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Oxidative stress in ENT Disease:
evidence for treatment of Craniofacial Neuralgia and Tinnitus

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CONTENTS

SPECIAL ISSUE: Oxidative stress in ENT Disease: evidence for treatment of Craniofacial Neuralgia and Tinnitus

Oxidative stress: general aspects and metabolic mechanisms

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Oxidative stress is characterized by the imbalance between production and accumulation of oxidants and the ability to detoxify these reactive products. Reactive oxygen species (ROS) and free radicals have a direct damaging effect on cellular functions and are involved in the pathogenesis of several diseases, including atherosclerosis, chronic obstructive pulmonary disease (COPD), diabetes, Alzheimer's disease and cancer. Recently, research has focused on the possible beneficial role of antioxidant treatments against oxidative stress-related diseases. However, their efficacy and mechanisms of action are still debated. This review aims to clarify the mechanisms underlying oxidative stress and the development and progression of several oxidative stress-related diseases.

Oxidative stress, defined as the imbalance between the production of oxidants and antioxidant defences, results in damage to biological systems, thus contributing to the etiology of both "normal" senescence and a wide range of diseases, such as atherosclerosis, chronic obstructive pulmonary disease (COPD), diabetes, Alzheimer disease and cancer (1). Low levels of oxidative stress are very common under normal physiological conditions and act synergistically with antioxidants to maintain cell homeostasis, playing an important role in host defence, gene transcription and apoptosis.

Since the extent to which oxidative stress participates in the pathophysiology of several diseases is quite variable, recent interest has focused on the intricate ways by which redox signalling maintains oxidative eustress and, therefore, on the possible role of antioxidant compounds in contributing to redox balance and preventing or treating these diseases (4, 5). In this context, understanding the mechanisms by which oxidative stress contributes to disease development is of utmost importance.

This brief review summarises the mechanisms involved in oxidative stress generation and the relationships between oxidative stress and the pathogenesis of several diseases.

Oxidative stress

Oxygen, a crucial component for life-sustaining aerobic pathways in humans, is consumed for about 95% as energy and ultimately transformed in water. The remaining 5% can turn into oxygen radicals, leading to the production of reactive oxygen species (ROS); in fact, the main source of ROS *in vivo* is represented by aerobic breathing and mitochondria are considered the most redox-active compartment in the cell, accounting for more than 90% of oxygen utilization. The generation of mitochondrial ROS mainly occurs at the electron transport chain (ETC), located on the inner mitochondrial membrane, during

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oxidative phosphorylation. Although the precise sites of ROS production within each complex and the mechanisms by which they are produced are not fully understood, complexes I (NADH: ubiquinone oxidoreductase) and III (ubiquinol: cytochrome c oxidoreductase) are recognized as the major sources of ROS within the respiratory chain (Fig 1) (6, 7).

Nevertheless, ROS are also generated by peroxisomal β-oxidation of fatty acids, microsomal cytochrome P450, metabolism of xenobiotic compounds, stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue-specific cellular enzymes (8).

Intracellular generation of ROS *per se* is an inevitable event, also involved in several physiological processes, such as redox signalling and the killing of bacteria, and human cells exhibit several defence systems against ROS. However, precisely the space-time imbalance of ROS production and detoxification determines oxidative stress rather than ROS generation *per se* (9). The triggering factors are numerous, ranging from hereditary or acquired genetic defects or environmental factors, such as toxins and radiation, to pure stochastic events, such as metabolic fluctuations (10).

ROS generation

ROS include superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), peroxyl radical ($RO_2^{\bullet}$), alkoxyl radical ($RO^{\bullet}$), hydroperoxyl radical (HO_2), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), reactive nitrogen species (RNS) and lipid radical/lipid peroxyl radical ($L^{\bullet}/LOO^{\bullet}$) (11) characterized by an imbalance between reactive oxygen species (ROS).

These ROS are generated in a sequence of reactions, where one type of ROS product is used for generating a new type of ROS (Fig. 2a). $O_2^{\bullet-}$, which is generated from the coupling of O_2 with an electron (e^-) from a donator represented by reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH), is typically the first ROS generating cascade. NADPH oxidase (NOX1/2/3/5 family), uncoupled NOS, xanthine oxidase (XO) and complex I/II/III/IV of mitochondria are the oxidases that typically catalyze $O_2^{\bullet-}$ production. $O_2^{\bullet-}$, in turn, is converted by mitochondrial superoxide dismutase (SOD) into H_2O_2, which in turn transmute into other oxidants, such as $HO^{\bullet}$. $HO^{\bullet}$ generation is catalyzed by Habor-Weiss and Fenton reactions. In

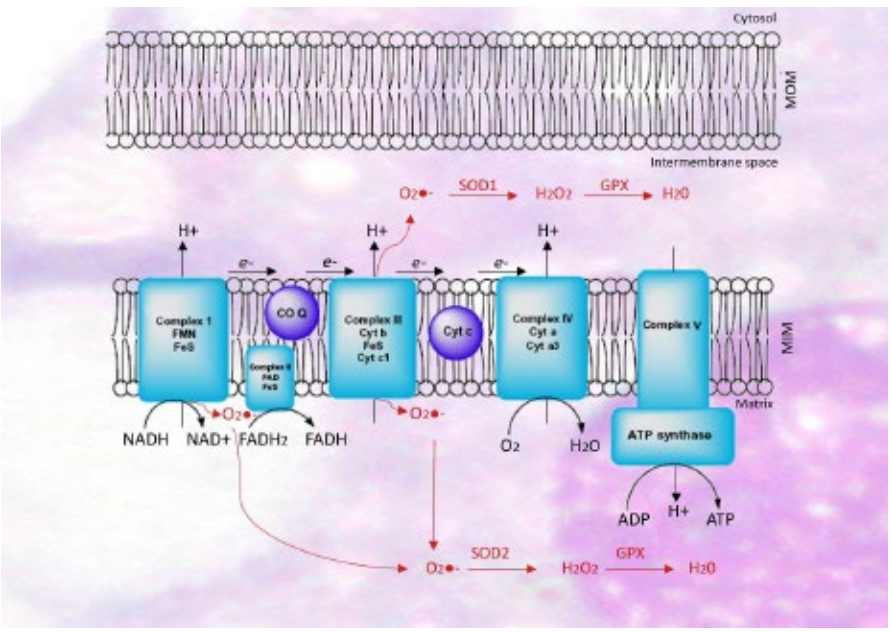

Fig. 1. *Major pathways of ROS mitochondrial production by the electron transport chain (ETC).*

the Habor-Weiss reaction, Fe3+ is reduced in Fe2+ by $O_2^{\bullet-}$, while, in the Fenton reaction, Fe^{2+} reacts with H_2O_2 to generate $OH^{\bullet}$ and OH^-. These two reactions can occur in sequence, amplifying ROS production (Fig. 2 b-c); this is the reason why oxidative stress could represent a major mechanism underlying diseases characterized by iron accumulation, such as atherosclerosis or sickle cell anaemia (8), (12). $O_2^{\bullet-}$ can also react with nitric oxide ($NO^{\bullet}$), which is generated from L-arginine (L-Arg) forming RNS, such as highly reactive peroxynitrite ($ONOO^-$)[13]and in particular from mithocondria. Morever, $OH^{\bullet}$ can attack lipids, forming $L^{\bullet}$ that in turn reacts rapidly with O_2 to generate $LOO^{\bullet}$. Although the molecular mechanisms responsible for mitochondria-mediated disease processes are unclear, oxidative stress seems to play an important role. ROS are essential to cell function, but adequate levels of antioxidant defenses are required in order to avoid the harmful effects that excessive ROS production can produce. Mitochondrial oxidative stress damage and dysfunction contribute to a number of cell pathologies that manifest themselves through a range of conditions. The antioxidants available until now have not proved to be particularly effective against many of these disorders. It is possible that these antioxidants do not reach the sites of free radical generation, especially when mitochondria are the primary source of ROS. Recent developments in mitochondria-targeted antioxidants have moved closer to providing protection against mitochondrial oxidative damage.

Mitochondrial antioxidant systems

Mitochondria are equipped with a multi-levelled ROS defence network of enzymatic and non-enzymatic antioxidants. Among the enzymatic antioxidants, mitochondria contain three superoxide dismutase isozymes (SOD), catalysing the dismutation of two $O_2^{\bullet-}$ to H_2O_2. Manganese SOD (Mn-SOD, SOD2) is considered a first-defence mitochondrial antioxidant enzyme, which is located in the mitochondrial matrix, whereas copper/zinc SOD (Cu, Zn-SOD, SOD1) is located in the intermembrane space of the mitochondria and the cytosol. SOD3 is localized in the extracellular matrix (7). Moreover, the removal of $O_2^{\bullet-}$ can be mediated by intermembrane cytochrome c. H_2O_2 can be transformed into H_2O by glutathione peroxidase (GPX). According to the glutathione redox cycle, GPX reduces H_2O_2 and becomes oxidized and inactive. Then, it can be converted back to the reduced form by glutathione (GSH), which in turn becomes glutathione disulfide (GSSG). GSS is then reduced to GSH after a reaction catalyzed by glutathione reductase (GSR). H_2O_2 conversion in H_2O and O_2 is also mediated by catalase (CAT), an iron-containing peroxidase (Fig 3). The reduction of H_2O_2 to H_2O is also catalyzed by PRDX, a family of thiol-specific peroxidases. PRDXs are oxidized during the

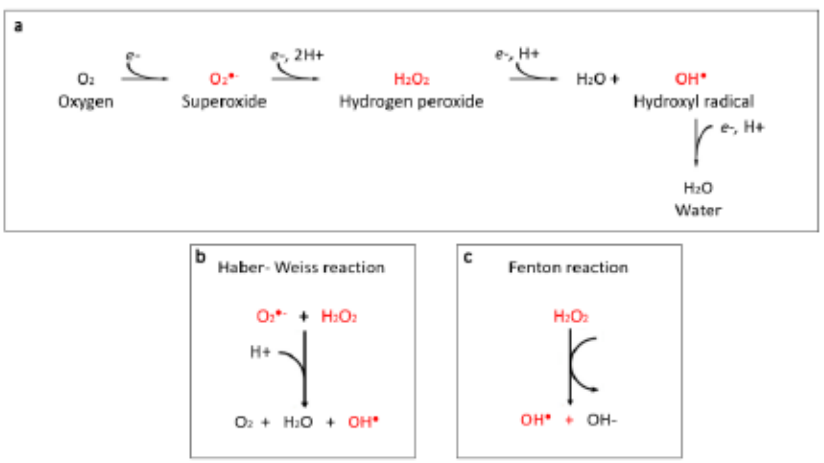

Fig. 2. *A: ROS are generated in a sequence of reactions, where one type of ROS product is used for generating a new type of ROS; B: Haber-Weiss reaction; C: Fenton reaction.*

detoxification of H_2O_2 into H_2O and then converted into reduced PRDXs by TRXs, which are non-enzymatic antioxidants. Then, oxidized TRX is reduced by TRX reductase using NADPH as a cofactor (14).

