

Effect Of Vitamin C On Tnf-Alpha Expression In Recurrent Tonsillitis At Rsud Dr. Moewardi

Putu Wijaya Kandhi¹

¹Department of Otorhinolaryngology – Head and Neck Surgery, Faculty of Medicine
Universitas Sebelas Maret / Dr. Moewardi Hospital, Surakarta, Indonesia
e-mail: 1putu_kandhi@yahoo.com

Abstract

Background: The immunopathogenesis of tonsillitis is thought to be due to the involvement of various proinflammatory cytokines including tumor necrosis factor α (TNF- α). Considering the role of TNF- α in the immunopathogenesis of tonsillitis, various interventions have been carried out both surgically and with drugs. Vitamin C, which is one of the important micronutrients in the body, has a role as an antioxidant and immunomodulator so that its administration is thought to be able to overcome the increase in TNF- α during the disease process. This study aims to determine the effect of vitamin C administration on TNF- α levels in patients with recurrent tonsillitis at Dr. Moewardi Hospital.

Methods: This study is an experimental quantitative study using Randomized Controlled Trials research design with non-blinding. Samples in the study were divided into two treatment groups, namely treatment with oral vitamin C and placebo 50mg/kg body weight/day which then measured serum TNF- α levels.

Results: The average TNF- α level in the Vitamin C group was smaller at 110.72 pg/mL compared to the control group which amounted to 148.96 pg/mL. The results of the independent t-test of TNF- α levels in both groups obtained a significant value <0.05 , which was 0.037. This means that there is a significant difference in TNF- α levels between the two treatment groups. Conclusions: Vitamin C administration before tonsillectomy in patients with recurrent tonsillitis can significantly reduce TNF- α levels compared to the control group. This opens the possibility of routine use of vitamin C in recurrent tonsillitis patients to get better treatment results.

Keywords: Tonsillitis, TNF- α , Vitamin C

Recurrent tonsillitis is an infection and inflammation of the tonsil with acute symptoms occurring at least 4-7 times in the past year, or five times in the last two years, or three times in the last three years (Anderson & Paterek, 2022). Immunopathogenesis of tonsillitis begins when the antigen enters the oropharyngeal space and hits the crypt epithelium. The body will subsequently respond to antigens with inflammation in the form of innate and adaptive immune reactions that are predominantly played by T cells. Antigenic stimulation of intraepithelial T cells will produce various kinds of proinflammatory cytokines such as tumor necrosis factor (TNF)- α , interferon (IFN)- γ , and interleukin (IL)-2. These various proinflammatory cytokines, especially TNF- α will be able to cause clinical manifestations of tonsillitis (Geißler et al., 2020). Given the important role of TNF- α in immunopathogenesis tonsillitis, it needs a therapy that can overcome this proinflammatory cytokine as adjuvant therapy for tonsillectomy which is the main management in cases of chronic tonsillitis.

Vitamin C is one of the micronutrients that is important for the body because it has many benefits not only as antioxidants, but vitamin C also has significant immunomodulatory, antimicrobial, antiparasitic, antiviral, and antioxidant properties. Several previous studies have indicated that vitamin C can reduce levels of proinflammatory cytokines such as IL-6, TNF- α , and IFN- γ and increase the production of anti-inflammatory IL-10 in a wide variety of inflammatory conditions and locations (Molina et al., 2014). It is revealed that similar studies have not been conducted in patients with inflammation of the tonsils. This study aims to determine whether there is an effect of vitamin C administration on TNF- α expression examined in patients with recurrent tonsillitis.

Introduction

Methods

This study was conducted at dr. Moewardi Regional General Hospital Surakarta, especially in Ear, Nose and Throat, Head and Neck Surgery (ENTHNS) polyclinic and the biomedical laboratory of the Faculty of Medicine, Universitas Sebelas Maret. The study was conducted during the period of June – September 2022.

This study was a Randomized Controlled Trials (RCT) study with a non-blinding method, by providing intervention in the form of oral vitamin C 50mg / kg body weight/day in two times of administration for 4 weeks to pre-tonsillectomy patients. The sampling process was conducted by simple random sampling technique.

The inclusion criteria in this study were the discovering of recurrent tonsillitis through physical and supporting examination and did not receive vitamin C supplementation or other immunomodulators within the past month. The exclusion criteria in this study were having undergone a previous tonsillectomy procedure, receiving steroids or other immunosuppressant therapy, suffering from severe chronic disease, and patients with other diagnostic findings on the tonsils other than recurrent tonsillitis during the period of the study.

