Research

Effect of N-Acetylcysteine and vitamin D supplementation on interleukin-1β level in chronic suppurative otitis media

Dewi Pratiwi, Astrid Gayatri
Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta

ABSTRACT

Background: Chronic suppurative otitis media (CSOM) is a major health problem in many populations around the world, causing significant morbidity and mortality. It is characterized by transformation and hyperplasia of the middle ear mucosa following infiltration of numerous inflammatory cells. Recent researches showed a significant impact of the anti-inflammatory and anti-oxidant effects of N-Acetylcysteine (NAC) and vitamin D independently, but their role in the pathophysiology of CSOM was still unclear. **Purpose:** To evaluate the effect of NAC and vitamin D supplementation on vitamin D and interleukine-1β levels in CSOM. **Method:** The research was an experimental study with randomized controlled trial design, and was conducted at ENT polyclinic, Dr Moewardi Regional General Hospital Surakarta on May-October 2023. Blood serum examination of vitamin D and IL-1β levels was done at Biomedical Laboratory of Medical Faculty Sebelas Maret University. **Result:** There were 36 CSOM subjects without cholesteatoma who met the inclusion and exclusion criteria. The NAC+vitamin D group had the highest mean vitamin D level (30.82±5.35 ng/mL) and the lowest mean IL-1β level (23.81±7.13 pg/mL) compared to the control group and the group that received only NAC or vitamin D alone. ($p<0.05$). **Conclusion:** N-Acetylcysteine and vitamin D supplementation had a synergistic effect in reducing oxidative stress and inflammation in CSOM.

Keywords: chronic suppurative otitis media, vitamin D, N-Acetylcysteine, interleukine-1β

ABSTRAK

Latar belakang: *Otitis media supuratif kronis (OMSK) adalah salah satu masalah kesehatan utama yang ditemukan pada populasi di seluruh dunia dengan morbiditas dan mortalitas tinggi. Hal ini ditandai dengan transformasi dan hiperplasi dari mukosa telinga tengah setelah infiltrasi dari sejumlah sel peradangan. Penelitian terbaru menunjukkan adanya efek anti-oksidan dan anti-inflamatori dari* N-Acetylcysteine (NAC) *dan vitamin D, namun peran keduanya pada patofisiologi OMSK masih belum banyak diketahui.* ***Tujuan:*** *Untuk mengevaluasi efek pemberian* NAC *dan vitamin D terhadap kadar* interleukine-1β *pada OMSK.* ***Metode:*** *Penelitian ini merupakan studi eksperimental dengan* randomized controlled trial*, di Poliklinik THT RSUD Dr. Moewardi Surakarta pada Mei–Oktober 2023. Pemeriksaan kadar vitamin D dan IL-1β serum darah dilaksanakan di Laboratorium Biomedik Fakultas Kedokteran Universitas Sebelas Maret.* ***Hasil:*** *Terdapat 36 pasien subyek penelitian dengan OMSK tanpa kolesteatoma yang memenuhi kriteria inklusi dan eksklusi penelitian ini. Kelompok NAC+vitamin D memiliki rerata kadar vitamin D tertinggi (30,82±5,35 ng/mL) dan rerata kadar IL-1β terendah (23,81±7,13 pg/mL) dibandingkan kelompok kontrol dan kelompok yang hanya mendapatkan NAC atau vitamin D saja ($p<0.05$).* ***Kesimpulan:*** *Pemberian NAC dan vitamin D memiliki efek sinergis dalam mengurangi stress oksidatif dan inflamasi pada OMSK.*

Kata kunci: *otitis media supuratif kronik, vitamin D,* N-asetilsistein*,* interleukin-1β

Correspondence address: Dewi Pratiwi. Department of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Surakarta. Email: pratiwidewi81@staff.uns.ac.id

