HIF-1 is a heterodimeric transcription factor that has been widely characterized. Levels of HIF-1 increase when cells are exposed to hypoxic conditions. HIF-1 consists of an unstable HIF-1α subunit, which is regulated by the amount of O_2 and determines HIF-1 activity, and a stable HIF-1β subunit, which is expressed in both hypoxic and normal O_2 levels [15–17]. Of these two subunits, HIF-1α has been extensively investigated under various disease conditions, as it plays important role in numerous cellular pathways, including glucose metabolism, erythropoiesis, angiogenesis, cell growth, and differentiation, acting on more than 40 genes in these processes [17,18]. In conditions where oxygen levels are normal, HIF-1β remains stable in all cells and HIF-1α is rapidly ubiquinoned and degraded. However, under conditions of O2 deficiency, HIF-1α degradation is prevented, which leads to the formation of a stable HIF-1α molecule resulting in activation of many downstream intracellular processes [17,19]. Erythropoietin (EPO), one of the HIF-1 sensitive proteins, is released from the kidneys in response to cellular hypoxia and increases erythropoiesis. Hypoxic conditions are responded by increased HIF-1 and increased erythropoiesis [18,20].

We hereby pioneer in investigating the plasma concentrations of HIF-1α in children with adenoidectomy.

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Experimental procedures

Study population

Research participants included the group of patients of the otorhinolaryngology polyclinic of the Konya Education and Research Hospital who visited between 1 July 2019 and 1 March 2020. Among the group there were 39 patients diagnosed with AH (without tonsillar hypertrophy) who were admitted to the Department of Otorhinolaryngology and 47 healthy volunteers without any known illness. Patients included those who were diagnosed with AH by clinical-radiological examination during this period. Adenoidnasopharyx ratio (ANR) was determined using an obstruction scoring ratio. Adenoid–nasopharynx ratio correlated with endoscopic and radiological findings.

Blood samples were obtained from the patients and controls during routine procedures with no further invasive procedures. Age, gender, hemoglobin (Hb) and ANR values of patients with AH were recorded based on information listed in hospital records.

The inclusion criteria included children of 4–15 years of age who required adenoidectomy, without tonsillectomy, due to hypertrophy of the adenoids causing upper airway obstruction, sleep apnea, snoring, and hyponasal speech. The control group comprised of children of the same ages who were brought to the polyclinic for vaccinations or health examinations and they had no history of upper airway obstruction, recurrent adenotonsillitis, adenoid hypertrophy any other reported health problems.

The exclusion criteria were: age of child (younger than four and older than fifteen years), cases with cleft palate, history of cleft palate repair, cases with bleeding or coagulation defects, cases that missed the follow-up appointments, cases with acute and chronic infection, cases with underlying congenital or chronic diseases (craniofacial anomalies, pulmonary, cardiac, or neurological diseases, etc.), and those with a history of allergies to anesthesia.

Ethical considerations

The study was approved by the ethics committee of the KTO Karatay University, Health Sciences Institute, Konya, Turkey (Ethics Committee No. 2019/0040, 18 June 2019).

Biochemical analysis

Peripheral venous blood samples were collected from patients before adenoidectomy. The blood samples were stored at −80 °C after centrifugation at 3000 rpm for 15 min. Serum HIF-1α levels were measured by an enzyme-linked immunosorbent assay (ELISA) (SEA798Hu, Cloud-Clone Corp. Export Processing Zone, Wuhan, Hubei, China). The ELISA assay was performed according to the manufacturer's instructions. The linearity of the kit was assayed by testing samples spiked with appropriate concentration of HIF1a and their serial dilutions. The results were calculated using the percentage of calculated concentration to the expected. Intra-assay and inter-assay coefficients of variation (CVs) were <10% and <12%, respectively. The minimum detectable concentration of HIF-1α was 0.059 ng/mL, and the assay diagnostic interval was 0.156–10 ng/mL.