TNF- α levels were examined using the enzyme linked immunosorbent assay (ELISA) method using tonsil tissue samples that had been previously acquired through tonsillectomy. Some of the materials needed include Phosphate Buffered Saline (PBS) buffer, incubator, glass homogenizer, ultrasonic cell disruptor, centrifugator, wash buffer, standard solution, blank solution, biotin-labeled antibody, HRP-Streptavidin Conjugate (SABC), 3,3',5,5'-tetramethylbenzidine (TMB) substrate, stop solution, and microplate reader. The steps for checking TNF- α levels by ELISA method were carried out according to the guidelines (MP Biomedicals©, USA).

Analysis of previously collected data was tested for data normality with Saphiro-Wilk. Data with normal and homogeneous distribution would be processed with paired t-

test and independent t-test. Mann-Whitney and Wilcoxon non-parametric tests were performed when the data was not normally distributed. All data processing analysis was performed using IBM SPSS program version 25 (Chicago, NY). This study has received approval from the Research Ethics Committee of Dr. Moewardi Hospital number 1,103/VIII/HREC/2022.

The minimum sample size was calculated using the Slovin formula. The symbol n is the minimum sample size, d is the absolute precision set at 0.1, p is the proportion of recurrent tonsillitis patients with high TNF- α (set at 96% or 0.96 based on previous research) (Aruan et al., 2015)), and q is the proportion of recurrent tonsillitis patients with normal TNF- α (obtained by the formula $q = 1 - p$ or 0.04).

$$n = \frac{4pq}{d^2}$$
$$n = \frac{4 \times 0.96 \times 0.04}{0.1^2}$$
$$n = 15,36$$

Based on the calculation with the Slovin formula, a minimum sample size of 15.36 was obtained, which was rounded up to 15 samples. Thus, the number of samples per group is at least 7 samples each.

Results

Characteristics of Research Subjects

This study was conducted at the ENT-HN polyclinic of Dr. Moewardi Hospital during June – September 2022. The subjects in this study were a total of 16 recurrent tonsillitis patients who were divided into two groups, namely the group receiving treatment with vitamin C 50mg / kg (Vit C) and the group that was receiving any treatment (control) with a total of 8 people in each group. The characteristics of the subjects in this study were subsequently summarized and presented in table 1

Table 1. Characteristics of Research Subjects

Characteristic	Vit C	Control
Age		
0-18	1 (14,3%)	2 (25%)
19-35	7 (85,7%)	6 (75%)

Sex		
Male	5 (62,5%)	4 (50%)
Female	3 (37,5%)	4 (50%)
BMI		
Underweight	1 (14,3%)	0 (0%)
Normal	4 (50%)	5 (62,5%)
Overweight	3 (35,7%)	3 (37,5%)

The majority of patients in this study were in the age group of 19 – 35 years, consisting of 7 patients (85.7%) in the Vit C group and 6 patients (75%) in the control group. In both groups the distribution of the sexes was almost the same. The most Body mass index (BMI) in the two groups was normoweight with 4 patients (50%) in the Vit C group and 5 patients (62.5%) in the control group.

Differences in TNF- α Levels

In order to find out whether there was a difference in the TNF α value between the control group and Vit C, an independent t-test was carried out. The results of the independent t-test of TNF α levels is presented in table 2.

Table 2. Differences in TNF- α Levels in Vit C and Control Groups

Variables	Groups		<i>p-value</i>
	Vit C	Control	
TNF- α (pg/mL)	110,72 \pm 22,17	148,96 \pm 41,37	0,037

The average TNF α level in the Vit C group was smaller at 110.72 pg/mL compared to the control group of 148.96 pg/ml. The results of the independent t-test of TNF α levels in both groups obtained a significant value of <0.05 , which was 0.037. This means that there was a significant difference in TNF- α levels between the two groups.

Discussion

The results of this study indicated that there was a significant difference in TNF α levels between patients who received vitamin C before underwent tonsillectomy compared to patients who immediately underwent tonsillectomy without previously receiving vitamin C. This is in line with a study by Chen et al. (2014) which also discovers that administration of vitamin C can reduce TNF α levels in patients suffering from community pneumonia (Chen et al., 2014). This study is the first study to report that giving vitamin C to recurrent tonsillitis patients who will undergo tonsillectomy can reduce TNF α level.

