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# The potential inflammatory role of arginase and iNOS in children with chronic adenotonsillar hypertrophy

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**KEYWORDS**
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**Summary**

*Objective:* Nitric oxide (NO) induced tissue damage has been implicated in the pathogenesis of several diseases. Although recurrent/chronic tonsillitis and hypertrophy are still the most frequent surgical procedures carried out on children in order to cure these pathologies, etiopathogenetic mechanisms underlying these entities are still unknown. We aimed to investigate the potential inflammatory role of NO regulatory enzymes, arginase and inducible nitric oxide synthase (iNOS), in children with adenotonsillar hypertrophy.
*Materials and methods:* The study consisted of 22 children with chronic adenotonsillar hypertrophy and 30 control subjects with similar age and sex. All the patients and/or their parents had complaints of snoring, mouth breathing and pausing of breathe during sleep at least 6 months. All patients underwent an adenotonsillectomy operation under general anesthesia with curettage and cold dissection methods. Venous blood samples were taken pre-operatively and 4 weeks post-operatively. iNOS activity was based on the diazotization of sulfanilic acid by nitric oxide at acid pH and subsequent coupling to *N*-(1-naphthtyl)-ethylenediamine. Arginase activity was measured by the spectrophotometric method.
*Results:* The mean pre-operative and post-operative arginase activities in patient group were $4283.7 \pm 1823.7$ and $2754.5 \pm 889.3$ IU/L, respectively. In the control group, mean arginase activity was $2254.7 \pm 903$ IU/L. When pre-, post-operative and control arginase values were compared with each other, the mean activity in pre-operative activity was significantly different from the post-operative and control values ($p < 0.001$). In the patient group, the mean levels of pre- and post-operative

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iNOS were $2.84 \pm 1.16$ and $1.99 \pm 0.78$ IU/ml, respectively. The difference was statistically significant ($p = 0.007$). Similarly, post-operative and control values were not significantly different ($p > 0.05$).
*Conclusion:* The results of the present study supports that L-arginine:NO pathway may be key the participant in the pathogenesis of chronic adenotonsillar disease; arginase and iNOS activities are altered in children with adenotonsillar hypertrophy and this alteration improves after tonsillectomy.


## 1. Introduction

Adenotonsillar diseases are one of the most common health problems in pediatric population. Although recurrent/chronic tonsillitis and hypertrophy are still the most frequent surgical procedures carried out on children in order to cure these pathologies, etiopathogenetic mechanisms underlying these entities are still unknown. In recent years, adenotonsillar hypertrophy has been attracting interest in regard of its pathological, immunological and clinical features.

Arginase is the final enzyme of the mammalian urea cycle and catalyzes the hydrolysis of arginine to urea and ornithine [1]. Recent studies have demonstrated that arginase is induced by cytokines in several cell types, especially in macrophages at inflammatory sites [2]. Macrophage infiltration and activation are frequently found in chronic tonsillar inflammation [3]. Arginase is located in the cystosol and can compete with other cytosolic enzymes such as nitric oxide synthase (NOS) that use arginine as a common substrate [4]. There is considerable interest in the possibility that arginase could limit nitric oxide (NO) production via inducible NOS (iNOS). Arginase contributes to wound healing by inhibiting NO production and increasing proline and polyamines [2]. The formation of polyamines leads to the hypertrophy of lymphoid tissue [3].

NO plays an important role in many biochemical processes such as regulation of blood vessel dilatation and immune response, and functions as a neurotransmitter [5,6]. In pathological conditions, NO has a bactericidal or tumoricidal functions; however, excess NO production may cause several diseases such as septic shock, autoimmune disease, cerebral infarction, diabetes mellitus, etc. [6].

In this study, we aimed to investigate the potential inflammatory role of the arginase and iNOS (NO regulatory enzymes) in children with chronic adenotonsillar hypertrophy.

## 2. Materials and methods

The study consisted of 22 children with adenotonsillar hypertrophy and 30 control subjects with similar age and sex. The ages ranged between 4 and 9 years old (mean age: 6). Male female ratio was 3:2. All the patients and/or their parents had complaints of snoring, mouth breathing and pausing of breathe during sleep at least 6-months. We performed a complete ear—nose—throat examination, which was supported with nasal and nasopharyngeal rigid endoscopy in appropriate cases for assessment of adenoid size. Nasopharyngeal air column was imaged with lateral skull radiography in all patients. Hypertrophy of the tonsils was graded according to the criteria of Brodsky et al. [7]. Only patients with Grade 3 and 4 tonsils were included into the study. All patients underwent an adenotonsillectomy operation under general anesthesia with curettage and cold dissection methods. Control subjects were selected among the children, who admitted to our outpatient clinic with no adenotonsillar disease, upper and lower respiratory tract infection and obstruction. Exclusion criteria included head and neck malformation, patients with identified syndromes and neurological or any other systemic diseases, allergy and infection. Also subjects with recent upper respiratory tract infection or ongoing infection were excluded from the study. All subjects and their parents gave written informed consent.

Venous blood samples were taken pre-operatively (1 week before the operation) and 4 weeks post-operatively and were put into anticoagulated (citrate, 3.5 mg/ml blood) glass tubes and, plasma and erythrocyte were separated. For preparation of erythrocytes from other blood cells, samples were washed and centrifuged with serum physiologic solution in three times as described by Beutler [8]. Erythrocytes were then hemolysed by diluting with deionized water (50-fold). Analyses were carried out in this hemolysed supernatant fraction. All the procedures were performed at +4 °C throughout the experiments. iNOS activity was based on the diazotization of sulfanilic acid by nitric oxide at acid pH and subsequent coupling to *N*-(1-naphthtyl)-ethylenediamine [9]. Results were expressed as IU/ml sediment.

Arginase activity was measured by the spectrophotometric method based on the hydrolysis of arginine by arginase yielding ornithine and urea.