Among the mitochondrial non-enzymatic antioxidants, GSH is worth mentioning as it is important due to its ubiquitous localization and potent antioxidant properties. GSH is considered the major antioxidant that maintains the whole body's redox status. Moreover, it acts as a reductant of a wide variety of antioxidants, including Vitamin C and Vitamin E (15). Other non-enzymatic antioxidants are represented by uric acid, which directly scavenges ROS and modifies the action of xanthine oxidase (XO) in human plasma, and Coenzyme Q and bilirubin, both of which may prevent lipid peroxidation.

Effects of oxidative stress

ROS are maintained at equilibrium levels in physiological conditions to facilitate physiological redox signalling; in fact, normal levels of $O_2^{\bullet-}$, H_2O_2, and $NOHO^{\bullet}$ are essential for cell metabolism, survival, proliferation and differentiation, angiogenesis, neurogenesis and immune response. However, impaired ROS production suppresses physiological redox signaling and directly damages proteins and lipids, hindering the mitochondrial bioenergetics function. Moreover, ROS directly have a deleterious effect on mitochondria DNA, caused by promoter inactivation and downregulation of mitochondrial gene expression (16).

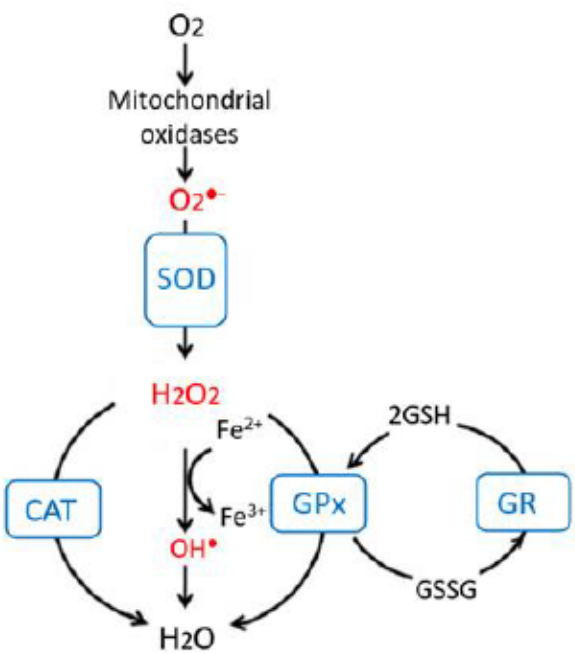

Fig. 3. *Main antioxidant pathways.*

Particularly, the negative effects of ROS are typically the damage of DNA or RNA, lipid peroxidation of polyunsaturated fatty acids, including membrane phospholipids, and oxidation of proteins. DNA damage caused by ROS can lead to mutations, carcinogenesis, apoptosis, necrosis and hereditary diseases. Moreover, DNA fragmentation, which is forced by the rupture of nucleosomes, alters the compaction and coiling of DNA within chromatin. Since chromatin plays an important role in the regulation of gene transcription, the latter alterations could result in mutagenesis (17).

Long-term oxidative stress can also cause the accumulation of mutations or deletions in the mitochondrial genome, which are involved in degenerative neuronal disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). In addition, ROS may cause DNA strand breakage due to free radical attacks on the DNA sugar-phosphate backbone, which are mutagenic or clastogenic. Lipid peroxidation, on the other hand, determines the generation of molecules with high biological activity, which disrupts DNA, proteins, and enzyme activity and activates signalling pathways initiating cell death (18).

Peroxidation occurring in blood vessels walls typically leads to atherosclerosis. Another worth mentioning effect of oxidative stress is the loss of structural integrity of several proteins that hinders the catalytic activity of several enzymes and alters the regulation of metabolic pathways. Unlike nucleic acids, to prevent their diffusion in metabolic pathways, the proteasome must hydrolyze or process oxidized proteins, which are involved in developing several diseases, including Alzheimer's disease and rheumatoid arthritis.

Biomarkers of oxidative stress

Oxidative stress biomarkers would be useful tools to assess the redox status of patients and evaluate the health-enhancing effects of antioxidants

therapies. However, there is a lack of consensus and validation regarding many oxidative stress markers (19). In addition, biomarkers and their assay should be simple, specific, accurate, sensitive, reproducible, non-invasive, high-throughput, and inexpensive. Nowadays, biomarkers that satisfy all these factors have not yet been identified. Nevertheless, research is moving in this direction, evaluating the substances most susceptible to oxidative stress as possible markers of redox unbalance.

Among the biological molecules, lipids and proteins are most susceptible to ROS attack. Thus, levels of lipid peroxidation products (such as isoprostanes, malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE) and 4-oxo-2-nonenal) or oxidized proteins (such as 3-nitrotyrosine, dityrosine and 3-chlorotyrosine) may be used as biomarkers measurement of oxidative stress status *in vivo*. Moreover, early glycation adducts and advanced glycation end products (AGEs) could be useful oxidative stress markers (20). Also, DNA alterations could be measured to evaluate the redox status, including base modification products. Furthermore, antioxidant levels, the ratio of their reduced forms to oxidized forms, oxidized antioxidants, and antioxidant enzymes have also been proposed as oxidative stress biomarkers (21).

From a practical point of view, oxidized proteins such as carbonyls or advanced oxidation protein products could be evaluated in plasma, while oxidized DNA (8-hydroxy-2'-deoxyguanosine and 8-nitroguanine) and oxidized lipids could be measured in urine (22), (23). In addition, plasmatic acylcarnitine, free carnitine and amino acids have been proposed as promising biomarkers for oxidative stress since they are readily detected by easy biochemical assays that could be implemented in clinical laboratories (24).

PRDXs could be used as biomarkers among the endogenous antioxidant defences since two oxidation products accumulate in cells under increased oxidative stress: an intermolecular disulfide and a hyperoxidized form. The levels of these products can be assessed in blood samples by Western blotting (25).

Notably, many studies have proposed the feasibility of salivary biomarkers in recent years by documenting their ability to differentiate diseased from healthy conditions (26).

Although no biomarkers have been considered effective in assessing redox status, there has been great progress in the development of biomarkers that may eventually be useful for early detection and prevention strategies for oxidative stress-associated human diseases in the last years. Thus, the validation of biomarkers represents an important challenge for the future.

DISCUSSION

By hindering cellular and metabolic functions, oxidative stress is implicated in the pathogenesis and progression of several chronic disorders and cancers. Therefore, understanding the cellular mechanisms that determine an imbalance between oxidizing agents and antioxidant defences is of fundamental importance for establishing the close relationship between impaired redox homeostasis and related disease etiology; this has significant therapeutic implications, as since to be effective, therapy requires the correct identification of the mechanisms to be targeted. In this context, identifying effective biomarkers of oxidative stress and ROS activity would be useful as diagnostic and prognostic tools and to monitor the effects of antioxidant therapies over time (27).

CONCLUSION

Further studies are needed to better clarify the correlation between oxidative stress and the etiology of related diseases and identify reliable and easily measured biomarkers of oxidative stress.

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Oxidative stress in ear nose and throat diseases: antioxidant agents and future perspectives

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Oxidative stress, defined as the imbalance between the production of reactive oxygen species (ROS) and antioxidant defences, is now known to be directly involved in the pathogenesis of numerous pathologies. Nevertheless, the extent to which redox imbalance participates in the development or progression of oxidative stress-related diseases is still not fully understood. Therefore, determining whether it plays a primary or secondary role remains challenging; this could have important therapeutical implications since understanding the mechanisms underlying the correlation between oxidative stress and the pathogenesis of the related pathologies could allow to correctly define therapeutic targets and address therapeutic choices. This brief review aims to summarize the relationship between oxidative stress and the development and progression of several diseases, focusing on ear, nose and throat (ENT) pathologies and highlighting the future perspectives of antioxidant treatments.

Oxidative stress plays an important role in the etiology of several diseases such as diabetes, atherosclerosis, hypertension, respiratory diseases, arthritis, cataract, cancer, neurodegenerative and cardiovascular diseases. The lack of vital nutrients that determines a decrease in antioxidant mechanisms in several diseases is one of the main mechanisms that explain the correlation between oxidative stress and their etiology. Another mechanism is represented by the increased generation of free radicals and reactive oxygen species (ROS), through intracellular mitochondrial dysfunction or through an extracellular source such as inflammation, which can act as second messengers, causing an aberrant redox signalling (1). Moreover, the accumulation of oxidative damage to DNA, proteins and lipids by free radicals leads to cellular functional losses, generating a vicious circle (2).

However, the extent to which redox imbalance participates in the development or progression of various oxidative stress-related diseases is still not fully understood. Therefore, research is recently focusing on the mechanisms underlying the etiology of these diseases and the possibility of using antioxidant agents to prevent or treat them.

This brief review aims to summarize the relationship between oxidative stress and the pathogenesis of various diseases, focusing on Otolaryngological pathologies, and to understand the limitations and potential of antioxidant treatments.

Oxidative stress-related diseases and therapeutic implications

Based on the contribution to the aetiology of the different pathologies, oxidative stress can be considered the primary cause of pathology (including toxicities caused by radiation and atherosclerosis) or the secondary contributor to disease progression (such as in COPD, hypertension and Alzheimer's disease). However, since the mechanisms by which oxidative stress contribute to the development or the progression of many diseases are not completely clear, determining whether it plays a primary or secondary role remains

Keywords: Oxidative stress; ROS; antioxidants; alpha-lipoic acid (ALA)

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challenging. Moreover, even when oxidative stress is considered a primary factor in the etiopathogenesis of disease, once the damage is established, other factors typically become dominant in the pathology and antioxidant therapy results ineffective (3).