Statistical analysis

The data analysis was performed using the Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, IL). A two-sided p value of less than .05 was considered statistically significant. Arithmetic mean ± standard deviation, and minimum and maximum values were used to summarize numerical data, and frequency distributions and percentages were used to summarize categorical data. The Shapiro–Wilk test was used in normality analysis to examine the distribution of numerical data. To determine statistically significant differences between groups of continuous variables, a Student's t test was used for variables Gaussian-distributed and a Mann Whitney U test was used for non-Gaussian variables. The presence of a correlation between the groups was analyzed with Spearman correlation test. The receiver operating characteristic (ROC) curve was used to determinate the sensitivity and specificity of HIF-1α, and the cut-off values in predicting AH.

Results

Eighty-six children (39 patients with AH and 47 healthy controls) fit the inclusion criteria and were included in the study. There were no age and gender differences between the patients with AH and the control group ($p > .05$). All patients had complaints of chronic nasal obstruction and the obstruction levels was above 0.70 in 74.3% of study group (Table 1).

There were significantly higher levels of HIF-1α in patients with AH (0.30 ± 0.47 ng/mL) as compared with healthy controls (0.27 ± 0.07 ng/mL). There was a statistically significant difference in HIF-1α levels ($p = .011$) between the study and control groups (Figure 1). HIF-1α levels were not significantly different in terms of gender between patients with AH ($p = .77$) and in the control group ($p = .97$) (Table 2).

The mean Hb value of patients with AH was 13.5 ± 1.0. There was no difference in Hb parameters in terms of gender in patients with AH ($p = .48$). In patients with AH, there was a moderately significant positive correlation between HIF-1α levels and Hb ($p = .000$) (correlation coefficient $r = 0.542$) (Figure 2). But there was no correlation between Hb and ANR ($p = .06$) (correlation coefficient $r = 0.28$).

In our study group, the mean ANR value of patients with AH was 0.80 ± 0.21. No correlation was seen between ANR and age or gender in patients with AH ($p = .62$, $p = .93$, respectively). In patients with AH, there was a

Table 1. Distribution of patients according to ANR values.

ANR (%)	<60	60–69	70–79	80–89	>90
n	3	7	8	5	16
%	7.7	18	20.5	12.8	41

moderately significant positive correlation between HIF-1α levels and ANR ($p = .005$) (correlation coefficient $r = 0.439$) (Figure 3).

On diagnostic performance analysis, the ROC curve demonstrated the cut-off value of HIF-1α as 0.275 or higher, the sensitivity level as 74.4%, the specificity as 55.3%, positive predictive value as 58.0% and negative predictive value as 72.2% in terms of AH [area under curve (AUC) = 0.659; 95% confidence interval (CI) = 0.543–0.775)] (Figure 4).

The medical records of patients with AH were scanned to assess whether malignancy was detected. All patients were diagnosed with reactive lymphoid hyperplasia and no malignancies were observed.

Discussion

Adenoid hypertrophy, causes snoring, mouth breathing, apnea, restlessness, sinusitis, anorexia, and other diseases, and seriously affects the growth, development, and quality of life in children [1,4,21]. HIF-1α plays a key role in oxygen homeostasis in mammalian cells, and is active under hypoxic conditions [15]. In our study, we hypothesized that hypoxia would increase in AH, resulting in evaluated levels of HIF-1α.

AH is a common condition in children and leads to hypoxia by blocking the upper airway. The main purpose of the adenoidectomy is to eliminate the nasopharyngeal reservoir of potential respiratory pathogens and to remove the cause of obstruction in the nasal airway [6,22,23]. In most children, enlarged adenoids can obstruct breathing patterns and can cause upper respiratory tract obstruction. Airway obstruction can be due to adenoid size alone and can lead to hypoxia [4,9,10,24]. ANR is used to determine nasopharyngeal obstruction ratio. Fujioka et al. found that in 34 out of 36 cases (94%) of AH, the ANR was above 0.80 [9]. In another study conducted by Eyibilen et al. examined adenoid tissue size and ANR values in 100 cases and compared these results with postoperative adenoid weight. The findings suggest a significant relationship between ANR and adenoid weight ($r = 0.46$, $p < .001$) [25]. In our study, the average ANR was 0.80 ± 0.21, similar to the results of other studies. As we are determining ANR values from data obtained from patient files, we were unable to measure adenoid weights and we could not make any comparisons.