INTRODUCTION

One of the main health issues affecting many people worldwide is chronic suppurative otitis media (CSOM), which is also the primary cause of significant morbidity and mortality. It is still difficult to explain how and why chronic otitis media happens that many research is still ongoing to elaborate the patho-physiology of CSOM.[1] Chronic suppurative otitis media is a middle ear inflammatory disease that lasts longer than three months, and is not entirely resolved with medication. It is characterized by three distinctive symptoms which are tympanic membrane perforation, ear discharge, and hearing loss.[2]

The World Health Organization (WHO) estimates that between 65 and 330 million people worldwide have CSOM, and 60% of those individuals have hearing loss. In comparison, there are nine cases for every 100,000 people. The national health survey data on hearing and vision indicate that 3.0 to 5.20% of Indonesians have CSOM. In Indonesia, CSOM is estimated to affect around 6.6 million persons. The etiology and pathophysiology of CSOM have an impact on its high incidence. Infection, anatomical or physiological malfunction, environment, allergies, or patient variables like immunity, gender, and others are some of the determining factors[3]. The number of CSOM outpatients at Dr. Moewardi Hospital was 2,276 in the year of 2022, with a total of 8,222 CSOM outpatients over the past 5 years.

The middle ear mucosa undergoes change and hyperplasia as a result of many inflammatory cells infiltrating it, which is a characteristic of chronic suppurative otitis media. Smoke exposure, bacterial and viral infections, compromised host immunity, and Eustachian tube (ET) dysfunction all contribute to induce CSOM.[4] Oxidative stress is the breakdown of the equilibrium between antioxidants and free radicals in biological systems. When oxidative stress escalates or the antioxidant defense system is compromised, elevated levels of reactive oxygen species (ROS) impact the chronicity of middle ear inflammation and the etiopathogenesis of chronic otitis media.[2] The antioxidant glutathione is found in large quantities in the body and assists in protecting cells from oxidative damage.[5] As a synthetic antioxidant, N-acetylcysteine (NAC) is an amino acid derivative of cysteine. NAC administration as "glutathione boosting treatment" can lower cytokines that promote inflammation. High dosages of NAC (600–2400 mg/day) have anti-inflammatory and antioxidant properties.[6-7] Despite not being an antibiotic, NAC contains antibacterial qualities and disrupts pathogen-associated bacterial biofilms.[8]

Vitamin D is notable for its function in controlling serum calcium and phosphate levels, promoting calcium absorption in the gut, and supporting bone health. Over the past thirty years, additional benefits of vitamin D have been identified. Not only does it control cell division and proliferation, but it also plays a critical part in immune responses. Nowadays, vitamin D has been shown to have the following benefits: detoxification from xenobiotics; decreased oxidative stress; neuroprotective properties; antibacterial properties; immunoregulation; anti-inflammatory; anti-cancer; and cardiovascular effects.[9]

Vitamin D deficiency affects around 50% of the global population, and its incidence is increasing in both tropical and

subtropical climates. Vitamin D deficiency can lead to bone abnormalities and increase the risk of some chronic diseases. According to recent research, vitamin D may have a significant immunomodulatory effect that raises the frequency and severity of viral and bacterial infections. Furthermore, numerous investigations have demonstrated that children with low serum 25(OH) vitamin D levels have high-risk respiratory infectious infections, which raises the possibility of otitis media.[10] According to one case-control study, children with recurrent otitis media had mean serum 25(OH)D levels that were considerably lower than those of controls (28.5 nmol/L vs. 72.9 nmol/L). Children with greater serum 25(OH)D levels had a decreased likelihood of developing chronic effusion otitis media, according to another case-control study.[4]

Vitamin D receptors are found in most immune system cells, and mutations in the DNA of these receptors have been related to an increased risk of infection. T and B lymphocytes, dendritic cells, macrophages, and other immune cells all express 25-hydroxyvitamin D (25(OH)D)-1α-hydroxylase, the enzyme that activates vitamin D. Numerous studies have also documented the mechanisms, such as the regulation of antimicrobial cytokine or peptide profiles, that underlie the immunoregulatory activity of vitamin D. Although there is little information available regarding the role of vitamin D in the pathogenesis of CSOM, a number of clinical studies have shown a substantial correlation between vitamin D sufficiency status and otitis media.[4,11]