In recent years research has focused on the role of antioxidant supplementation in the management of oxidative-stress-related diseases, highlighting its limits and potential (4). Indeed, since organisms have evolved defences against oxidative injury, primarily dependent upon antioxidant enzymes, which could be repressed in a state of oxidative stress, external sources of antioxidants are required to supplement the endogenous antioxidant defences. Nevertheless, it should be noted that free radicals and antioxidants participate in several physiological pathways and have beneficial effects on the body. Therefore, the goal of therapy should not be the elimination of free radicals but rather the achievement of a subtle balance between oxidizing agents and antioxidants.

Among other things, the action of antioxidants substances varies according to the surrounding environment. Thus, many substances with beneficial effects in a laboratory do not show the same properties *in vivo*, as well as many antioxidants can act as pro-oxidants in particular conditions (5), (6). Moreover, the effectiveness of antioxidant strategies is directly related to the contribution of oxidative stress in disease development and progression. Indeed, when oxidative stress is a contributing factor but not the main cause of a disease, the increase in antioxidant defence and the reduction of oxidative stress markers are not sufficient in curing the disease. Therefore, research should focus on the possibility of using these therapeutic strategies as symptomatic rather than curative treatment.

Ear, nose, and throat (ENT) oxidative-stress related diseases

Several ENT conditions have been associated with oxidative stress and low antioxidant defences. Nasal mucosa constitutes a first barrier between the host and its environment that includes mechanical, innate, and adaptive systems, collaborating in the defence against inflammation and infection of the upper respiratory system caused by inhaled pathogens allergens, and other irritants (7). The first layer of mucosal defence includes glycoproteins and lysosomal contents, which form a physiologic barrier of nonimmunologic molecules against exogenous agents. When these agents bypass the initial barrier, innate and adaptive immunological defence systems act together to remove them (8). The innate response includes phagocytic cells such as macrophages, dendritic cells, neutrophils and the complement system.

Interestingly, neutrophils play a pivotal role in host defence by exhibiting potent microbicidal activity by generating reactive oxygen species (ROS). In particular, neutrophils and, to a lesser extent, monocyte express a heme-containing peroxidase known as myeloperoxidase (MPO) that catalyzes the formation of reactive oxygen intermediates, including hypochlorous acid (HOCl). The MPO/HOCl is directly involved in microbial killing, local inflammation and oxidative tissue damage(9). Also, an adaptative response is classically associated with inflammatory responses, including ROS generation, which cause additional stress by directly or indirectly breaking covalent bonds in DNA, proteins, and lipids (10).

Therefore, it is deductible that, to obtain the efficient defence of the airways such as to prevent infections and inflammation without causing oxidative damage, a balanced response between the mechanic, innate and adaptive systems is required. The imbalance of these mechanisms determines the development or progression of inflammatory, infectious and oxidative stress-related pathologies.

Sino-nasal disorders

Recent studies have shown that oxidative stress contributes to allergic inflammation mechanisms underlying asthma and allergic rhinitis (AR). Eosinophils of allergic patients are considered the main source of ROS, an important pathogenic factors of AR, that may be involved in increased mucosal permeability and mucus production, decreased number and function of epithelial cilia, impaired expression of adhesion molecules and the release of inflammatory mediators. In particular, increased ROS may upregulate the expression of IL-1β, affecting IL-17 production and serving a role in the pathogenesis of AR (11, 12). Similarly, several studies have shown the correlation between oxidative

stress and the pathogenesis of chronic rhinosinusitis with nasal polyps (CRSwNP), probably due to the increased expression of several oxidases, including Dual oxidases (DUOX1 and DUOX2), the H_2O_2-producing isoforms of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase family, and thioredoxin-interacting protein (TXNIP), which is considered an important trigger of oxidative stress (13, 14). This results in increased ROS levels and, therefore, in tissue damage in CRSwNP. On the contrary, a recent study has excluded the role of oxidative stress in the etiology of antrochoanal polyps, which seems to have a different pathophysiology than other nasal pathologies have (15).

Otologic disorders

Chronic otitis media with effusion (OME) is a common pathological condition, defined as fluid in the middle ear without signs or symptoms of acute ear infection, which leads to a moderate conductive hearing loss and a flat tympanogram (Type B tympanogram). Recent studies have shown a close relationship between oxidative stress and OME, characterized by thiol/disulfide unbalance (16). Moreover, among the factors directly implicated in otitis media, there are, in addition to genetic factors, Eustachian tube malfunction, autoimmunity, infections, osteoclastic activity, cytokines, endotoxins, even lipid peroxidation products (17). In addition, a significantly increased expression of TFIIB-related factor 2 (BRF2), a redox sensor that is overexpressed in oxidative stress, has been shown in acquired middle ear cholesteatoma (18).

Some hearing disorders, including tinnitus and sudden or noise-induced hearing loss, have been attributed to oxidative stress over time, although the underlying mechanisms are not fully understood (19, 20). In addition, oxidative stress and ROS have been proposed as mediators for microvascular damage also in endolymphatic hydrops and Meniere's Disease, characterized by the loss of integrity of labyrinthine blood barrier (BLB) and by a significant increase in the expression of ROS in the utricular macula, corroborated with an upregulated expression of mRNA for ROS (21).

Pharyngolaryngeal disorders

Advanced oxidation protein products levels are elevated in tonsillar tissue and serum of patients suffering from chronic tonsillitis, demonstrating the correlation between oxidative stress and tonsillitis (22). Similarly, patients suffering from PFAPA syndrome, a clinical entity of unknown etiology, which consists of recurrent periodic fever, aphthous stomatitis, tonsillitis or pharyngitis, and cervical lymphadenopathy, show high levels of antioxidants. Viral and autoimmune mechanisms are probably involved in the etiology of PFAPA. However, studies on PFAPA syndrome have recently revealed an increase in pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines (23).

Moreover, excessive ROS levels have been shown in patients suffering from gastroesophageal reflux disease since they are produced by mucosal epithelial cells under stress caused by the reflux of gastric contents. In particular, increased levels of TNF-α produced by macrophages and immune cells stimulates neutrophils to produce large amounts of ROS, leading to peroxidation of the esophageal mucosa and predisposing for the development of Barrett's esophagus and esophageal adenocarcinoma (24), (25). Regarding laryngeal carcinoma and other head and neck cancers, oxidative stress and free radicals play crucial roles in carcinogenesis. Indeed, pro-oxidant molecules modulate genes related to differentiation and cell growth and may cause structural DNA changes, triggering the onset and progression of cancer (26, 27).

Other ENT diseases

Growing evidence demonstrates that mitochondrial dysfunction is involved in neuropathic pain, such as trigeminal neuropathy and Bell's palsy (28, 29).

Another common disease directly correlated with redox unbalance is represented by obstructive sleep apnea syndrome (OSAS), which is highly associated with higher body mass index (BMI), male gender, and older age. Among the mechanisms involved in the etiology of OSAS, oxidative stress, the activation of inflammatory molecular pathways, endothelial dysfunction and metabolic dysregulation contribute directly to the syndrome's pathogenesis. Chronic intermittent hypoxia leads to repetitive cycles of hypoxia and reoxygenation, enhancing systemic

oxidative stress and leading to the rise of systemic inflammatory-related biomarkers (30, 31).

In addition, an emerging environmental disease known as multiple chemical sensitivity (MCS) has been correlated with oxidative stress. MCS, characterized by phobosmia and hyperosmia, is frequently related to a toxicant-induced loss of tolerance that develops in response to acute toxic exposure or cumulative exposure of non-threshold low-dose and long-term acting contaminants. MCS development is associated with hypoxia, oxidative stress, and inflammation due to sensitization to N-methyl-D-aspartate (NMDA), increased levels of nitric oxide and the pro-inflammatory cytokine, decreased glutathione, altered redox enzymes, and cytochrome P450 metabolism, altered serotonin receptors, neural sensitization, and neurogenic inflammation (32).

Antioxidant compounds

Given the involvement of oxidative stress in the pathogenesis of several diseases, in recent years, antioxidants have been used in addition to the traditional pharmacological therapies (33). The goal of antioxidant therapies is to support alternative energy production pathways, reduce oxidative stress, remove toxic metabolites, and stabilize residual mitochondrial respiratory complexes.

Nowadays, several dietary supplements and drugs with antioxidant effects are available. However, the extensive heterogeneity of oxidative stress-related diseases, along with a lack of biomarkers to evaluate redox unbalance and health-enhancing effects of antioxidants therapies, make it difficult to identify suitable and effective therapeutic agents. Typically, a general antioxidant regimen may include multivitamins, one or more antioxidants, and coenzyme Q10, with additional nutrient supplements selected based on the individual patient's endotype and phenotype (34).

Vitamins

The vitamin B complex comprises eight water-soluble constituents that serve as cofactors: thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folic acid (B9), and cobalamin (B12). These substances are functionally related and cooperate in protein, lipid, nucleic acid synthesis, energy production, and immune defence (35). Furthermore, since each vitamin B participates in the antioxidative response, several studies have evaluated the role of vitamin B complex in the treatment of oxidative-stress related diseases (36, 37) and insufficient amounts of such vitamins have been associated with higher levels of neural inflammation and oxidative stress, as marked by increased blood plasma homocysteine. Indeed, although the effectiveness of vitamin B is still debated, numerous dietary supplements containing this vitamin, combined with other compounds, are available today. Therefore the effects of vitamin B supplements have been evaluated in patients suffering from various pathologies, including neuropathies, tinnitus, diabetes and cardiovascular or neurodegenerative diseases (38).

According to data available today, B vitamins or their metabolites may be involved in the prevention rather than the therapy of these pathologies, preventing the formation of oxyradicals and lipid peroxidation due to H_2O_2 and showing anti-inflammatory actions (39). Similarly, vitamin C has been proposed as an extremely promising compound to modulate the overwhelming oxidative stress in severe sepsis, trauma, and reperfusion damage and to support standard treatment regimens. However, the use of vitamin C requires firm and unequivocal support of the results of clinical trials and tests since it is an active substance that is also burdened with detriment effects and potential toxicity at high levels (40), (41). Vitamin E could also be supplemented, a potent lipophilic antioxidant, which can prevent or reduce mitochondrial oxidative dysfunction. Indeed, oxidized vitamin E is a stable free radical, which can itself be reduced again by ascorbate to continue the antioxidant cycle (42).

