



# Is There a Difference between the Preoperative and Postoperative Serum Levels of Interleukin-6 and Tumor Necrosis Factor- $\alpha$ in Children Submitted to Adenotonsillectomy?

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## Abstract

**Introduction** Palatine and pharyngeal tonsils are the first line of defense against pathogens. Clinically, two alterations may require surgical removal of the tonsils: hypertrophy and recurrent tonsillitis. The two conditions probably result from a dysfunction of the immune system.

**Objective** To evaluate possible differences in the plasma levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and interleukin-10 (IL-10) in patients submitted to adenotonsillectomy.

**Methods** Prospective, longitudinal study with 25 children undergoing adenotonsillectomy separated into 3 different groups: recurrent tonsillitis (RT), composed of 7 patients; recurrent hypertrophy tonsillitis (RTTH), with 8 patients; and the tonsillar hypertrophy (TH) group, with 10 patients. Ten healthy control children (SD) were also included in the study. Peripheral blood was collected, and plasma was separated to measure the levels of TNF- $\alpha$ , IL-6, and IL-10. The Mann-Whitney test was used for statistical analysis.

**Results** The plasma level of IL-6 was higher in the RT ( $p = 0.0394$ ) and TH ( $p = 0.0009$ ) groups, compared with the control group. The TH group also had higher levels of IL-6 than the RT group ( $p = 0.039$ ). The IL-6/IL-10 ratio was higher in the RT ( $p = 0.029$ ) and TH ( $p = 0.0005$ ) groups compared with the control group. Between the RT and RTTH groups, the IL-6/IL-10 ratio was higher in the RT group, with a statistically significant difference ( $p = 0.0091$ ).

**Conclusion** Patients with a history of chronic tonsillitis had higher levels of IL-6, compared with the control group.

## Keywords

- ▶ chronic tonsillitis
- ▶ tonsillar hypertrophy
- ▶ adenotonsillectomy
- ▶ cytokines

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## Introduction

The tonsils form part of the mucosa-associated lymphoid tissue (MALT), in particular the nasopharynx-associated lymphoid tissue (NALT), and are rich in B and T lymphocytes, which are core components of the immune system.<sup>1-4</sup> The tonsil's histological structure is, therefore, closely related to its immunological role. In children, the density of lymphocytes in the tonsils is greater than in adults,<sup>5</sup> with T-cells (helper and cytotoxic) and B cells being greatly increased when the tonsils are affected by pathological processes.<sup>6</sup>

There are two main clinical manifestations of an affected tonsil: tonsillar hypertrophy and recurrent tonsillitis.<sup>7</sup> In cases of hypertrophy, the tonsils increase in size, with no signs of inflammation, the main symptom being obstruction of the upper airways like in obstructive sleep apnea syndrome (OSAS), characterized by repetitive upper airway occlusion that causes intermittent hypoxia. Conversely, tonsillitis presents as an infectious/inflammatory process, often caused by viral or bacterial agents. These infections trigger local and systemic reactions that are often mediated by cytokines.<sup>8</sup>

Cytokines are produced in a cascade by various types of cells at the site of the lesion, that is, a cytokine may stimulate its target cells to produce more cytokines. These substances bind to specific receptors, activating intracellular messengers that regulate gene transcription. They affect the activity, differentiation, proliferation, and survival of different cells, including the cells of the immune system, and also regulate the production and activity of other cytokines. Some cytokines may exert proinflammatory (Th1 profile) or anti-inflammatory activities (Th2 profile) according to the microenvironment in which they are located. Those considered proinflammatory include IL-6 and TNF- $\alpha$ , while those considered antiinflammatory include IL-10.<sup>9,10</sup>

Under antigenic stimulation, the intraepithelial T lymphocytes present in the tonsillar crypts produce Th1 proinflammatory and Th2 antiinflammatory responses. The proinflammatory Th1 cytokines that are then released include interleukin-2 (IL-2), interferon- $\gamma$  (IFN- $\gamma$ ), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). The antiinflammatory Th2 cytokines include IL-4, IL-5, IL-6, and IL-13. All of these cytokines can be measured in peripheral blood.<sup>11</sup> The production of Th1 cytokines is dominant in immunocompetent patients. In acute tonsillitis, either viral or bacterial, the production of cytokines begins with type Th1, including IFN- $\gamma$  and TNF- $\alpha$ , and later progresses to the secretion of Th2 cytokines. This allows a balance to be reached in the defense against external agents, with subsequent control of the immunological response to ensure that the inflammatory process does not perpetuate.<sup>12,13</sup>