Deficiency in vitamin D is defined as serum 25(OH)D levels below 20 ng/mL (50 nmol/L) and serum 25(OH)D levels between 21-29 ng/mL (52.5-72.5 nmol/L) is defined as vitamin D insuficiency. in accordance with American Endocrine Society recommendations. Vitamin D intake should be increased and sun exposure should be sufficient to maintain adequate levels of the hormone (40–60 ng/mL).[12,13] In order to prevent vitamin D insufficiency, experts advise consuming at least 4000 IU of vitamin D daily. The biological explanation for this can be attributed to three factors: the expression of vitamin D receptors in the majority of human tissues; the significantly lower levels of vitamin D in northern latitudes compared to hominids that evolved in equatorial regions of Africa; and the way that calcitriol stimulation of vitamin D receptors changes the expression of over two hundred genes, supporting a wide range of physiological responses that may prevent the onset of multiple pathologies.[12]

Based on scientific research, it is suggested that having enough vitamin D is essential for preventing a variety of diseases since it affects many genes, the immune system, and inflammatory reactions. The discovery that most immune system cells, such as neutrophils, dendritic cells, macrophages, and T lymphocytes, have vitamin D receptors lends credence to the possible significance of vitamin D in immune system activity. Therefore, the level of vitamin D is thought to be one of the most important factors that affects how well the immune system of the body functions, including the control of cytokines.[13]

Cell signaling molecules called cytokines are crucial for regulating every type of immunological response. In reaction to inflammation, cytokines can grow by almost a factor of 1,000 and are found in the blood at picomolar concentrations. One significant member of the IL-1 family, which is essential to innate immune responses, is interlukine-1β (IL-1β). By inhibiting the synthesis of the cytokine IL-1β, which is crucial for controlling both local and systemic inflammation, vitamin D regulates the inflammatory response. Data from the literature suggests that elevated levels of IL-1β inflammation may be linked to low vitamin D levels.[14]

METHOD

This study was an experimental research with randomized controlled trial test, and was conducted at Dr Moewardi Regional General Hospital, Surakarta, in the polyclinic of Ear Nose and Throat, from May to October 2023. Data that were collected including identity, age, gender, occupation, body mass index (BMI), smoking history, frequency of otorrhea, duration of CSOM, degree and type of hearing loss. Serum blood samples were collected and sent to the Biomedical Laboratory of Faculty of Medicine Sebelas Maret University for examination of vitamin D and IL-1 β levels. The inclusion criteria were CSOM patients without choleteatoma in active phase, and signed informed consent. The exclusion criteria were CSOM patients who received topical antibiotic ear drops therapy in the last 7 days, patients with systemic infectious diseases, immunocompromised and using immunosuppressant drugs, congenital ear abnormalities, external ear infections, CSOM patients with complications, and those who were not willing to participate in the study.

The data obtained were then statistically analyzed using Statistical Package for Social Science (SPSS) 26 for Window. The normality test of this study used the Shapiro Wilk test because the number of samples of each group was small (<50), and the homogeneity test of the variants of this study used the Levene test. This research used a randomized controlled trial (RCT) test analysis if the data met the assumptions of normality and homogeneity using the ANOVA test, then proceed with post hoc Schefee; for data that met the assumptions of normality but did not meet the assumptions of homogeneity would be analyzed using the Brown Forsythe test, then proceed with post hoc Dunnet T3; and for data that did not meet the assumptions of normality using the Kruskal Wallis test, then proceed with post hoc Dunn Test.

RESULT

Subject Characteristics

In this study, 36 research subjects were obtained, who were CSOM patients without cholesteatoma at Dr. Moewardi Surakarta Regional General Hospital during May-October 2023 and met the inclusion and exclusion criteria of this study. Based on the characteristics of the research sample presented in Table 3, it was found that gender (p=0.801) and age (p=0.291) had almost the same distribution in each treatment group and did not show significant differences (p>0.05), thus the characteristics of the subjects of this study were equivalent or homogeneous.