Coenzyme Q10, Alpha-Lipoic Acid, Acetyl L-Carnitine and other compounds

Alpha-lipoic acid (ALA) is a dithiol short-chain fatty that has recently received attention as a dietary supplement because of its antioxidant and therapeutic properties. In particular, the antioxidant capacity depends on its two sulfhydryl moieties, enabling it to

scavenge free radicals and chelate metals (43). Several dietary supplements containing various dosages of ALA, eventually combined with other compounds, are nowadays available (44). Although the results of the efficacy of this molecule in improving the redox balance are promising, the posology and method of administration are not yet clear (45).

Coenzyme Q10 (CoQ10), specifically in its reduced form, ubiquinol, is a commonly used antioxidant. However, the efficacy of CoQ10 as a supplement for the treatment of diseases associated with mitochondrial dysfunction is not completely clear (46). Nevertheless, several studies have shown that CoQ10 reduces inflammation and restores antioxidant and mitochondrial activities, involving many metabolic and antioxidant processes. Therefore, the supplementation with CoQ10 in many oxidative-stress-related diseases could improve metabolic activities and decrease oxidative damage (47). Another thiol-containing compound with strong antioxidant properties is represented by N-acetyl cysteine (NAC). Indeed, several studies have shown that dietary NAC could be considered an effective candidate for the treatment and prevention of oxidative stress-related diseases (48).

In addition, Acetyl L-Carnitine (ALCAR), an endogenous molecule with antioxidant and neurotrophic properties, could be used alone or in combination to reduce oxidative stress (49).

Other nutrients that could be considered to supplement the diet to prevent and/or ameliorate oxidative stress-related disease are riboflavin, thiamine, magnesium ions, niacin, melatonin, pyridoxine, folate, and cobalamin. They can supplement a normal, healthy diet, which should be adjusted to individual needs determined mainly by the physiological constitution of an organism (50).

Future perspectives

Further studies and randomized controlled trials will allow defining which are the most effective antioxidant molecules, the possible combinations, dosages, and administration methods.

However, in the era of Precision Medicine, a personalized approach is needed to guarantee patients effective and tailored treatments. In this context, several antioxidant treatments, defined as mitochondrial medicine regimes, will be developed in the coming years (34). These treatments will be adapted to patients' endotype and phenotype and their specific molecular diagnosis, allowing the prevention of several chronic diseases and cancers. Identifying biomarkers of redox imbalance will be useful not only to understand the etiopathological mechanisms underlying the various pathologies but also to choose the most suitable treatment and evaluate its effectiveness over time.

DISCUSSION

Redox unbalance is directly involved in the pathogenesis and progression of several oxidative stress-related diseases. Despite the absence of randomized controlled trials evaluating antioxidant therapies to prevent or cure oxidative stress-related disease and the lack of data concerning administration and posologies, progress has been made to identify potentially beneficial compounds with a high benefit-to-risk ratio that are generally well-tolerated by patients (26).

Identifying biomarkers of redox unbalance and understanding the extent to which oxidative stress contributes to the development and progression of these pathologies would be useful for correctly addressing therapeutic choices and monitoring therapeutic targets.

CONCLUSION

Further studies are needed to understand the real efficacy of these therapeutic interventions and the prospects: considering the involvement of oxidative stress in numerous ENT pathologies, antioxidant therapies will probably be an integral part of therapeutic strategies in the future.

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The effectiveness of dietary supplements based on alpha-lipoic acid, acetyl l-carnitine and vitamin B complex in the treatment of craniofacial neuralgia

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Background: Craniofacial neuralgias are considered a highly unmet medical need. The understanding of neuropathic pain pathophysiology remains challenging, hindering the development of new therapeutic strategies. Recent evidence has shown that oxidative stress plays a crucial role in neuralgias pathogenesis. Therefore, antioxidant compounds have been proposed as possible treatments.

Methods: We conducted a national survey involving 13 Italian ENT centres to analyse current craniofacial neuropathic pain treatments and evaluate their efficacy.

Results: Craniofacial neuralgias negatively affected the patients' Quality of Life (QoL), causing sleep disturbances, reduced work performance and the impediment of regular meals. Three months after the start of the treatments, patients treated with dietary supplements based on alpha-lipoic acid, acetyl L-carnitine and vitamin B complex (Tioneural Retard®) showed the greatest reduction in the VAS score attributed to neuralgia discomfort ($p<0.001$)

Conclusions: Antioxidant compounds, due to their neuroprotective effects, effectively reduce neuropathic pain. Therefore, oxidative stress reduction appears to be a promising direction of neuropathic pain treatment and gives hope to patients currently forced to live with chronic, often disabling pain.

Neuropathic pain of the craniofacial territory can be associated with several diseases, including trigeminal neuralgia, nerve entrapments and chemical, tumoral or viral neuropathies, which typically cause debilitating and difficult to treat painful conditions (1). Non-headache, non-dental craniofacial neuralgias have extremely heterogeneous expressions of variable intensity, intermittent or continuous, spontaneous or aroused by various stimuli. Among these disorders, idiopathic trigeminal neuralgia (TN) is the most common neuralgic disorder involving the craniofacial region (2).

The understanding of neuropathic pain pathophysiology remains challenging due to the complex mechanisms underlying neuropathy and the lack of standardised diagnostic pathways (3); this hinders the development of new therapeutic strategies and makes neuropathic pain a still highly unmet medical need, as most of the available treatments have modest efficacy or dose-limiting side effects.

Keywords: Craniofacial neuralgia; Neuropathic pain; Oxidative stress; Nutraceuticals; Alpha lipoic acid; Acetyl L-carnitine; Vitamin B

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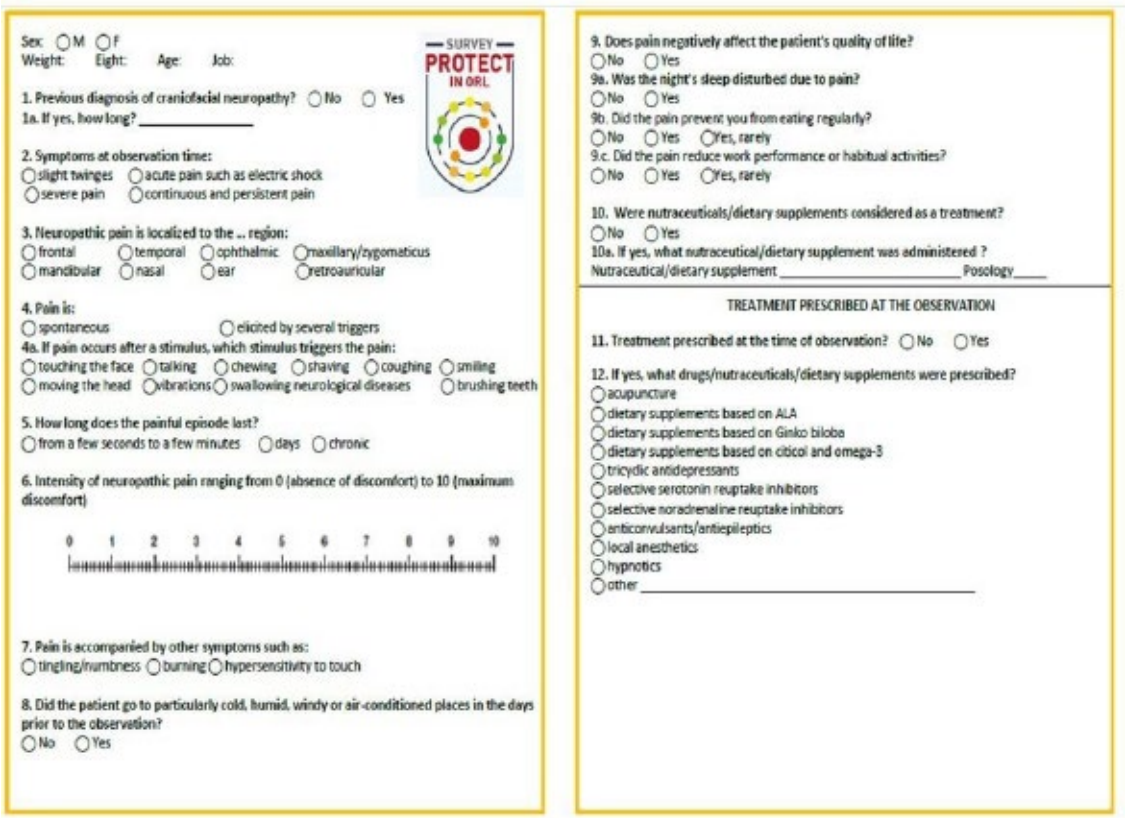
0393-974X (2020)

Several compounds that act on new pain targets have been proposed and are currently being tested with encouraging results (4). In addition, since oxidative stress has been shown to play an important role in neuralgias, recent evidence has indicated that antioxidant agents can induce a neuroprotective state and attenuate functional deficits (5, 6). In particular, antioxidant non-pharmacological pain relievers such as Vitamins, alpha-lipoic acid (ALA), *N-acetylcysteine*, acetyl *L-carnitine* (ALCAR), *Boswellia serrata*, coenzyme Q10 and palmitoylethanolamide, have several properties, including protection from oxidative stress, modulation of brain neurotransmitters and neurotrophic factors, and also long-term neurotrophic and analgesic activity in experimental models of chronic inflammatory and neuropathic pain (7), (8).

The present review aims to analyse current craniofacial neuralgias management strategies and explore the potential therapeutic effect of dietary supplements based on alpha-lipoic acid (ALA), acetyl L-carnitine (ALCAR) and vitamin B complex in reducing neuropathic pain.

