JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2015. Vol. **9**(3), p. 2127-2130

# Serum Level of Nitric Oxide in Patients with Otitis Media with Effusion, Chronic Otitis Media, and Healthy Subjects

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(Received: 15 July 2015; accepted: 01 September 2015)

**Otitis Media is a common problem among the subjects attending to otolaryngology Clinics. Hence in this study the serum level of nitric oxide (NO) was determined and compared between patients with otitis media with effusion, subjects with chronic otitis media, and healthy subjects. In this observational case-control study, 29 patients with otitis media with effusion, 28 subjects with chronic otitis media, and 30 healthy subjects attending to Imam-Khomeini Hospital of Tehran in 2013 and 2014 were enrolled and the serum level of nitric oxide among them were determined and compared. There was no significant difference between NO level in otitis media with effusion (12.1 ± 4.1) and chronic otitis media (13.4 ± 8) groups (P > 0.05); but normal subjects (6.8 ± 1.3) had significantly lower serum NO levels in comparison with patients with otitis media with effusion (P = 0.003) and those with chronic otitis media (P = 0.001). Totally, according to the obtained results, it may be concluded that Serum level of nitric oxide is significantly higher among subjects with otitis media with effusion and patients with chronic otitis media compared with healthy normal subjects.**

**Key words:** Nitric Oxide, Otitis media with effusion, Chronic otitis media.

Otitis media with effusion is the most common cause of hearing impairment in children resulting from inflammatory conditions in middle ear characterized by inflammatory cells infiltration in sub-mucosal layer, cellular proliferation in mucosal layer and epithelium of middle ear, and aggregation of neutrophils and macrophages and lymphocytes in middle ear fluid[1, 2]. Late fluid accumulation after acute process in middle ear is due to mucocilliary dysfunction[3, 4]. Otitis media may be purulent (acute), serous, mucoid, and chronic[5]. Chronic otitis media (COM) is inflammation of mucosal layer of middle ear and temporal bone and is defined with tympanic membrane perforation for more than three months[6]. Numerous factors are integrated in tissue destruction and chronic course but the pathophysiology is yet unclear. Among these factors, osteoclastic activity, cytokines, chronic inflammations, endotoxins, and lipid peroxidation products may be mentioned but recently the focus is increased on free oxygen radicals and nitric oxide[6, 7]. Nitric oxide as an oxidant agent is especially increased in inflammatory processes[8, 9] with an important role in chronic phase of many diseases[10]. Also in multiple studies, the association of nitric oxide and oxidative stresses with chronic otitis

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media and sinusitis is proposed[11]. But this role is not compared in different types of otitis media. In this study, the serum level of nitric oxide in patients with chronic otitis media and otitis media with effusion and healthy subjects were compared.

## MATERIALS AND METHODS

In this case-control study, 30 healthy subjects, 28 patients with chronic otitis media, and 29 subjects with otitis media with effusion attending to a training hospital in Ahwaz, Iran in 2013 and 2014 were enrolled. Those with acute/ systemic infections, those with use of alcohol and drug and smoking, the subjects regularly using antioxidant vitamins such as C and E were excluded. After 12 hours of fasting, blood sampling was performed and the sample was put on ice in 4 centigrade degree. The serum was extracted with 3000 cycle centrifuge for 10 minutes. The serum samples were kept in -20 ˚C up to test time. The reactant was Griese according to Moshage method. Data analysis was performed among 87 subjects in by SPSS (version 18.0) software [Statistical Procedures for Social Sciences; Chicago, Illinois, USA]. Kolmogorov-Smirnov and one-way ANOVA tests were used and were considered statistically significant at P values less than 0.05.

## RESULTS

Totally, 44 subjects (60.6%) were male and 43 patients (49.4%) were female. The gender distribution in three groups was alike (Table 1). The mean NO level had not significantly difference between men and women (Table 2). The mean NO concentration according to gender in groups was significant for two case groups but not control group (Table 3). However the serum NO level was not differed between those with chronic otitis media and subjects with otitis media with effusion ($P > 0.05$) the normal groups showed significant difference with both groups ($P < 0.05$).

**Table 1.** Gender distribution among groups

| | | | Sex | | Total |
|---|---|---|---|---|---|
| | | | Male | Female | |
| Group | Normal | Count | 15 | 15 | 30 |
| | | % within Group | 50.0% | 50.0% | 100.0% |
| | VT | Count | 15 | 14 | 29 |
| | | % within Group | 51.7% | 48.3% | 100.0% |
| | COM | Count | 14 | 14 | 28 |
| | | % within Group | 50.0% | 50.0% | 100.0% |
| Total | Count | | 44 | 43 | 87 |
| | % within Group | | 50.6% | 49.4% | 100.0% |

**Table 2.** Mean NO level in men and women

| | Sex | Mean | Std. Deviation |
|---|---|---|---|
| No Concentration | Male | 9.8343 | 4.74196 |
| | Female | 11.5351 | 6.77270 |

## DISCUSSION

In this study, the serum level of nitric oxide in patients with chronic otitis media and otitis media with effusion and healthy subjects were compared. We found non-significant difference between serum levels of NO among two case groups but significant difference between these groups with control subjects. In Donor *et al*.,[10] animal study, the ear and serum levels of Malondialdehyte were measured and it was increased in those who had otitis media demonstrating the role of free oxygen radicals[11] in accordance with our findings. Li *et al*. selected 40 patients to evaluate effusion in middle ear as an in-vivo. The addition of interleukin 13 and TNF-Alpha resulted in increased expression of nitric oxide synthetase in these groups. This shows that cytokines would result in increased

**Table 3.** The mean No concentration according to gender in groups no Concentration

| Group | Sex | Mean | Std. Deviation |
|---|---|---|---|
| Normal | Male | 6.8240 | 1.04678 |
| | Female | 6.7853 | 1.63222 |
| | Total | 6.8047 | 1.34740 |
| VT | Male | 11.0193 | 2.85166 |
| | Female | 13.1771 | 4.93687 |
| | Total | 12.0610 | 4.07260 |
| COM | Male | 11.7900 | 6.94583 |
| | Female | 14.9821 | 8.90061 |
| | Total | 13.3861 | 8.00088 |
| Total | Male | 9.8343 | 4.74196 |
| | Female | 11.5351 | 6.77270 |
| | Total | 10.6749 | 5.86310 |

activity of nitric oxide synthetase and increased NO in middle ear [13].

Takoudes performed first human study among 35 children with otitis media and found that lipid hydroperoxide level was increased[14] as well as NO in our study. The serum oxidative level was measured by superoxide dimutase, xantin oxidase, and malondialdehyde (MDA) in six animal models with serous otitis media and six healthy models showing significant increase in free oxygen radical and revealing the role of these radicals in tissue destruction in otitis media[15]. The study by Baysal et al. showed the increased anti-oxidants and oxidative stress in case group in comparison with control group[16].

Totally, according to the obtained results, it may be concluded that serum level of nitric oxide is significantly higher among subjects with otitis media with effusion and patients with chronic otitis media compared with healthy normal subjects. However further studies should be carried out as multi-center assays with larger sample size, and comparison with other markers.

## ACKNOWLEDGEMENTS

The authors would like to thank the research deputy of vice-chancellor for research for their valuable coopera-tion. This research work was the result of a funded ENT specialist thesis.

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