Table 1. Subject characteristics

Variables	NAC n = 9 (%)	Vit. D n = 9 (%)	NAC + Vit. D n = 9 (%)	Control n = 9 (%)	*p Value*
Age (mean)[a]	40.44±15.53	33.56± 9.36	41.78± 8.86	33.22± 12.33	0.291
Gender[b]					0.801
Male	3 (33.3)	4 (44.4)	3 (33.3)	2 (22.2)	
Female	6 (66.7)	5 (55.6)	6 (66.7)	7 (77.8)	
Smoking history (active/passive) [b]					0.747
Yes	4 (44.4)	5 (55.6)	4 (44.4)	6 (66.7)	
No	5 (55.6)	4 (44.4)	5 (55.6)	3 (33.3)	
CSOM[b]					0.272

Unilateral	4 (44.4)	5 (55.6)	7 (77,8)	3 (33.3)	
Bilateral	5 (55.6)	4 (44.4)	2 (22.2)	6 (66.7)	
Types of hearing loss[b]					0.178
Conductive	4 (44.4)	8 (88.9)	4 (44.4)	5 (55/6)	
Mixed	5 (55.6)	1 (11.1)	5 (55.6)	4 (44.4)	
Degree of hearing loss[b]					0.376
Mild	0	1 (11.1)	4 (44.3)	2 (22,2)	
Moderate	2 (22.2)	3 (33.3)	3 (33.3)	1 (11.1)	
Moderately Severe	5 (55.6)	4 (44.4)	2 (22.2)	4 (44.4)	
Severe	2 (22.2)	1 (11.1)	0	2 (22,2)	
Profound	0	0	0	0	
Duration of CSOM[b]					0.107
<5 year	1 (11.1)	5 (55.6)	6 (66.7)	1 (11.1)	
5-10 year	5 (55.6)	2 (22.2)	2 (22.2)	4 (44.4)	
>10 year	3 (33.3)	2 (22.2)	1 (11.1)	4 (44.4)	
Otorrhea frequency in last 3 months[b]					**0.010***
<3 x	1 (11.1)	3 (33.3)	6 (66.7)	0	
3-6 x	2 (22.2)	5 (55.6)	2 (22.2)	4 (44.4)	
>6 x	6 (66.7)	1 (11.1)	1 (11.1)	5 (55.6)	

[a] Anova test; [b] Chi square test

It was found that the control group (16.05±3.91ng/mL) had the lowest mean vitamin D levels, and NAC + Vitamin D group (30.80±6.11 ng/mL) had the highest mean vitamin D levels compared to the NAC only group (22.96±5.07 ng/mL) and the vitamin D only group (26.36±4.79 ng/mL). The results of the Anova test obtained a value of $p<0.05$.

The results of this study showed that the control group had the highest mean IL-1β level (37.94±7.30 pg/mL), and in NAC only group and Vit D only group, the mean of IL-1β level were not much different (30.54±6.13 and 30.23± 3.53 pg/mL). The lowest IL-1β level was in the NAC + Vit D group (23.81±7.13 pg/mL). The results of the Anova test obtained a p value <0.001.

Table 2. Anova test of Vitamin D

	Vitamin D			
Groups	**Mean±SD**	**Min**	**Max**	**p-value**
NAC	22.96±5.07	12.05	35.94	**< 0.001***
Vit. D	26.36±4.79	18.97	34.17	
NAC + Vit. D	30.80±6.11	22.75	39.50	
Control	16.05±3.91	11.08	19.50	

Table 3. Anova test of IL-1 β

	IL-1 β			
Groups	**Mean±SD**	**Min**	**Max**	**p-value**
NAC	30.54±6,13	20.50	38.90	**<0.001***
Vit. D	30.23±3,53	25.65	36.20	
NAC + Vit. D	23.81±7,13	19.14	42.13	
Kontrol	37.94±7,30	29.90	52.75	