MATERIALS AND METHODS

We conducted this survey involving 13 Italian Otorhinolaryngological centres that endorsed the Project PROTECT in ORL (Integration strategies in oxidative stress pathologies) to ensure complete national coverage. ENT specialists enrolled were asked to recruit consecutive patients suffering from craniofacial neuralgia and to fill in for each patient a "survey book" aimed at collecting information relating to neuralgia and its characteristics, exposure to risk factors, drug history and any treatment prescribed at the time of observation (Fig. 1). At the same time, each recruited patient had to fill in a diary for self-assessment of neuralgia discomfort, assigning a score to neuralgia discomfort according to a Visual Analogue


Sex: ○M ○F
Weight: Eight: Age: Job:

SURVEY
PROTECT
IN ORL

1. Previous diagnosis of craniofacial neuropathy? ○No ○Yes
1a. If yes, how long? _____

2. Symptoms at observation time:
○slight twinges ○acute pain such as electric shock
○severe pain ○continuous and persistent pain

3. Neuropathic pain is localized to the .. region:
○frontal ○temporal ○ophthalmic ○maxillary/zygomaticus
○mandibular ○nasal ○ear ○retroauricular

4. Pain is:
○spontaneous ○elicited by several triggers
4a. If pain occurs after a stimulus, which stimulus triggers the pain:
○touching the face ○talking ○chewing ○shaving ○coughing ○smiling
○moving the head ○vibrations ○swallowing neurological diseases ○brushing teeth

5. How long does the painful episode last?
○from a few seconds to a few minutes ○days ○chronic

6. Intensity of neuropathic pain ranging from 0 (absence of discomfort) to 10 (maximum discomfort)
0 1 2 3 4 5 6 7 8 9 10

7. Pain is accompanied by other symptoms such as:
○tingling/numbness ○burning ○hypersensitivity to touch

8. Did the patient go to particularly cold, humid, windy or air-conditioned places in the days prior to the observation?
○No ○Yes

9. Does pain negatively affect the patient's quality of life?
○No ○Yes
9a. Was the night's sleep disturbed due to pain?
○No ○Yes
9b. Did the pain prevent you from eating regularly?
○No ○Yes ○Yes, rarely
9.c. Did the pain reduce work performance or habitual activities?
○No ○Yes ○Yes, rarely

10. Were nutraceuticals/dietary supplements considered as a treatment?
○No ○Yes
10a. If yes, what nutraceutical/dietary supplement was administered ?
Nutraceutical/dietary supplement _____ Posology____

TREATMENT PRESCRIBED AT THE OBSERVATION

11. Treatment prescribed at the time of observation? ○No ○Yes

12. If yes, what drugs/nutraceuticals/dietary supplements were prescribed?
○acupuncture
○dietary supplements based on ALA
○dietary supplements based on Ginko biloba
○dietary supplements based on citicol and omega-3
○tricyclic antidepressants
○selective serotonin reuptake inhibitors
○selective noradrenaline reuptake inhibitors
○anticonvulsants/antiepileptics
○local anesthetics
○hypnotics
○other _____

Fig. 1. *Observational form.*

Scale (VAS) ranging from 0 (absence of discomfort) to 10 (maximum discomfort). This score was attributed at time 0 (T0), before the start of the assigned therapy, and monthly until T3, 3 months later (Fig. 2).

This survey was based on real-world practice. Thus, Otolaryngologists chose the pharmacological strategy freely, according to their best practice. We recorded treatments prescribed at the observation time and compared

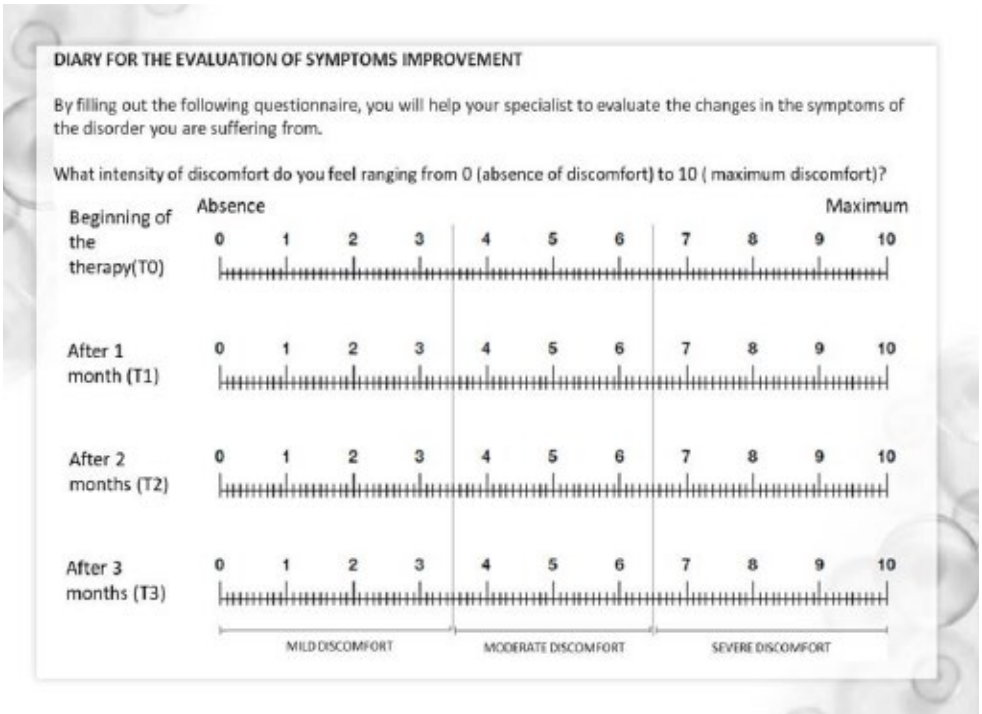


Fig. 2. *Diary for self-assessment of the improvement of the neuropathic pain following the therapy.*

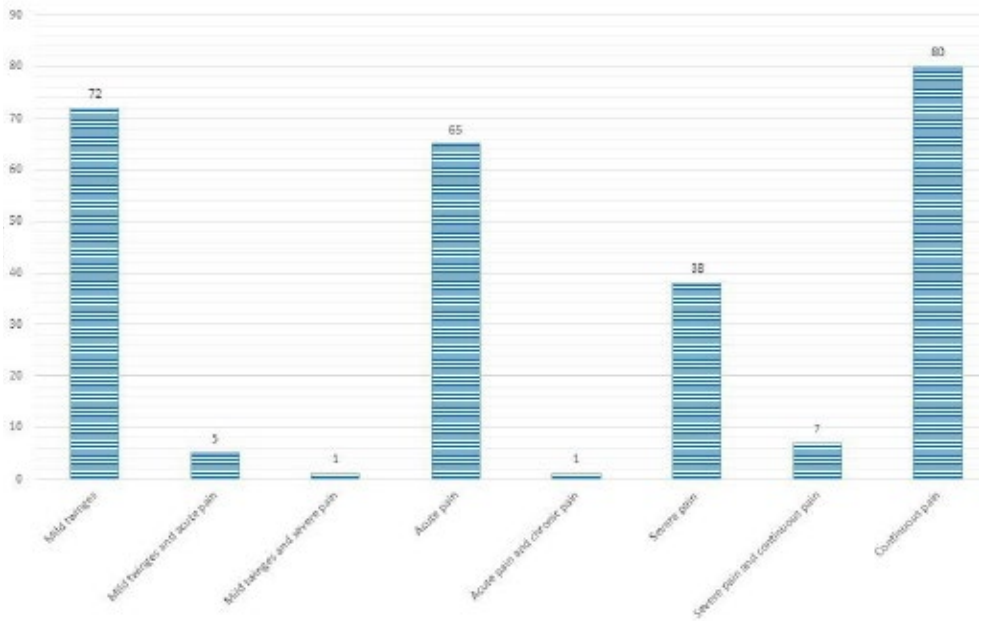


Fig. 3. *Symptoms complained by patients are variably associated with neuropathic pain.*

the VAS scores attributed to neuropathic pain by patients prior and after treatment, using paired t-Test. Statistical analyses were performed using GraphPad Prism software, GraphPad Software Inc, CA, USA. A p-value <0.05 was considered statistically significant.

RESULTS

All 13 identified ENT Centers joined the survey with 270 observational forms and 120 diaries filled out and returned by patients. Patients' sex was indicated in 268 forms: 108 (40.3%) were males, and 160 (59.7%) were females. Table I shows patients' mean age, weight, height and BMI. Two hundred thirty-four forms reported information regarding the previous diagnosis of neuralgia. In particular, 190 patients (81%) had previously been diagnosed with craniofacial neuralgia, while 44 (19%) patients had never been diagnosed before. They complained of several symptoms, variably associated, summarised in Fig.3. Moreover, Fig. 4 shows the different anatomical sites where patients localised the neuropathic pain. The latter was described as spontaneous by 194 patients, while 63 patients described it as elicited by various stimuli, including touching the face, talking, brushing teeth, smiling, swallowing, chewing, shaving, coughing, moving the head and vibrations. Moreover, in 236 patients, pain was accompanied by other symptoms such as tingling/numbness (n= 106, 45%), burning (n=72, 30%) or hypersensitivity to touch (n=58, 25%). These symptoms negatively affected the patients' Quality of Life (QoL), causing sleep disturbances, reduced work performance and the impediment of regular meals (Fig. 5).

Regarding treatments, 249 forms indicated the therapeutic approaches adopted prior to observation. In particular, nutraceuticals, including ALA, Boswellia, Bromelain, Ginko Biloba, and Vitamin B, had been considered in 50 patients. On the other hand, 258 forms reported treatments prescribed at the time of observation: 253 (98%) patients were prescribed treatments to relieve neuropathic pain, while 5

Table. I. *Patients' demographic characteristics.*

n=264

Age, years (DS)	49 (15.7)
Weight, Kg (DS)	69.5 (11.5)
Height, cm (DS)	167.7 (8.5)
Body Mass Index (DS)	24.7 (3.17)

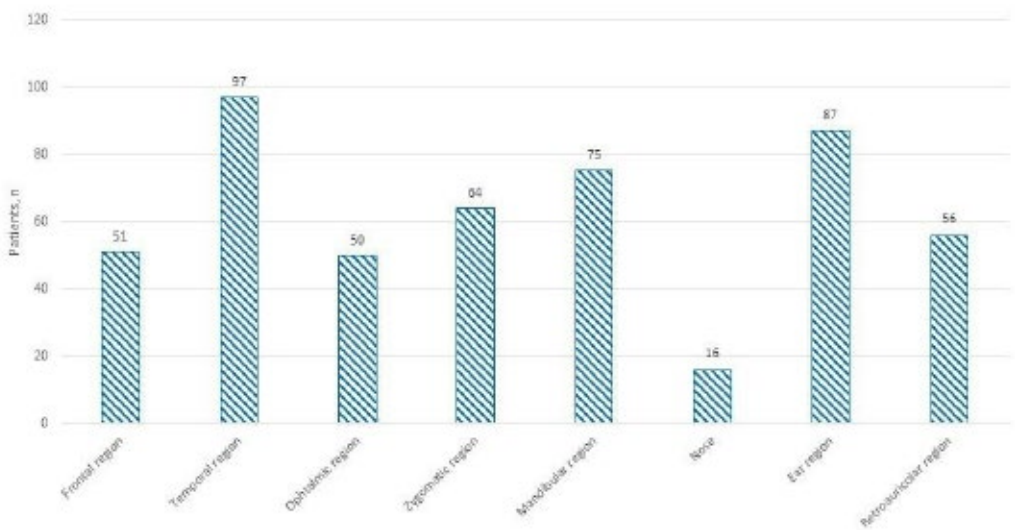


Fig. 4. *Different anatomical sites where patients localised the neuropathic pain.*