A significant increase in the levels of the inflammatory cytokines IL-6 and TNF- $\alpha$  was found in patients with chronic tonsillitis compared with a healthy control group.<sup>14</sup> Tumor necrosis factor  $\alpha$  and IL-6 are considered potent inflammatory cytokines, since they play an important role in the acute phase of the inflammation. The quantification of these

cytokines allows the extent of the inflammation to be calculated.<sup>13</sup>

Therefore, the objective of the present study was to evaluate possible differences in the plasma levels of TNF- $\alpha$ , IL-6, and IL-10 in patients submitted to adenotonsillectomy with clinical conditions of tonsillar hypertrophy and recurrent tonsillitis, either alone or in combination.

## Methods

### Ethical Aspects and the Study Population

The institute's internal review board approved the protocol of this prospective, longitudinal study under reference CAAE 28210014.1.0000.5078. The parents or guardians were given information on the study, and those willing to allow their children to participate read and signed the informed consent form. The children who were able to read signed an assent form.

Children receiving care at the otorhinolaryngology outpatient clinic at Hospital das Clínicas/Universidade Federal de Goiás, between January 2015 and January 2017, who were to undergo tonsillectomy or adenotonsillectomy due to tonsillar hypertrophy and were complaining of obstruction of the upper airways with rhonchi and/or breathing through the mouth or because of recurrent infection of the tonsils, were admitted to the study. Children with any other comorbidity were excluded. The patients were distributed into three different groups according to their clinical symptoms, medical history of tonsil infections, and the size of the tonsils.<sup>11</sup>

**Group 1 - Recurrent tonsillitis (RT):** Tonsil size grade I or II in children with a history of recurrent tonsillitis and at least 3 episodes/year for 3 consecutive years, or at least 5 episodes/year for 2 consecutive years, or more than 6 episodes of tonsillitis within a single year.

**Group 2 - Recurrent tonsillitis with hypertrophy (RTH):** Hypertrophied tonsils grade III or IV according to the Brodsky grading scale, associated with recurrent infections.

**Group 3 - Tonsillar hypertrophy (TH):** Tonsil grade III or IV with no history of infection of the tonsils.

**Group 4 - Control:** No history of upper respiratory tract infection or adenotonsillar hypertrophy.

### Peripheral Blood Sample Collection

A 3-ml sample of peripheral blood was collected into ethylenediaminetetraacetic acid (EDTA) vacuum tubes for both patients and controls. In the case of the patients, that sample was taken immediately prior to the surgical procedure and a second sample was collected 4 to 6 months after the surgery. The blood samples were sent to the immunoregulation laboratory of the Institute of Tropical Pathology and Public Health to measure cytokine levels.

### Preparation of the Samples

After centrifuging the blood samples at 2,000 rpm for 7 minutes, the separated EDTA plasma was frozen at -80°C until measurement of the cytokine levels.

### Cytokine Level Measurement

The levels of IL-6 and IL-10 were measured in plasma using the immunoenzymatic method called enzyme-linked immunosorbent assay (ELISA) (BD Bioscience, San Diego, CA, USA) in accordance with the manufacturer's instructions. Readings were performed using a Multiskan microplate spectrophotometer (Thermo LabSystems, Milford, MA, USA) at a 450 nm wavelength. The range of detection of the IL-6 kit used was 4.68 to 300 pg/mL, while the range of the IL-10 kit was from 7.8 to 500 pg/mL.

The TNF- $\alpha$  cytokine was measured using the Human TNF- $\alpha$  Standard ABTS ELISA Development Kit (Peprotech, Cranbury, NJ, USA) in accordance with the manufacturer's instructions. After 25 minutes, optical density was read using the Multiskan microplate spectrophotometer (Thermo Labsystems, USA) at 405 nm, using the 620 nm filter as reference wavelength. The detection range of the test was 62.5 to 4,000 pg/mL.

### Statistical Analysis

The data were analyzed using the GraphPad Prism statistical program, version 5.0. (GraphPad Software, San Diego, CA, USA). Medians were calculated and presented as a horizontal line. The groups were compared using the nonparametric Mann-Whitney U-test. Statistical significance was defined as  $p < 0.05$ .