DISCUSSION

Compared to the supplementation of NAC and Vitamin D independently, it was discovered in this study that the combination of vitamin D and NAC could raise serum vitamin D levels. The result of this study was in accordance with other prior researches that indicated the co-administration of vitamin D and L-cysteine (LC) was regarded more efficacious than vitamin D supplementation only. This is explained by the several benefits of the increased glutathione (GSH) cell state brought on by LC. The genes related in vitamin D metabolism (VDBP/CYP2R1/CYP27A1/VDR), which are essential for the efficient transit and hydroxylation of cholecalciferol, will first exhibit increased expression in response to LC. Furthermore, LC will trigger the VDR/PGC-1α/GLUT-4 pathway, which controls the 1,25(OH)2 vitamin D metabolism.[15] Furthermore, lipids and proteins are essential parts of the membrane bilayer and are needed to keep different organs structurally intact and physiologically functioning. Since LC is hydrophilic and vitamin D is lipophilic, these two micronutrients work well together. As a result, the combination of vitamin D and LC/GSH will be more successful in preventing oxidative damage to proteins and lipids and will offer more robust anti-inflammatory and antioxidative defense against oxidative stress brought on by infection. Consequently, consuming GSH precursors and vitamin D together, as opposed to just high-dose vitamin D, is a novel and useful method for achieving more efficient bioavailability than cholecalciferol ingestion only. Studies on animals have demonstrated that the combination of vitamin D and LC is more beneficial than vitamin D only in terms of raising GSH levels and vitamin D regulatory genes at the cellular or tissue level, raising 25(OH)-vitamin D levels, and lowering blood levels of inflammatory biomarkers, TNF-α, and oxidative stress. Additionally, by boosting GSH and antioxidant capacity, combining vitamin D supplements with GSH precursor LC can enhance the gene status of vitamin D metabolism. It is advised that more clinical study be done to see whether vitamin D and LC together can offer a less expensive way to maximize continuous 25(OH)-vitamin D levels and strengthen immunity.[16]

In the current study, raising serum vitamin D levels with NAC only was similarly successful. There had not been any research or written material that explains this. Nevertheless, research indicated that taking NAC along with 1,25(OH)2D, or calcitriol, will boost GSH together and lessen oxidative stress. In animal and human models with different oxidative stress etiologies, NAC raised intracellular GSH concentration, crossed the blood-brain barrier, and enhanced cell survival. It also supplied a rate-limiting substrate for GSH synthesis. Apart from its extensively documented anti-inflammatory properties, 1,25(OH)2D also stimulates the production of glutathione reductase, an enzyme accountable for restoring GSH from oxidized glutathione disulfide, thereby elevating GSH levels.[17] Thus, we assumed that the supplementation of NAC could

enhance the effects of vitamin D and work in collaboration with it.

We also looked at the impact on IL-1β levels in CSOM patients of using NAC and vitamin D only, as well as vitamin D and NAC concomitantly. Compared to the supplementation of either vitamin D or NAC only, the combination of NAC and vitamin D had the greatest efficacy in lowering IL-1β levels. NAC might have anti-inflammatory properties through blocking nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which is crucial for immunological responses and inflammatory cascades related to oxidative stress. This had been suggested by past studies. NAC inhibits the nuclear activity and translocation of the transcription factor NF-κB, which regulates the expression of genes that support inflammation. It has been demonstrated that NAC prevents lipopolysaccharide-stimulated macrophages from releasing the inflammatory cytokines TNFα, IL-1β, and IL-6.[18]

Additional research had demonstrated the function of NAC as a precursor of glutathione and its ability to protect neurons from oxidative damage. Apart from its intended application, NAC possesses the ability to eradicate biofilm layers generated by diverse bacteria. Numerous studies had demonstrated the impact of NAC on the biofilm layer linked to CSOM formed by the primary pathogen *P. aeruginosa.*[19]