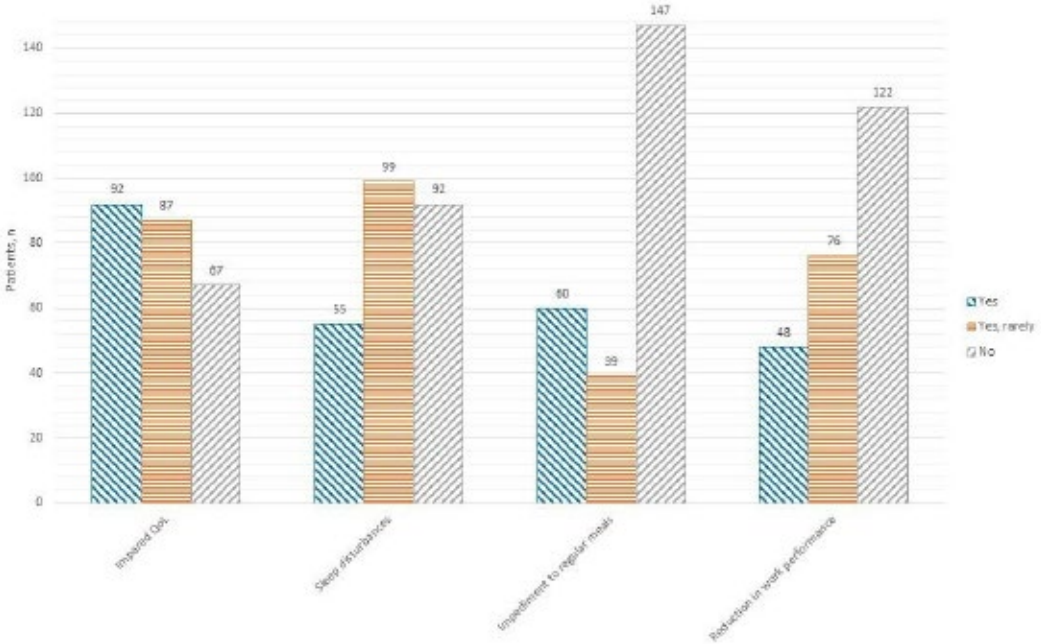


Fig. 5. *The graph shows whether neuropathic pain has resulted in a deterioration in the patients' QoL, sleep disturbances, decreased work performance, and the impediment of regular meals.*

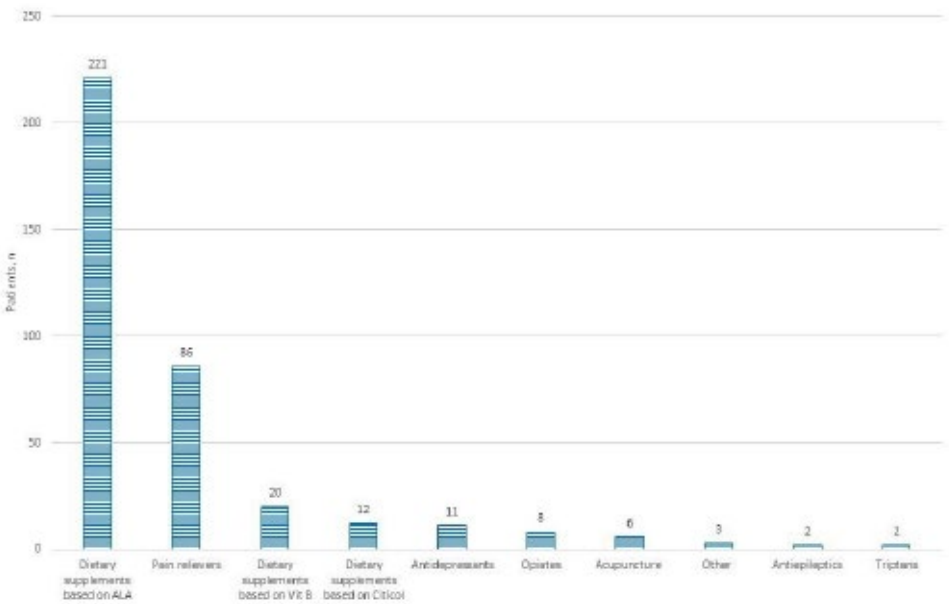


Fig. 6. *Treatments prescribed after observations were indicated in 240 patients.*

(2%) patients did not receive any treatment. Fig. 6 summarises the treatments indicated in 240 patients. Notably, dietary supplements based on alpha-lipoic acid (ALA), acetyl L-carnitine (ALCAR) and vitamin B complex were prescribed alone in 121 patients and combined with other treatments in 100 patients.

The average VAS score attributed to pain caused by craniofacial neuralgias by 268 patients at T0 was 6.6. However, this value decreased significantly up to T3. In particular, 91 forms reported VAS scores attributed at T0, T1, T2 and T3. Fig. 7 shows how scores decreased over time, from T0 to T3, in this sample ($p<0.001$). Furthermore, Fig. 8 shows changes in the VAS scores at T0 and T3, comparing the most adopted therapeutic strategy, represented by the association of alpha-lipoic acid (ALA), acetyl L-carnitine (ALCAR) and vitamin B complex, to other therapeutic alternatives ($p<0.001$).

DISCUSSION

Neuropathic pain, the most common clinical

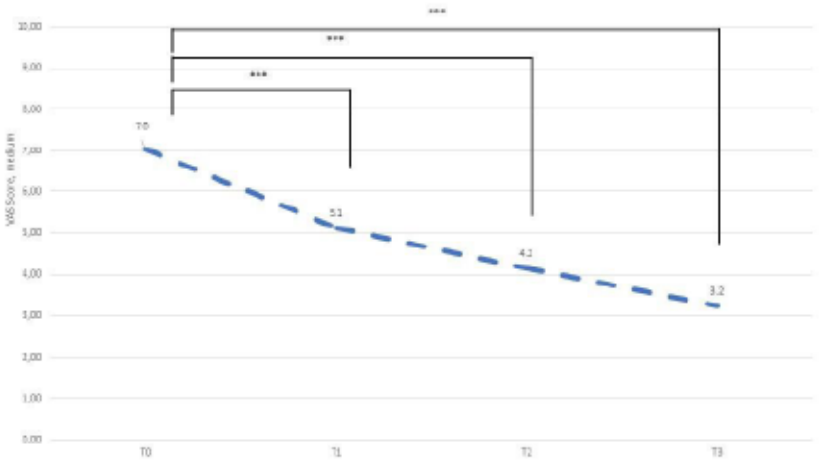


Fig. 7. *The graph shows how VAS scores decreased over time, from T0 to T3, in 91 patients. *** p<0.001 vs T0, paired t-Test.*

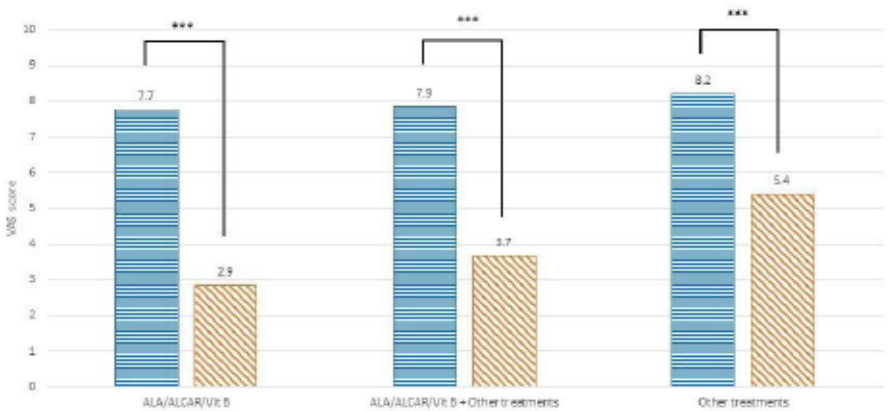


Fig. 8. *Comparison of changings in the severe VAS score (from 7 to 10 at T0) from T0 to T3 as a function of the chosen treatment (Association of ALA, ALCAR and Vitamin B complex versus association of ALA, ALCAR and Vitamin B combined with other treatments versus other treatments). *** p<0.001 vs T0, paired t-Test.*

manifestation of peripheral neuropathy, has a prevalence of approximately 19% of the adult European population and usually requires multidisciplinary treatment (9).

According to the International Classification of Orofacial Pain (ICOP), neuropathic pain of the craniofacial territory is attributed to injury or disease of the cranial nerves. Neuropathic pain of this area has unique problems compared to neuropathic pain in the spinal cord, especially due to anatomical boundaries and associated medical speciality demarcations in the orofacial area. Craniofacial neuropathic pain can be easily misdiagnosed and misdirected, mimicking, for example, odontogenic toothache or cephalalgia. Moreover, the subjective nature of neuralgic pain hinders its objective evaluation and measurement (10). Difficulties in the clinical evaluation of the disorder, together with the incomplete understanding of the pathophysiological mechanisms underlying neuropathy, prevent the development of effective medical strategies. To date, chronic neuropathic pain remains one of the most debilitating and difficult-to-treat conditions, resistant to available pharmacological and non-pharmacological treatments, which only improve pain-related symptoms, temporarily and/or moderately, producing often unbearable adverse reactions or causing drug resistance (11, 12).