## Results

### Sociodemographic Characteristics of the Study Groups

The overall study sample consisted of 35 children of 2 to 14 years of age (mean 7.04 years). Fourteen of the children were female and 21 were male. The RT group consisted of 7 patients, the RTTH group of 8, and the TH group of 10. Another 10 children formed the healthy control group ( $\rightarrow$ Table 1).

### Cytokine Profile in the Plasma of the Individuals Included in the Study

No statistically significant difference was found in the plasma levels of TNF- $\alpha$  between any of the study groups in relation to the control group ( $\rightarrow$ Fig. 1A). The plasma levels

**Table 1** Characteristics of the patients according to study group

Study group	Females	Males	Age (mean $\pm$ SD)	Total number of individuals
RT	3	4	7.8 $\pm$ 2.5	7
RTTH	3	5	6.5 $\pm$ 1.5	8
TH	4	6	6.9 $\pm$ 3.1	10
Control	4	6	10.0 $\pm$ 3.0	10

Abbreviations: RT, recurrent tonsillitis; RTTH, Recurrent tonsillitis with hypertrophy; SD, standard deviation; TH, tonsillar hypertrophy.

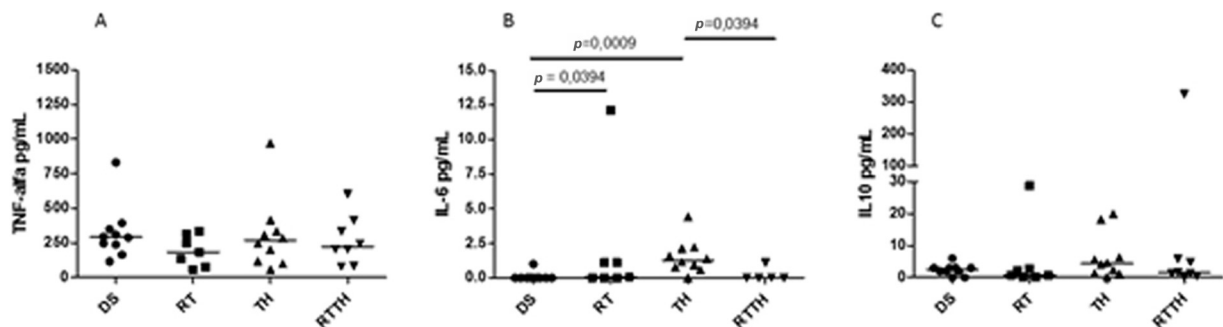
of IL-6 were higher in the RT ( $p = 0.0394$ ) and TH ( $p = 0.0009$ ) groups compared with the control group. The levels of IL-6 were also higher in the TH group compared with the RTTH group ( $p = 0.0394$ ) ( $\rightarrow$ Fig. 1B). No statistically significant difference was found in the plasma levels of IL-10 between any of the study groups in relation to the control group ( $\rightarrow$ Fig. 1C).

There was no statistically significant difference in the TNF- $\alpha$ /IL-10 ratio between any of the groups in the present study ( $\rightarrow$ Fig. 2A). The IL-6/IL-10 ratio was greater in the RT ( $p = 0.0293$ ) and TH groups ( $p = 0.0005$ ) compared with the control group ( $\rightarrow$ Fig. 2B). Between the RT and RTTH groups, the IL-6/IL-10 ratio was greater in the RT group ( $p = 0.0091$ ) ( $\rightarrow$ Fig. 2B).

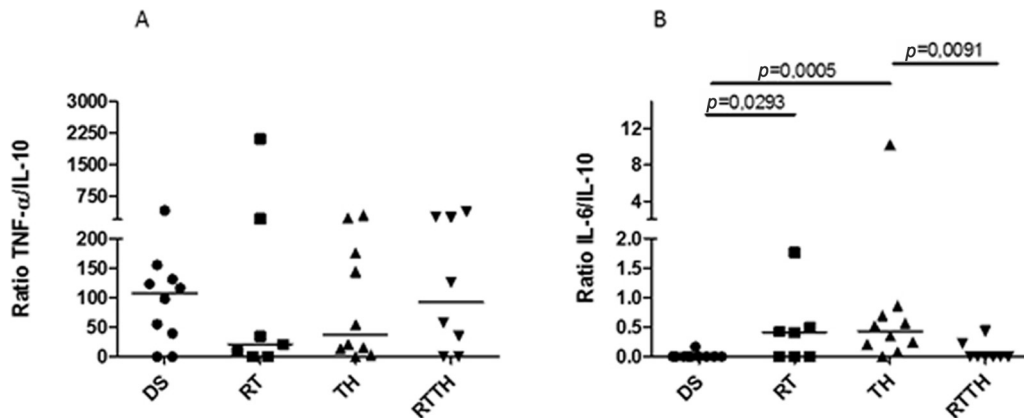
In the patients to be submitted to adenotonsillectomy, the samples taken preoperatively did not differ from those collected 4 to 6 months after the surgical procedure (late postoperative period) with respect to the plasma levels of TNF- $\alpha$  ( $\rightarrow$ Fig. 3A) ( $p = 0.2799$ ), IL-6 ( $\rightarrow$ Fig. 3B) ( $p = 0.9684$ ), or IL-10 ( $\rightarrow$ Fig. 3C) ( $p = 1.000$ ).

