Is There a Relationship Between Serum Levels of Vitamin A, Vitamin E, Copper and Zinc and Otitis Media with Effusion in Children?

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Abstract The aim of this study is to determine whether the serum levels of vitamin A, vitamin E, copper and zinc have a role in the development of otitis media with effusion (OME) in childhood. This prospective study was conducted between February 2010 and February 2011 at a tertiary Otorhinolaryngology Clinic. There were 113 subjects and subdivided into three groups. Group 1 consisted of 44 patients who underwent adenoidectomy with ventilation tube placement due to OME. Group 2 consisted of 43 patients underwent adenoidectomy alone and group 3, called control group, included 26 healthy children. Serum values of vitamin A, vitamin E, copper and zinc were measured preoperatively. Comparison of the patient groups showed that group 1 patients had significantly lower serum zinc levels than group 2 patients ($p = 0.002$), although differences between both patients groups and controls were

not significant. We found that the differences among the three groups in terms of serum levels of vitamin A, vitamin E and copper were not statistically significant ($p > 0.05$). The results of this study showed that serum levels of vitamin A, vitamin E and copper may not play a role on development of OME. We postulated that high serum zinc status may have the preventive effect in the predisposition to OME which may related to adenoid hyperplasia.

Keywords Zinc · Copper · Vitamin A · Vitamin E · Antioxidant · Otitis media with effusion

Introduction

Otitis media with effusion (OME) is the most common ear disease in children. It causes a conductive type hearing loss as a common morbidity [1]. Hearing loss can have serious effects on a child's linguistic and educational development [2]. The precise pathogenesis of the disease remains unknown [1, 3]. Multiple factors including infection, eustachian tube dysfunction, allergy, and barotrauma may responsible for the pathogenesis of OME [1].

Oxygen radicals are also blamed in the pathogenesis of inflammation. Infections are one of the reasons of increased reactive oxygen species production [4]. Excessive production of reactive oxygen species which is known as oxidative stress, leading to damage of nuclear and mitochondrial DNA, cell membrane lipids and intracellular proteins, resulting in cell death [5]. When the balance between reactive oxygen species production and the antioxidative defense mechanisms are disturbed, the level of reactive oxygen species increases finally lead to tissue damage [3]. Free radical damage to the membrane lipids of leukocytes results in decreased their immune function. Also, DNA

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damage decreases synthesis of certain critical factors by leukocytes and reproductive capacity of leukocytes [1].

Aerobic organisms are protected against free radicals by enzymatic and non enzymatic antioxidant defenses [6]. One of the most important enzymatic antioxidants is superoxide dismutase (SOD) [7]. The cytoplasmic copper/zinc-SOD, one of three forms of SOD, contains copper and zinc as cofactors and is believed to play a predominant role in the first step of antioxidant defense [6]. Ceruloplasmin, the copper-containing protein of extracellular fluids, has been shown to have important antioxidant properties towards peroxidising lipids [4]. Vitamin A and vitamin E are known among non enzymatic antioxidant defense systems [5]. They react with free radical molecules to prevent tissue injury [1].

In OME, infection leads to middle ear inflammation, which causes excess production of free radicals by inflammatory cells. Also, hypoxia and ischemia in OME causes oxidative stress by increasing oxygen radicals and by inhibiting oxidative phosphorylation, and therefore cell death occurs by decreasing ATP levels. The low level of antioxidants may be both the cause and the result of chronic infection [1].

Particularly in developing countries, research on micro-nutrient status and vitamin deficiency and their influence on middle-ear disease may provide a valuable information on the knowledge of the pathogenesis of middle-ear infection, prevention and treatment. Our hypothesis is that low serum values of vitamin A, vitamin E, copper and zinc may be a contributory factor in the development of OME.

There are limited data on the relationship between antioxidant status and the pathogenesis of OME in humans [1, 3, 8]. The aim of this study is to determine whether the serum levels of vitamin A, vitamin E, copper and zinc have a role in the development of OME in childhood.

Patients and Methods

This prospective study was conducted between February 2010 and February 2011 at a tertiary Otorhinolaryngology Clinic. The study was approved by the hospital ethics committee (approval no. 0357) and informed consent was obtained from the parents of children. One-hundred thirteen subjects were enrolled and subdivided into three groups. Group 1 (OME-adenoid hyperplasia) included 44 patients who had adenoidectomy with ventilation tube placement due to OME. Group 2 (adenoid hyperplasia-alone) consisted of 43 patients who had adenoidectomy without ventilation tube insertion. Group 3 (healthy control) included 26 healthy children without any complaint in the head and neck region, without any infection, any other systemic disease or any history of previous operation concerning the tonsils, adenoids and ears. Controls were

randomly selected among healthy children visiting hospital, children of hospital workers, and schoolchildren. Patients were excluded if they had adenoidectomy with tonsillectomy, acute otitis media, acute upper respiratory track infection within the last 3 months, nasal polyp, history of atopy, repeated ventilation tube insertion, immune deficiency and metabolic diseases. None of them had taken any medication or dietary vitamin supplements for at least 3 months prior to the study.

