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EVALUATION OF OXIDATIVE STRESS AND VITAMIN A IN PATIENTS OF CHRONIC OTITIS MEDIA WITH AND WITHOUT CHOLESTEATOMA.

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ABSTRACT:

Background: Chronic otitis media is a serious clinical condition characterized by an accumulation of fluid behind the tympanic membrane, while lacking the signs and symptoms of acute infection. The aim of this study was to investigate the effect of oxidative stress and antioxidant situation on chronic otitis media (COM) with and without cholesteatoma.

Methodology: The study included a total of 75 cases of COM and further subcategorised based on cholesteatoma, 35 cases COM with cholesteatoma (20 females and 15 males) and 35 COM without cholesteatoma (16 females and 19 males). Serum specimens were taken prior to surgery and diseased tissue specimens from the ear were obtained during surgery from all patients. A proper audiometry testing was done. serum specimens were taken from every individual. The malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GHPx) were measured in the serum samples of the patient of COM with and without cholesteatoma. Vitamin A were determined by HPLC methodology.

Results: The patients in this study, who might be of either gender, ranged in age from 25 to 40. Patients with chronic otitis media were subdivided based on their cholesteatoma status, as well as the levels of different oxidative indicators and vitamin A. Table 1 displays information about each groups' age, gender, and BMI. Table 2 displays antioxidant enzymes as well as additional biochemical information for both groups.

Conclusion: The present study could help clarify the fundamental causes of the pathophysiology of COM. A finding of vitamin A does not show any direct role in occurrence of diseases but it shown significant difference in COM with and without cholesteatoma.

Introduction:

The rupture of the ear drum and inflammation of the mucosa lining the middle ear hollow and the airy spaces of the temporal bone that last longer than three months are known as chronic otitis media (COM). A growing tumor made up of keratinizing squamous epithelium in the middle ear and/or mastoid process is a characteristic of COM with cholesteatoma [1]. Traditionally, the mucoperiosteum has been the exclusive site of COM pathology. Any pathology that surpasses this threshold may lead to consequences such meningitis, osteitis, and bone deterioration [2].

Under normal physiological settings, reactive oxygen species (ROS) are significant pathogenic mediators of many illnesses. Not only are some reactive forms of oxygen necessary for human life,

but they can also be harmful to the body. ROS can be harmful to many tissues because they are produced during chemical and metabolic interactions involving molecules like lipid peroxides, hydrogen peroxide, hydroxyl free radicals, and many more derivatives. Two examples of oxidative enzymes are myeloperoxidase (MPO) and nitric oxide (NO) [3].

Free oxygen radicals, or FORs, are critical for both metabolic processes and immunological responses. The body produces FORs as defense cells such macrophages, neutrophils, and monocytes combat antigens [4]. However, an overabundance of FORs results in tissue damage, which impedes the body's normal healing process and lengthens the duration of inflammation [5]. Free radicals are transformed into less dangerous forms by antioxidants, which also stop new free radicals from being created. After antioxidants have neutralized the ROS in a tissue, damage to the tissue, lipids, proteins, and DNA results, ultimately leading to cellular death [6].

An imbalance between increased reactive oxygen species (ROS) and antioxidant defence mechanisms interferes with the healing process of wounds and encourages persistent inflammation, which facilitates the development of cholesteatoma in COM. Antioxidant enzymes like glutathione peroxidase, catalase, and superoxide dismutase not only play a fundamental but indispensable role to fend off free radical damage via antioxidant defense system.[5]

In this study, Antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GHPx), malondialdehyde (MDA), and vitamin A, were evaluated in serum samples of COM patients with and without cholesteatoma. These samples showed mucosal inflammation in the middle ear.