Recently, the knowledge on the pathophysiological processes underlying neuropathic pain has evolved, highlighting a complex scenario in which multiple cellular and molecular actors contribute to the development of the disease. Recent evidence suggests that an increase in oxidative stress plays a crucial role in the etiology of neuropathic pain. Indeed, chronic pain is associated with plastic changes in the central and peripheral nervous system and, through thermal hyperalgesia and allodynia, triggers a cascade of events that contribute to the development of reactive oxygen and nitrogen species (ROS; RNS) and cytokines involved in persistent painful states (9, 13). Oxidative stress activates intracellular signalling pathways resulting in tissue and cell damage and leading to neuropathic pain. Moreover, oxidative stress also fuels a vicious circle, changing the structure and function of mitochondria, which aggravate the oxidative state and promote the development of neuropathic pain (14).

Several studies have proposed antioxidants compounds as effective therapeutic strategies in relieving neuropathic pain (6). Demonstrating this growing awareness, the Otolaryngologists involved in this review freely chose therapies based mainly on nutraceutical agents. Notably, in most cases, dietary supplements based on ALA, ALCAR and vitamin B complex (TIONEURAL – Fenix Pharma) were chosen as the therapeutic strategy, alone or in association with other treatments. Although no data on the efficacy of the association these antioxidant compounds are currently available in the literature, each component had already been shown to act as a neurotrophic agent and relieve neuropathic pain. In particular, ALA is considered an essential cofactor for mitochondrial oxidative metabolism, which enhances cellular glutathione synthesis, protecting peripheral nerves from oxidative stress and improving nerve conduction velocity (15).

Several studies have shown that ALA non-invasive administration through the oral route can be considered a valid adjuvant for treating pain symptoms, improving patients' QoL (16, 17). Similarly, ALCAR plays a crucial role in energy metabolism and has demonstrated neurotrophic and analgesic activity in experimental models of chronic inflammatory and neuropathic pain[18]. Moreover, a recent review has highlighted ALCAR's beneficial effects on nerve conduction parameters and nerve fibre regeneration, with a notable safety profile (19). However, another review has debunked the effectiveness of ALCAR, compared to placebo, in reducing pain, due to the lacking data on both symptoms' improvement and adverse events (20). In addition, the intake of vitamin B complex has also been supposed to relieve neuropathic pain (21). Nevertheless, it is still unclear whether vitamin B supplements change pain intensity or impairment in the short or long term in patients with neuropathy (22).

As shown in the results, prior to observation, only 50 patients had been treated with antioxidant agents, and most of them complained of considerable pain, such as worsening their QoL. ENT specialists who endorsed the project, aware of the importance of treating any condition that causes an impairment of the patient's QoL, have prescribed treatments to relieve pain to most patients (98%). We believe that chronic neuropathic pain is an extremely debilitating condition

that deserves to be treated. Although the therapeutic strategies available today often have temporary and incomplete effects, it is of utmost importance that both doctors and patients do not become discouraged: the clinical results of nutraceutical-based treatments are encouraging. Regardless of the compound used, we found a reduction in the VAS score attributed by patients to neuropathic pain, from T0 to T3, reflecting their overall satisfaction. Patients who complained of a severe grade of pain reported a moderate discomfort at the end of the therapy, while patients who complained of a mild disturbance reported complete disappearance of pain at T3.

Furthermore, we compared the VAS scores at T0 and T3 according to the treatment the patients had undergone. With a greater reduction in the VAS scores, the best results were obtained from the association of ALA, ALCAR and Vitamin B complex, the most prescribed treatment. On the other hand, albeit the small sample, patients who had not been treated with the latter combination had less satisfactory results in reducing the VAS scores. Alternative treatments included over-the-counter pain relievers and opioids, burdened with major side effects, and acupuncture which has been shown to reduce pain (23). However, it is worth mentioning that most of the previous studies investigated the efficacy of antioxidant agents in the treatment of diabetic neuropathy and that craniofacial neuropathies, given the limited extent of this anatomical district and the associated medical speciality demarcations in the orofacial area, have not been adequately investigated or have been traced solely to trigeminal neuralgia.

Studies on a larger scale on the efficacy of the nutraceutical agents and, in particular, on the specific association of ALA, ALCAR and vitamin B complex, in the treatment of craniofacial neuralgias would be useful to standardise the therapeutic approaches and, possibly, to develop a definitive cure. Our results are encouraging: oxidative stress reduction appears to be a promising direction of neuropathic pain treatment and gives hope to patients currently forced to live with chronic, often disabling pain.

Authors' contribution

Matteo Gelardi designed the study and contributed to the final revision of the manuscript. Pier Gerardo Marano directed the study by coordinating the ENT Centers involved in the survey and analysing data. Rossana Giancaspro wrote the manuscript. Michele Cassano revised the final version of the manuscript. Those included in the Protect Italian Group participated in collecting data in the various Centers involved in the survey.

Conflict of interest statement

The authors have no conflict of interest and no funding to declare.

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The effect of dietary supplements-based alpha-lipoic acid, acetyl l-carnitine and vitamin B complex in patients with tinnitus

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BACKGROUND: Tinnitus affects more than 10% of the population, with a high social and economic burden. Although several hypotheses have been proposed to explain tinnitus development, the aetiology remains unclear, hindering the development of effective therapies. However, recent evidence has shown that oxidative stress plays a crucial role in tinnitus pathogenesis, sparking a growing interest in nutraceuticals as potentially effective treatment approaches.

METHODS: We conducted a national survey to analyse the tinnitus management strategies and evaluate their efficacy.

RESULTS: Our results showed that tinnitus is still not considered a full-fledged pathology and is not adequately treated. Among the treatments, dietary supplements based on alpha-lipoic acid, acetyl L-carnitine and vitamin B complex (Tioneural Retard®) have proven effective in reducing the discomfort induced by tinnitus.

CONCLUSIONS: The growing attention paid to oxidative stress as a cause of tinnitus and a possible therapeutic target seems to represent a promising treatment direction.

Tinnitus, defined as a subjective perception of noise in the absence of an external sound, is a common auditory condition that affects between 10 and 15% of the adult population (1).

The prevalence of tinnitus increases in the elderly population, reaching 40%. Moreover, approximately 10% of subjects with tinnitus develop mild to severe disabilities. Tinnitus can be classified into objective when associated with an identifiable organic cause other than sensorineural hearing loss and subjective, when considered an idiopathic symptom eventually associated with sensorineural hearing loss (2). Subjective tinnitus is the most common type of tinnitus complained by adults. Disabling tinnitus is typically associated with hearing loss, cognitive dysfunction, depression, anxiety and sleep disorders (3). Therefore, the burden of tinnitus to society is substantial, reflecting both the negative impact on patients' quality of life (*QoL*) and high social costs due to lost productivity, missed days of work, and the expense of palliative treatments (4 5).

Despite the widespread diffusion of this condition,

Keywords: tinnitus; thioctic acid; oxidative stress.

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many aspects remain unclear, including the underlying mechanisms and effective treatment strategies. Several hypotheses have been proposed to explain subjective tinnitus development. In particular, the degeneration of the outer hair cells in the peripheral auditory, changes in biochemical systems, spontaneous hyperactivity of the auditory tract, neural plasticity, cortical reorganisation and imbalances between inhibitory and excitatory transmitter activities of the central auditory tracts are associated with the generation of tinnitus (6). However, the incomplete understanding of these mechanisms hinders the development of an effective cure. Furthermore, even if several therapeutic strategies have been proposed to cure tinnitus (including medications, cognitive behavioural therapy, neurobio feedback, neuromodulation, tinnitus retraining therapy, sound therapy, and hearing aids), most of them aim at reducing the tinnitus-associated distress, and none of them offers completely satisfactory solutions (7). Thus, tinnitus treatment remains challenging.

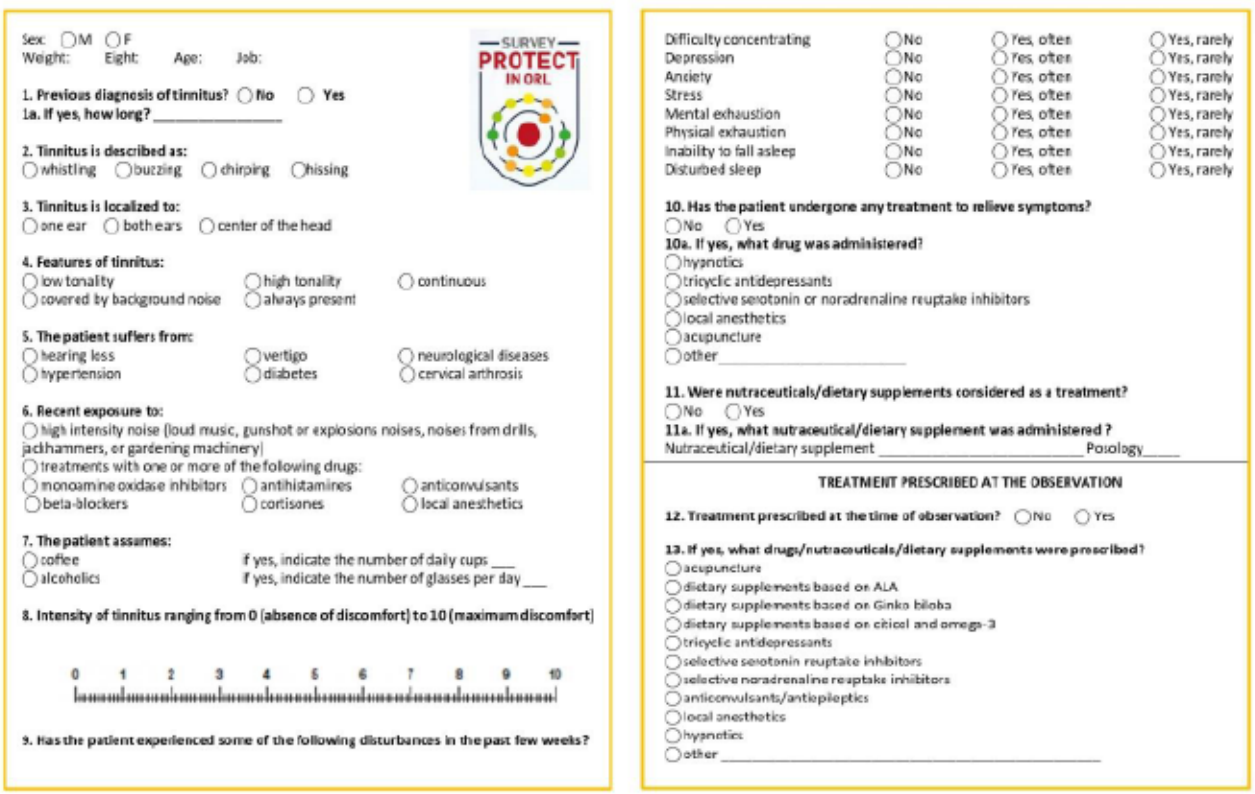
Recently, oxidative stress has been sown to play a role in the pathogenesis of tinnitus. The oxidative metabolism of patients with tinnitus shows an imbalance with the transition from the preponderance of antioxidant enzymes to the predominance of oxidative stress (8). Therefore, several antioxidant compounds have been studied as potential treatment approaches (9).