The presence of OME was verified at least three successive monthly examinations with otomicroscopy and tympanometry. Patients with an effusion lasting more than 3 months were included in this study. The indications for ventilation tube insertion with adenoidectomy was the lack of response to medical treatment including amoxicillin-clavulanate, systemic decongestants and anti-inflammatory agents for 3 months. In group 2 (adenoid hyperplasia-alone), there was no suspected OME finding by otoscopic, otomicroscopic and tympanometric evaluation. Adenoid hyperplasia was confirmed with cephalometry, and the adenoid to nasopharynx ratio was calculated. Children who had an adenoid to nasopharynx ratio greater than 80 % were included in the study.

Venous blood samples of all subjects were taken before operation for routine preoperative investigations (in the morning, following 12 h fasting). To avoid isomerization of vitamin A and E, blood samples were protected from light as soon as they were drawn. Serum zinc and copper measurements were obtained calorimetrically using commercial diagnostic kits (respectively QCA, Far) in an oto-analyser (Beckman Coulter AU 2700). Vitamin A and E measurements were done by using the high performance liquid chromatography (HPLC) (Agilent 1100 HPLC system) with original chromsystems kit.

Statistical Analysis

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) 11.5 software (SPSS Inc., Chicago, IL, United States). Whether the continuous variables were normally distributed or not was determined by using Shapiro–Wilk test. Homogeneity of variances was evaluated by Levene test. Data were expressed as mean $\pm$ SD or median (minimum–maximum) for continuous data and number of cases and percentages for nominal variables. While the mean differences among groups were compared by One-Way ANOVA, otherwise, Kruskal–Wallis test was used to compare the median levels. When the *p* value from the Kruskal–Wallis test are statistically significant to know which group differ from which others by using Conover's multiple comparison test were used. Nominal data were evaluated by Pearson Chi-square test. A *p* value <0.05 was considered statistically significant.

Results

Group 1 (OME-adenoid hyperplasia) consisted of 44 patients (23 males and 21 females) with a mean age of 6 ± 2 (range 3–11 years). Group 2 (adenoid hyperplasia-alone) included 43 patients (22 males and 21 females) with a mean age of 7.2 ± 3.4 (range 3–15 years). Group 3 (healthy control) made up 26 subjects (18 males and 8 females) with a mean age of 7.7 ± 2.6 (range 3–13 years). The differences between all groups regarding distributions of age and gender were statistically insignificant ($p > 0.05$).

There were no statistically significant differences among the three groups in terms of serum levels of vitamin A, vitamin E and copper (respectively $p = 0.188$, $p = 0.948$, $p = 0.709$).

Comparison of the patient groups showed that group 1 patients (OME-adenoid hyperplasia) had significantly lower serum zinc levels than group 2 patients (adenoid hyperplasia-alone) ($p = 0.002$), although differences between both patients groups and controls were not significant (for OME-adenoid hyperplasia $p = 0.316$, for adenoid hyperplasia-alone $p = 0.077$). Statistical results are presented in Table 1.

Discussion

The close interactions among nutrition, infection, and health have been recognized since many decades. Adequate intake of vitamins and micronutrients is required for the immune system function [9]. Nutritional factors may play a contributory role in the middle ear diseases [2, 5].

Vitamin A has anti-infective, anti-inflammatory and antioxidant activities. It is a fat soluble substance and essential for the immune system, growth, reproduction, vision, cellular differentiation and the maintenance of the respiratory epithelium [6, 7, 10]. The effects of vitamin A

deficiency have been linked to immunological functions, included increased risks of mortality and morbidity from measles and respiratory tract infections. Retinoids and their derivatives are required for the maintenance of the normal epithelial mucociliary phenotype [10]. Elemraïd et al. [2] stated that vitamin A deficiency may have effects on the eustachian tube, tympanic membrane and middle ear mucosa by various possible mechanism such as effects on epithelial function and sub-epithelial oedema, oxidant-antioxidant effects, luminal narrowing of eustachian tube, and altered secretion of mucin, lysozyme and secretory immunoglobulins. This deficiency in children leads to an increase in frequency of otitis media and the impairment of closure of tympanic membrane perforation by inhibiting epithelial regeneration, thus contributes to chronicity of otitis media. Additionally, it leads to middle ear epithelial metaplasia [1]. Lassisi [10] reported that hyporetinolaemia have a significant etiological factor for acute and chronic suppurative otitis media, and suggested retinol supplementation in control of suppurative otitis media. Vitamin A supplementation reduces severe morbidity and mortality from infectious diseases in children and also decreases the incidence of otitis media [2, 7, 11].