Materials & methods:

A type of analytical and comparative study conducted on confirm diagnosis of chronic otitis media (COM) of total 75 patients after obtaining the ethical approval from institutional ethical committee. A confirmed cases of COM were enrolled from different ENT clinic of Cuttack and Balasore, Odisha, India. A total of 75 cases were further divided based on their cholesteatoma. 75 patients were further comprised 35 patients with COM with cholesteatoma (20 females and 15 males) and 35 COM without cholesteatoma (16 females and 19 males). Pure tone audiometry and temporal bone computed tomography (CT) were used to evaluate each patient. Participants in the study were those whose CT scans revealed soft tissue density, which indicated granulation tissue in the middle ear and mastoid cells. Antioxidant vitamin supplements, including vitamins A were not being regularly administered to any of the patients. Patients with systemic illness or acute infections were not included. Furthermore, the patients were not on any medications, nor were they smokers or drinkers. After an overnight fasting the blood samples were drawn with aseptic precaution. The serum was then separated by centrifugation at 3,000 RPM for 10 minutes. Serum samples used for the measurement of antioxidant enzymes levels such as CAT, GHPx, and SOD. For the measurement of MDA and vitamin E, serum sample were stored at –40°C till the analysis. Every patient had a mastoidectomy. During surgery, tissue samples were extracted from problematic locations in the middle ear or mastoid cells. After splitting the specimens into two groups, one for each type of cholesteatoma the specimens were frozen at -40°C in dry tubes.

For vitamin A determination, 75 µL of serum was transferred into a 1.5 mL tube. Isopropanol (300 µL) and an internal standard (0.75 mg/L acetic retinyl) were added to the tube, and the mixture was vigorously vortexed for 2 min. Following this, the mixture was centrifuged for 15 min at 8500 revolutions per minute. The supernatant was collected, and the determination of vitamin A levels was conducted using the high-performance liquid chromatography (HPLC) method.

Statistical analysis:

Version 22.0 of the SPSS software was used to assess each data set's statistical analysis. A Pearson chi-squared test was run on the group's gender evaluation statistics. Numbers and percentages were used to report the comparison results that were obtained. The continuous variables between the groups were compared using an independent sample t-test. The results of this comparison are given as mean and standard deviation.

Results:

The age ranged from 25to 40 years of the patients with either gender was enrolled in this study. A chronic otitis media patients were subgroup on the basis of their cholesteatoma condition and levels of various oxidative markers and vitamin A were determinised. Age, gender and BMI data of both the groups are presented in Table 1. Antioxidant enzymes and other Biochemical data for both the groups are presented in Table 2.

Table-1: Statistical analysis of general characteristic

Characteristic		COM with cholesteatoma (n=35)	COM without cholesteatoma (n=35)	P value
Age (Mean ± SD)		31.0 ± 11.4	35.1 ± 10.9	0.34
sex	Male	20	16	0.27
	Female	15	19	
BMI (kg/m2)		24.8 ± 4.5	25.8 ± 4.6	0.83

Table-2: Analysis of oxidative stress and vitamin E in COM with & without cholesteatoma

Characteristic	COM with cholesteatoma (n=35)	COM without cholesteatoma (n=35)	P value
CAT (U/L)	20.55 ± 3.01	25.64 ± 5.01	<0.05
GHPx (EU/mL)	34.32 ± 3.02	34.48 ± 2.98	<0.05
SOD(EU/mL)	19.98 ± 1.98	21.65 ± 2.00	<0.05
MDA (nmol/mL)	35.24± 2.03	45.38 ± 2.43	<0.05
Vitamin A (ug/L)	504.66 ± 165.23	531.22 ± 170.23	<0.05

Discussion:

A timeline of treatment illness course and its potential complication are the main cause for the chronic condition of otitis media. COM is still significant in the practice of otologists. The pathophysiology includes recurrent upper respiratory tract infections, immunosuppressive diseases, allergies, malnutrition, nasopharyngeal lymphatic tissue hypertrophy, and craniofacial abnormalities. The chronicity of infection and tissue damage in COM have lately been attributed to ROS, [7]. Endogenous ROS production occurs during a variety of physiological events related to oxygen consumption. By chemically altering proteins, carbohydrates, nucleotides, and lipids, excessive ROS synthesis damages tissue and may contribute to the etiology of a number of illnesses. The body nonenzymatic antioxidants include glutathione, tocopherole (vitamin E), ascorbic acid (vitamin C), beta-carotene (vitamin A), albumin, bilirubin, and uric acid [8]. The most significant antioxidant enzymes are SOD, GHPx, and CAT. Oxidative stress is linked to reductions in antioxidant levels or elevations in oxidant production; this state results in phospholipid peroxidation, which damages essential bodily components such lipids, lipoproteins, proteins, and DNA [9]. Polyunsaturated fatty acids are hydrolyzed into physiologically active substances at the conclusion of the process. The most significant of these substances is MDA, which signifies the body's lipid peroxidation [10]. Martanegara et al (2020) also observed that most (64.72%) cases were in the age range of 21-30 years. [11] Pratama et al conducted a study at Sanglah General Hospital, reporting that 43.2%, 18.9%, and 18.6% of CSOM cases occurred in adults, elderly, and teenagers [12]. similarly, Gaurano and Johaarjy reported that most cholesteatoma patients were aged between 20 and 35 [13] In this study, a similar trend was identified in both CSOM with and without cholesteatoma, with the highest incidence occurring in the age between 25 and 35 years. This age group appears particularly susceptible to CSOM, likely due to their productive age and potentially lower attention to hygiene, sanitation, as well as overall health.[11]

Yilmaz et al. [14] demonstrated high levels of serum MDA preoperatively, but low levels of serum SOD, GHPx, retinol, beta-carotene, alpha-tocopherole, lycopene and ascorbic acid in children with ventilation tubes due to adenoidectomy and otitis media with effusion. They showed that antioxidant

levels normalized after one month postoperatively and emphasized the requirement of antioxidant therapy in these patients. Garcia Callejo et al. [15] found that the MDA value in the effusion fluid of COM patients with effusion was approximately 10 times higher than that in the middle ear fluid of OME patients. Baysal et al. [16] reported a low total antioxidant capacity and high oxidative stress index in COM patients with and without cholesteatoma.

In this study, the oxidative balance was evaluated in patients with and without cholesteatoma were compared. When the serum values of all COM patients with and without cholesteatoma were compared, then the levels of antioxidant enzyme such as MDA, SOD & CAT in the patient of COM without cholesteatoma was found to be significantly higher ($P<0.05$). The present study did not find any such differences in the mean levels of GHPx in patients of COM with and without cholesteatoma but somehow it has shown a significant difference between these two groups ($P<0.05$). These findings indicate that evaluating the values of only serum oxidative stress, excluding assessment of tissue values, may suffice for the evaluation of oxidative stress in COM patients. Also, this indicates that the severity of the disease is not parallel to the stress oxidation, in other words, oxidative stress does not reflect the severity of the disease.

Vitamins A serve as secondary or exogenous antioxidants, effectively inhibiting and neutralizing oxidation reactions.[17] Study conducted by Baysal et al identified a significant increase in serum oxidant status and oxidative stress index among CSOM patients. Furthermore, changes in diet and nutritional intake can impact the levels of these vitamins in the blood. In this context, Vitamin A plays a role in cell differentiation and epithelial proliferation.[18] Additionally, Vitamin E, being a fat-soluble antioxidant, effectively inhibits peroxyl radicals and halts the oxidation of polyunsaturated fatty acids (PUFA). According to a study by Greaves, et al, an advantage of vitamin A is its effectiveness at low concentrations of oxygen. [19] Therefore, it can complement the antioxidant properties of vitamin E that is effective at high concentrations.[20]

In this study, it was observed that patients with low levels of vitamins A did not increase the risk of CSOM with cholesteatoma. Furthermore, the mean serum level of vitamin A was slightly lower in CSOM with cholesteatoma, and found significantly different ($P<0.05$). The study by Arulselvan et al stated that otitis media is a manifestation of vitamin A deficiency [20].

Conclusion:

Oxidative stress due to FORs plays a role in the pathogenesis of both COM with and without cholesteatoma. Assessing the values of only serum oxidative stress, excluding assessment of tissue values, may suffice for the evaluation of oxidative stress in COM patients. However, oxidative stress values may not reflect the severity of the disease. However, Vitamin A levels does not show any impact on occurrence of COM and cholesteatoma. But it has role in neutralizing the ROS, so it may have potent role in pathophysiology of diseases. For the reliable conclusion a large number of participants and details daily utility of vitamin A by patient would emphasize it role in clear outlook.

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