By reducing the synthesis of IL-1β, vitamin D regulates the inflammatory response and is crucial for both systemic and local inflammatory control. Based on the available data, there might be a correlation between elevated levels of inflammation and IL-1β and low vitamin D levels.[20]

Long-term low-dose N-acetylcysteine (NAC) administration boosted the expression of pro-inflammatory cytokines, interleukin 1β (IL-1β) and interleukin-6 (IL-6), in lipopolysaccharide (LPS)-activated macrophages. Long-term exposure of cells to low-dose NAC boosted LPS-induced phosphorylation of AKT and ERK, which in turn activated the transcription factor AP-1, resulting in enhanced activation of the IL-1β and IL-6 gene promoters. Furthermore, short-term low-dose NAC therapy was linked to negative control of pro-inflammatory cytokine expression through enhanced expression of the p53tumor suppressor protein.[21]

After cellular stimulation, NAC dramatically reduced the synthesis of IL-10 by PBMCs and considerably raised the expression of IFN-γ, IL-1β, IL-5, IL-12, and IL-12p40. Numerous researches had looked into how NAC affected the production of cytokines by various cell types, such as PBMCs, alveolar macrophages, and alveolar epithelial cells. The study by Viora et al. cited by Al-Shukaili et al.[22] revealed a significant increase in IL-1β, IL-2, IL-12, and IL-15 production and an insignificant increase in IL-10 and IFN-γ production by PBMCs activated by PHA in the presence of NAC. Some of the results of our study (increased IL-1β production and decreased IL-10 production) partially compliant with this study. Genetic variances between individuals might be the cause of these discrepancies in IL-10 production, which could lead to significant variability in responsiveness to different stimuli and cytokine production.

The main sources of inflammatory mediators are T cells and monocytes, which are also capable of interacting with other cells. Both direct cell-to-cell contact and soluble substances mediate this communication. Strong cytokine polypeptides, as has long been known, exhibit pleiotropic activity and functional redundancy. They function in intricately linked networks in which a single cytokine can affect the synthesis of numerous other cytokines as well as how they react to them. For instance, Th1 cells stimulate proinflammatory responses by releasing IFN-γ and, to a lesser extent, IL-2 and IL-

12. Th2 cells stimulate anti-inflammatory reactions by secreting IL-4 and, on occasion, IL-5, IL-6, IL-10, and IL-13. Therefore, the study was unable to demonstrate that the direct impact of NAC was the cause of the rise in other inflammatory cytokines or the decrease in IL-10.[22] Furthermore, the data indicate that the activating impact of T cells in this system is probably responsible for the rise in IL-1β production following anti-CD3 activation. There is an ongoing debate over NAC's impact on cytokine production. On the one hand, research indicates that NAC may boost the synthesis of cytokines that promotes inflammation. However, in other research, NAC was shown to reduce the generation of proinflammatory cytokines such IL-6, TNF-α, IL-1β, and IL-8. Furthermore, it was discovered that the induction of TNF-α, IL-1β, and IFN-γ was decreased by NAC therapy. It seems from the explanation above that the type of cell determines how NAC affects cytokine production.[22]

Consequently, we considered that when NAC and vitamin D were administered together, their synergistic effect could be greater and improve the ability of both medications to significantly lower IL-1β levels in comparison to when the medications were given separately.

There were further limitations to this study, such as the fact that the samples were unicentric or only collected from one health center. Therefore we proposed carrying out a multicentric study with a bigger sample size, which could have an impact on the study's findings. Moreover, we limited our analysis of NAC and vitamin D effects to CSOM individuals who did not have cholestoma. Thus, further investigation into the two medications' therapeutic benefits on CSOM patients with and without choleastoma is necessary.

In conclusion, this study had shown that, in comparison to single supplementation of both medications and control, combined supplementation of NAC and vitamin D was beneficial in lowering serum IL-1β levels and raising serum vitamin D levels in CSOM patients without cholesteatoma by lowering oxidative stress and inflammatory levels.

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CONFLICT OF INTEREST

There is no conflict of interest in this study.

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