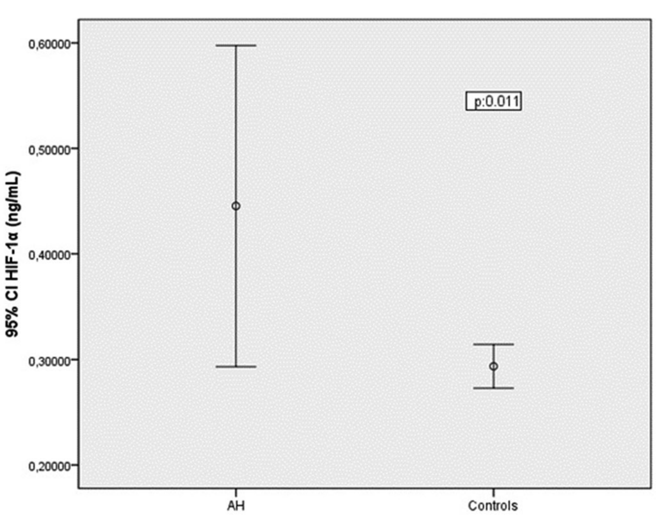

Figure 1. HIF-1α levels in patients with AH and control group.

Hypoxia occurring in the body also increases the oxidative stress markers. There are also studies showing that malondialdehyde, one of which is used in the evaluation of oxidant damage, is increased in AH [26,27]. Akçay et al. observed a positive correlation between adenoidectomy and/or tonsillectomy and asthma [28]. Several studies in Nigeria have also validated these findings and noted that tonsillectomy and adenoidectomy are performed due more to obstructive hypertrophy [29,30].

Hypoxia is a fundamental stimulus that impacts cells, tissues, organs, and physiological systems. The discovery of hypoxia-inducible factor-1 (HIF-1) and subsequent identification of other members of the HIF family of transcriptional activators has provided insight into the molecular underpinnings of oxygen homeostasis [31,32]. HIF-1α is one of them and hypoxia is expected in adenoid hypertrophy depending on the size of the adenoids. In our study, there was a positive correlation between HIF-1α levels and ANR, supporting the hypothesis that hypoxia is associated with AH. It has been supported with studies that hypoxia increases HIF-1 and HIF-1 activates the EPO pathway to correct hypoxic conditions and increases hemoglobin. The positive correlation between HIF-1α and Hb levels in patients with AH in our study also supports this information. In our study, there are no blood gas values of the patients group. However, we think that the increase in HIF-1α and Hb values is due to hypoxia caused by adenoids, since we excluded most diseases before surgery in children who underwent adenoidectomy. In a retrospective study comparing HIF-1 gene polymorphism with Hb concentrations, it was reported that there was no relationship between HIF-1 polymorphism and Hb [33]. However, the study was conducted retrospectively and other factors that could cause hypoxia were not excluded. In order to support the findings in our study, studies with larger numbers of patients and using blood gas values are needed.

The current study is the first to evaluate the serum HIF-1α levels in patients with AH. Consistent with these findings, HIF-1α levels in patients with AH were higher than the control group ($p = .011$) supporting the hypothesis that hypoxia is caused by obstruction of the airway. HIF-1α

Table 2. Comparison of age, gender and HIF-1α parameters of the AH patients and controls.

Characteristics	AH ($n = 39$) (median ± SD ± IQR)	Controls ($n = 47$) (median ± SD ± IQR)	p value
Age	8.0 ± 2.85 (5–8)	7.9 ± 2.75 (6–11)	>.05
Gender (F/M)	20/19	24/23	>.05
HIF-1α (ng/mL)	0.30 ± 0.47 (0.27–0.42)	0.27 ± 0.07 (0.24–0.32)	.011*

*Indicates statistical significance $p < .05$. IQR: interquartile range.