## Discussion

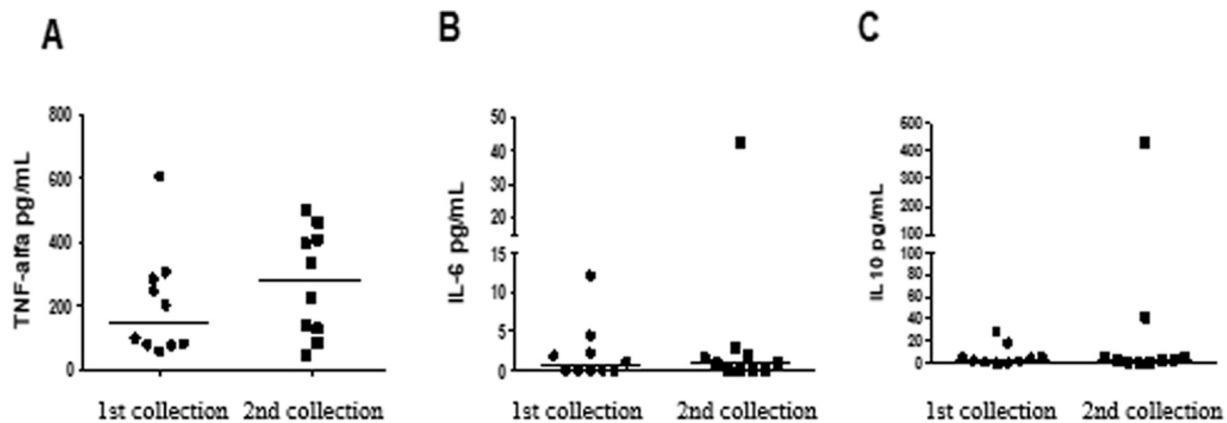
The present prospective study sought to evaluate the plasma levels of the TNF- $\alpha$ , IL-6, and IL-10 cytokines in patients submitted to adenotonsillectomy due to tonsillar hypertrophy and/or recurrent tonsillitis, both preoperatively and postoperatively.



**Fig. 1** Plasma levels of TNF- $\alpha$  (A), IL-6 (B) and IL-10 (C) to each group in the present study (preoperatively). Plasma levels of TNF- $\alpha$ , IL-6 and IL-10 were quantified in the recurrent tonsillitis (RT), tonsillar hypertrophy (TH), recurrent tonsillitis with hypertrophy (RTTH) and controls (DS) groups by the ELISA method. The horizontal bars indicate medians. The Mann-Whitney statistical method was used and values less than 0.05 were considered significant.



**Fig. 2** Ratio between plasma levels of TNF- $\alpha$  (A) and IL-6 (B) in relation to IL-10. Plasma levels of TNF- $\alpha$ , IL-6 and IL-10 were quantified in the recurrent tonsillitis (RT), tonsillar hypertrophy (TH), recurrent hypertrophy tonsillitis (RTTH) and healthy controls (DS) groups by the ELISA method. The ratios of pro-inflammatory and anti-inflammatory cytokine concentrations were calculated for each individual in each group. The horizontal bars indicate the medians. The Mann-Whitney statistical method was used and values less than 0.05 were considered significant.



**Fig. 3** Plasma levels of TNF- $\alpha$  (A), IL-6 (B) and IL-10 (C), in preoperatively (1st collection) and postoperatively (2nd collection) between 4 and 6 months after the surgery. No significant difference. The horizontal bars indicate the medians. The Mann-Whitney statistical method was used and values less than 0.05 were considered significant.