Vitamin E is known as one of the most important lipid soluble antioxidant which exists within lipoproteins and cell membranes and was shown to optimize and enhance the immune response due to its antioxidant activity [5, 6, 9]. It suppresses the oxidative damage of the membranes [5]. In a recently study by Cemek et al. [5] nonenzymatic antioxidants such as vitamin A and E were determined lower in children with acute otitis media compared with healthy control subjects. Yılmaz et al. investigated the blood levels of antioxidants and oxidants in 24 children with OME-adenoid hyperplasia before and after operation and 23 healthy subjects. They found that preoperative blood levels of retinol, β -Carotene, vitamin E in the OME-adenoid hyperplasia group were lower than control group [1]. These findings are not in accordance with our results. We found that the differences among the three groups in terms of serum levels of vitamin A, vitamin E were not statistically significant. The controversial results may be due to variations in the number and gender of the patients. Additionally, developed preventive health services, increased socioeconomic status of the people and altered nutrition habits during past years may be responsible for this result.

Copper has been shown to play a role in the development and maintenance of the immune system. A large number of experimental studies have demonstrated that copper status alters several aspects of neutrophil, monocyte, and T-cell function in the immune system [9]. In a comprehensive review of the literature, no human study specifically examining the association between copper

Table 1 Serum levels of copper, zinc, vitamin A and E in the subjects

Variables	Group 1 (n = 44)	Group 2 (n = 43)	Group 3 (n = 26)	p value
Copper	16.3 $\pm$ 4.6	16.3 $\pm$ 5.3	17.2 $\pm$ 3.1	0.709 ^b
Zinc	11.6 (6.6–19.7) ^a	14.5 (7.6–31.4) ^a	12.8 (7.1–18.2)	0.013 ^c
Vitamin A	1.0 (0.3–2.2)	1.1 (0.5–1.6)	1.0 (0.5–1.7)	0.188 ^c
Vitamin E	24.0 $\pm$ 5.0	23.9 $\pm$ 4.6	23.6 $\pm$ 3.8	0.948 ^b

Group 1 (OME-adenoid hyperplasia), Group 2 (adenoid hyperplasia-alone), Group 3 (control)

OME otitis media with effusion

^a Difference between groups 1 and 2 was statistically significant ($p = 0.002$)

^b One-way ANOVA

^c Kruskal–Wallis test

status and middle ear diseases was identified. In our study, we did not find a significant difference among groups concerning serum copper levels.

Dietary zinc deficiency impairs immune function and resistance to infection by suppressing thymic function, T-lymphocyte development, lymphoproliferation, and T cell dependent B-cell functions. Its deficiency has been reported to increase the time for tissue repair [8]. In a large, well controlled trial in Bangladeshi children, supplementation with zinc showed a significant protective effect in reducing suppurative otitis media [2]. Lasisi [8] found that serum zinc levels of the patients with mucoid otitis media were significantly lower than those of patients with purulent otitis media and healthy control subjects. Our results showed that patients in group 1 (OME-adenoid hyperplasia) had significantly lower serum zinc values than those of group 2 patients (adenoid hyperplasia-alone), although there were no statistically significant differences between both patient groups and controls.

In OME, middle ear drainage is disturbed due to the functional blockage of the eustachian tube such as mucosal edema and hyperplasia, thick secretions, ciliary dysfunctions, pressure differences between the middle ear and nasopharynx [1]. Adenoid hyperplasia may contribute to the formation of OME in several ways. It may lead to physical obstruction or local inflammatory reaction in the eustachian tubes and middle ear caused by release of inflammatory mediators. The inflammation of adenoid tissue is important rather than its volume in the formation of OME. It is known that OME does not develop in every patient with adenoid hyperplasia [3]. The antioxidant status of these patients may be a significant factor in predisposition to OME. We found that serum zinc values in group 2 (adenoid hyperplasia-alone) were significantly higher than group 1 (OME-adenoid hyperplasia). However, there were not statistically significant differences between group 2 (adenoid hyperplasia-alone) and other groups regarding other three parameters. We speculated that high serum zinc values of group 2 (adenoid hyperplasia-alone) may be a contributory factor in prevention of OME in patients with adenoid hyperplasia.

Conclusion

The results of this study showed that serum levels of vitamin A, vitamin E and copper may not play a role on development of OME. On the other hand, we found that the

serum levels of zinc were higher in group 2 patients (adenoid hyperplasia-alone) than group 1 patients (OME-adenoid hyperplasia). We postulated that high serum zinc status may have the preventive effect in the predisposition to OME which may related to adenoid hyperplasia. Further controlled clinical studies are needed to elucidate the effects of micronutrient and vitamin status in pathogenesis of OME.

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Conflict of interest The authors declare that they have no conflict of interest.

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