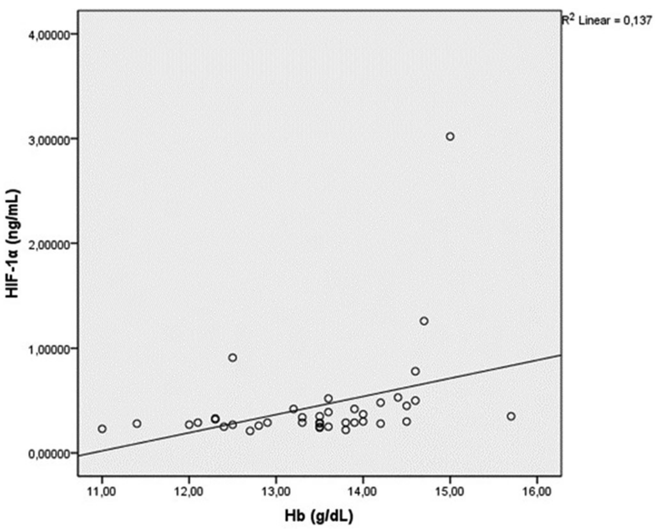

Figure 2. HIF-1α and Hb correlation graph in patients with AH.

levels were previously examined in adenoid cystic carcinoma [17]. S100 Calcium Binding Protein B known to be elevated in hypoxia was studied in chronic adenotonsillar hypertrophy and statistically significant differences between the groups were obtained [12]. However, HIF-1α levels in AH have not been previously reported. For this reason, we could not find an article to compare we attempted to do it in our work.

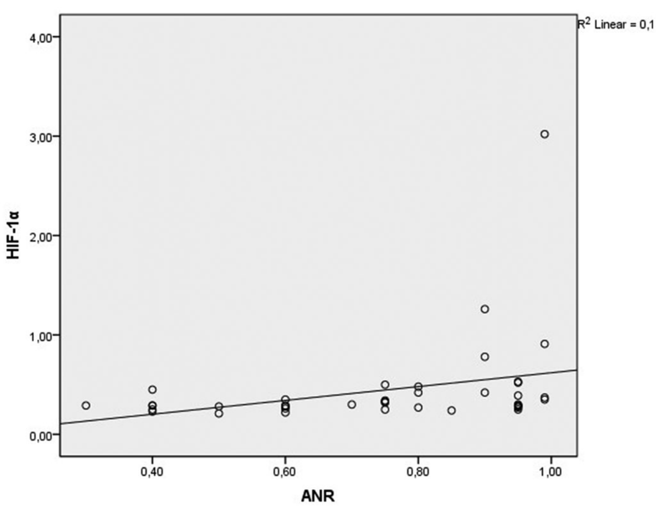

Figure 3. HIF-1α and ANR correlation graph in patients with AH.

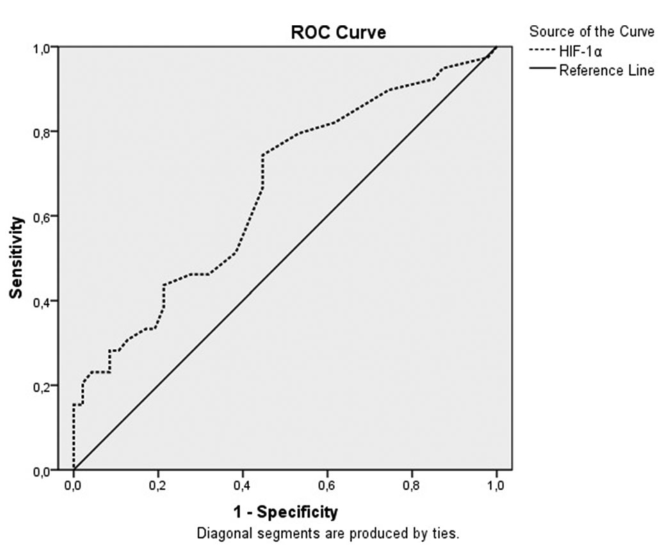

Figure 4. ROC analysis for HIF-1α (AUC = 0.659).

Limitations of the study

This study has limitations due to a small sample size and lack of measurements other than ANR in the patients records studied. Although we attempted to include more patients in this study, a number of patients who had adenotonsillectomy surgery were excluded from the study. Furthermore, if the HIF-1α concentrations before and after surgery had be compared, the design had been substantially better. Unfortunately, we were unable to contact the patients for renewed sampling and analysis after the operation.

Conclusion

Treating AH prevents infections and protects children from hypoxia. This study shows that HIF-1α level increase in patients with AH. A moderately significant positive correlation was found between HIF-1α and ANR values in these patients. Based on the information we have obtained, we hypothesize that HIF-1α level can be used as an additional diagnostic marker for the AH.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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