The tonsils and adenoids are the first line of defense against pathogenic agents in the host. Located in the upper aerodigestive tract, these structures act through both humoral and cell immunity. Clinically, there are two situations in which the surgical removal of the tonsils may be necessary: hypertrophy and recurrent tonsillitis.<sup>15</sup> Both conditions exert a negative effect on patients' quality of life and health and, when indicated, surgery to remove the tonsils represents a good solution for improving the child's quality of life.<sup>15,16</sup>

The present results showed higher IL-6 levels in the RT and TH groups. There was a significant increase in inflammatory cytokine IL-6 levels in the patients with chronic tonsillitis compared with the healthy control group. Together with TNF- $\alpha$ , IL-6 is considered to be one of the most potent anti-inflammatory cytokines, since it exerts an important role in the acute phase of inflammation, such as in the differentiation of lymphocytes, cell proliferation, and survival, and in potentiating apoptotic signaling. The measurement of these cytokine levels allows the extent of the inflammation to be estimated.<sup>15</sup>

Interleukin-6 is secreted by many different types of cells including macrophages, monocytes, eosinophils, hepatocytes, and glial cells, with TNF- $\alpha$  and IL-1 being potent inducers of this cytokine. Interleukin-6 causes fever and activates the hypothalamic-pituitary-adrenal axis through its  $\alpha$ -receptors (IL-6R $\alpha$ ) and the gp130 subunit (glycoprotein 130, member of the class I cytokine receptor family).<sup>17</sup> As a proinflammatory cytokine, IL-6 promotes neutrophil maturation and activation, macrophage maturation, and differentiation/maintenance of cytotoxic T lymphocytes and natural killer (NK) cells. In addition, it activates astrocytes and microglia and regulates the expression of neuropeptides following neural lesion, contributing to regeneration.<sup>18</sup>

In the RT group, the increase in IL-6 levels could be the result of repeated stimulation by pathogenic agents, with this increase occurring 24 hours after the contact of these agents with the host.<sup>19,20</sup>

In the HT group, the increase in IL-6 levels could be the result of OSAS, a pathological condition that can elevate the serum levels of inflammatory cytokines, including IL-6. The intermittent hypoxia increases oxidative stress and

generates reactive oxygen species (ROS), which activates oxidant-sensitive transcription factors.<sup>21</sup> One of them is nuclear factor  $\kappa$ B (NF- $\kappa$ B), which is known to induce many proinflammatory cytokines, including IL-6.<sup>22</sup>

In the present study, the ratios of the serum levels of the inflammatory/antiinflammatory cytokines were determined. The IL-6/IL-10 ratio was higher in the RT and TH groups compared with the control group, which is justifiable, since there is an increase in proinflammatory activity in relation to anti-inflammatory activity, increasing the ratio between the interleukins. There was a significant increase in the levels of inflammatory cytokines IL-6 in the patients with recurrent tonsillitis compared with the healthy control group. The higher IL-6/IL-10 ratio in the RT and TH groups represents the predominant inflammatory profile in the plasma of these patients.

Both hypertrophy and the presence of recurrent infections in the tonsils are probably due to immune system dysfunction. An abnormal regulatory mechanism in lymphocyte activation must cause these deviations in the function of the tonsils, which then go on to harm the individual, leading to the need for their surgical removal.<sup>17,18</sup>

No statistically significant differences were found in the TNF- $\alpha$  levels between the study groups, as previously shown in some studies.<sup>14,23</sup> Tumor necrosis factor- $\alpha$ , also known as cachetin, is a proinflammatory cytokine produced mainly by monocytes, macrophages, and T lymphocytes. Following a surgical procedure, trauma or during infections, TNF- $\alpha$  is one of the earliest and most potent mediators of inflammatory response. It triggers important metabolic and hemodynamic changes and is able to activate different cells to produce other cytokines.<sup>7,9</sup> Some studies have reported serum TNF- $\alpha$  values 10 to 20 times higher than normal in patients with chronic tonsillitis, with TNF- $\alpha$  production being higher, particularly in patients with recurrent tonsillitis.<sup>24</sup> In the present study, blood sampling was not performed during the acute phase of tonsillitis, either preoperatively or in the late postoperative period. This fact could explain the absence of any statistically significant difference between the groups in relation to TNF- $\alpha$  levels. Nonetheless, another explanation may lie in the small sample size.

## Conclusion

Patients with a history of chronic tonsillitis, in particular those allocated here to the RT and TH groups, showed higher levels of IL-6 plasma when compared with healthy controls, highlighting a more inflammatory profile in these patients.

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