Vitamin C is a powerful antioxidant, able to protect important biomolecules from the damaging effects of endogenous produced oxidants, during metabolism and inflammatory processes as well as oxidants originating from the environment (Bozonet &

Carr, 2019). Vitamin C can reduce TNF- α levels by suppressing the activation of nuclear factor κ B (NF- κ B) which is the main inflammatory regulator in the body (Mussa et al., 2023).

Vitamin C blocks phosphorylation of the nuclear factor of kappa light polypeptide gene enhancer in the B-cells inhibitor, alpha (I κ B α), a key step that allows translocation of NF- κ B to the cell nucleus to activate gene expression. Phosphorylation of I κ B α is mediated by several kinases, and vitamin C inhibits activation of NIK and IKK β kinases independent of p38 MAP kinase. Activation of p38 by AA is induced by prolonged exposure to AA with very high concentrations. The inhibitory activity of vitamin C on TNF- α -induced phosphorylation of I κ BR was observed in several cellular systems, including HeLa (human cervical adenocarcinoma), MCF7 (human breast adenocarcinoma), U937 (monocytic) and HL60 (myeloid leukemia) cell lines and in primary cultures of human umbilical vein endothelial cells (HUVECs), showing generality of the effect (Bozonet & Carr, 2019; Cerullo et al., 2020). This may explain why patients who received vitamin C before tonsillectomy were found to have lower levels of TNF α than those who did not receive vitamin C,

Conclusion

The administration of vitamin C 50mg/kg in two times of administration for 4 weeks before tonsillectomy in patients with recurrent tonsillitis can reduce TNF- α levels significantly compared to the control group. This reveals the possibility of routine use of vitamin C in patients with recurrent tonsillitis to obtain better results. In this study, tonsillectomy has been carried out before, so that the effect of vitamin C administration on TNF- α could possibly be influenced by the treatment that have previously been undertaken, therefore it is recommended for future studies to compare standard therapy with vitamin C therapy or a combination of both. In addition, it is necessary to identify other pro-inflammatory cytokines so that they can be better understood regarding the effect of vitamin C administration.

References

- Anderson, J., & Paterek, E. (2022). Tonsillitis. [cited July 27th 2022]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK544342/>
- Aruan, S.Y., Aboet, A., Zahara, D., Aliandri, A., Saragih, A.R. (2015). Hubungan ekspresi Tumor Necrosis Factor Alpha (TNF- α) dengan destruksi tulang akibat kolesteatoma. *Oto Rhino Laryngologica Indonesiana*, 45(1), 36-42.
- Bozonet, S. M., & Carr, A. C. (2019). The Role of Physiological Vitamin C Concentrations on Key Functions of Neutrophils Isolated from Healthy Individuals. *Nutrients*, 11(6), 1363.
- Cerullo, G., Negro, M., Parimbelli, M., Pecoraro, M., Perna, S., Liguori, G., Rondanelli, M., Cena, H., & D'Antona, G. (2020). The Long History of Vitamin C: From Prevention of the Common Cold to Potential Aid in the Treatment of COVID-19. *Frontiers in immunology*, 11, 574029. <https://doi.org/10.3389/fimmu.2020.574029>
- Chen, Y., Luo, G., Yuan, J., Wang, Y., Yang, X., Wang, X., Li, G., Liu, Z., & Zhong, N. (2014). Vitamin C mitigates oxidative stress and tumor necrosis factor-alpha in severe community-acquired pneumonia and LPS-induced macrophages. *Mediators of Inflammation*, 2014, 426740. <https://doi.org/10.1155/2014/426740>
- Geißler, K., Weigel, C., Schubert, K., Rubio, I., & Guntinas-Lichius, O. (2020). Cytokine production in patients with recurrent acute tonsillitis: Analysis of tonsil samples and blood. *Scientific Reports*, 10(1), 13006. <https://doi.org/10.1038/s41598-020-69981-1>
- Molina, N., Morandi, A. C., Bolin, A. P., & Otton, R. (2014). Comparative effect of fucoxanthin and vitamin C on oxidative and functional parameters of human lymphocytes. *International Immunopharmacology*, 22(1), 41-50. <https://doi.org/10.1016/j.intimp.2014.06.026>
- Mussa, A., Afolabi, H. A., Syed, N. H., Talib, M., Murtadha, A. H., Hajissa, K., Mokhtar, N. F., Mohamud, R., & Hassan, R. (2023). The NF- κ B Transcriptional Network Is a High-Dose Vitamin C-Targetable Vulnerability in Breast Cancer. *Biomedicines*, 11(4), 1060. <https://doi.org/10.3390/biomedicines11041060>