This survey aimed to analyse the tinnitus management strategies used in clinical practice and evaluate the effectiveness of nutraceuticals in reducing the discomfort associated with tinnitus.

MATERIALS AND METHODS

We conducted a national survey involving 33 Italian Otorhinolaryngological centres, which endorsed the Project PROTECT in ORL (Integration strategies in oxidative stress pathologies), to investigate the state-of-art of tinnitus management.

Each centre received a "survey book" containing observational forms that otolaryngologists had to fill in for each patient, at the time of the first outpatient visit,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SURVEY PROTECT IN ORL

1. Previous diagnosis of tinnitus? ◯No ◯Yes
1a. If yes, how long? _____

2. Tinnitus is described as:
◯whistling ◯buzzing ◯chirping ◯hissing

3. Tinnitus is localized to:
◯one ear ◯both ears ◯center of the head

4. Features of tinnitus:
◯low tonality ◯high tonality ◯continuous
◯covered by background noise ◯always present

5. The patient suffers from:
◯hearing loss ◯vertigo ◯neurological diseases
◯hypertension ◯diabetes ◯cervical arthrosis

6. Recent exposure to:
◯high intensity noise (loud music, gunshot or explosions noises, noises from drills, jackhammers, or gardening machinery)
◯treatments with one or more of the following drugs:
◯monoamine oxidase inhibitors ◯antihistamines ◯anticonvulsants
◯beta-blockers ◯cortisones ◯local anesthetics

7. The patient assumes:
◯coffee if yes, indicate the number of daily cups ___
◯alcoholics if yes, indicate the number of glasses per day ___

8. Intensity of tinnitus ranging from 0 (absence of discomfort) to 10 (maximum discomfort)

0 1 2 3 4 5 6 7 8 9 10

9. Has the patient experienced some of the following disturbances in the past few weeks?

Difficulty concentrating	◯No	◯Yes, often	◯Yes, rarely
Depression	◯No	◯Yes, often	◯Yes, rarely
Anxiety	◯No	◯Yes, often	◯Yes, rarely
Stress	◯No	◯Yes, often	◯Yes, rarely
Mental exhaustion	◯No	◯Yes, often	◯Yes, rarely
Physical exhaustion	◯No	◯Yes, often	◯Yes, rarely
Inability to fall asleep	◯No	◯Yes, often	◯Yes, rarely
Disturbed sleep	◯No	◯Yes, often	◯Yes, rarely

10. Has the patient undergone any treatment to relieve symptoms?
◯No ◯Yes
10a. If yes, what drug was administered?
◯hypnotics
◯tricyclic antidepressants
◯selective serotonin or noradrenaline reuptake inhibitors
◯local anesthetics
◯acupuncture
◯other _____

11. Were nutraceuticals/dietary supplements considered as a treatment?
◯No ◯Yes
11a. If yes, what nutraceutical/dietary supplement was administered?
Nutraceutical/dietary supplement _____ Posology_____

TREATMENT PRESCRIBED AT THE OBSERVATION

12. Treatment prescribed at the time of observation? ◯No ◯Yes

13. If yes, what drugs/nutraceuticals/dietary supplements were prescribed?
◯acupuncture
◯dietary supplements based on ALA
◯dietary supplements based on Ginko biloba
◯dietary supplements based on citicol and omega-3
◯tricyclic antidepressants
◯selective serotonin reuptake inhibitors
◯selective noradrenaline reuptake inhibitors
◯anticonvulsants/antiepileptics
◯local anesthetics
◯hypnotics
◯other _____

Fig. 1. *Observational form that ENT specialists had to fill in for each patient.*

investigating information regarding the possible presence of tinnitus and its characteristics, exposure to risk factors, drug history and any treatment prescribed at the time of observation (Fig. 1). Furthermore, each recruited patient had to fill in a diary for self-assessment of the improvement of the tinnitus following the assigned therapy, assessed monthly according to a Visual Analogue Scale (VAS) ranging from 1 (absence of discomfort) to 10 (maximum discomfort), from the beginning of the therapy assigned (T0) up to the third month (T3) (Fig. 2).

Since the survey was based on real-world practice, ENT specialists had the complete liberty of choosing the pharmacological strategy based on the best practice. Past or current treatments were recorded and analysed. Patients' demographic and clinical features were expressed as mean values ± deviation standard (DS). The differences in the VAS scores attributed to tinnitus discomfort by patients before and after treatment were evaluated using a non-parametric Wilcoxon test. Statistical analyses were performed using GraphPad Prism software, GraphPad Sotware Inc, CA, USA. P-value <0.05 was considered statistically significant.

RESULTS AND ANALYSIS

All 33 identified ENT Centers joined the survey with 748 observational forms (average per centre: 22.6 forms) and 467 diaries completed and returned by patients (average per centre: 14 diaries). Among the 467 diaries, 306 (65.5 %) were filled out and collected all the required information. Therefore, the final sample we analysed consisted of 306 subjects.

The diagnosis of tinnitus was already known in 282 patients (92.2%). Among them, 115 (40.8%) had been diagnosed with tinnitus for less than a year, 113 (40.1%) 1 to 5 years earlier, 29 (10.2%) for more than 6 years, while 25 (8.9%) patients reported having been diagnosed with tinnitus for an unspecified time (Table I). In particular, patients complained of hearing sounds described as whistling, buzzing, chirping and hissing, variously associated. These sounds were heard by one (42.5%) or both ears (51.3%) or described as coming from the centre of the head (6.2%).The most frequent comorbidities were hearing loss (n= 163), hypertension(n= 104), cervical arthrosis (n= 74), dental malocclusion (n=47), diabetes (n= 32), dizziness (n=25), and neurological disorders (n=6). Moreover, 71 of the 306 patients (23.2%) had been exposed to high-intensity noise prior to the onset of tinnitus.

Prior to observation, 62 (20.2%) patients underwent treatment to relieve symptoms (including hypnotics

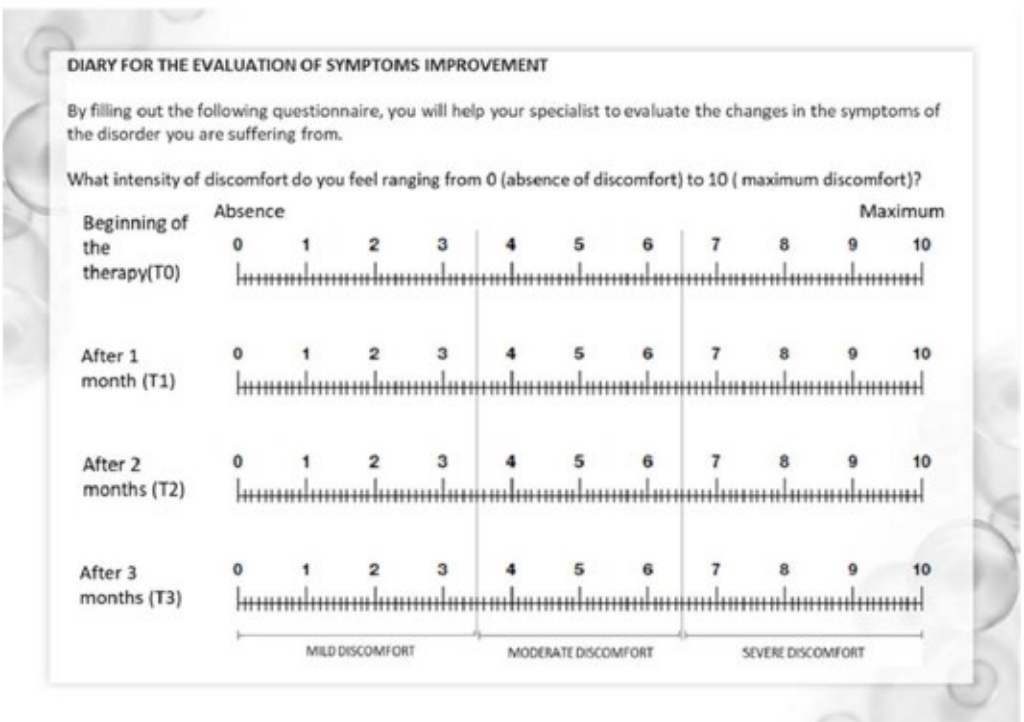

Fig. 2. *Diary for self-assessment of the improvement of the tinnitus following the therapy.*

43,5%, antidepressants 9,6%, selective serotonin reuptake inhibitors (SSRIs) 3,4%, acupuncture 11,3% or others 32,2%) and 231 (75.5%) underwent no treatment, while 13 (5.6%) did not provide any indication in this regard. The use of nutraceuticals for the tinnitus treatment was considered in only 48 patients; the totality of the combination had antioxidant activity.

At the observation time, 272 patients (88.9%) were prescribed treatments to relieve tinnitus, while 16 (5.2%) patients did not receive any treatment. In 18 cases (5.9%), no information was provided regarding the treatment (Fig. 3).

Patients attributed an average VAS score of 6.2 to tinnitus distress at T0. However, this value decreased significantly up to T3, three months after the start of therapy, reducing from 6.2 to 3.9 ($p<0,0001$) (Fig. 4). In particular, Fig. 5 shows how VAS scores decreased

Table I. *Patients' demographic characteristics.*

	Total Sample	*Male*	*Female*
Sex, number (%)	306 (100)	172 (56.2)	134 (43.8)
Age, years (DS)	55.88 (13.93)	56.59 (13.94)	55.10 (13.9)
Weight, Kg (DS)	73.09 (14.74)	78.74 (13.06)	65.78 (12.84)
Height, cm (DS)	169.28 (8.92)	174.04 (7.31)	163.12 (6.73)
Body Mass Index (DS)	25.38 (4.08)	25.90 (3.66)	24.70 (4.48)

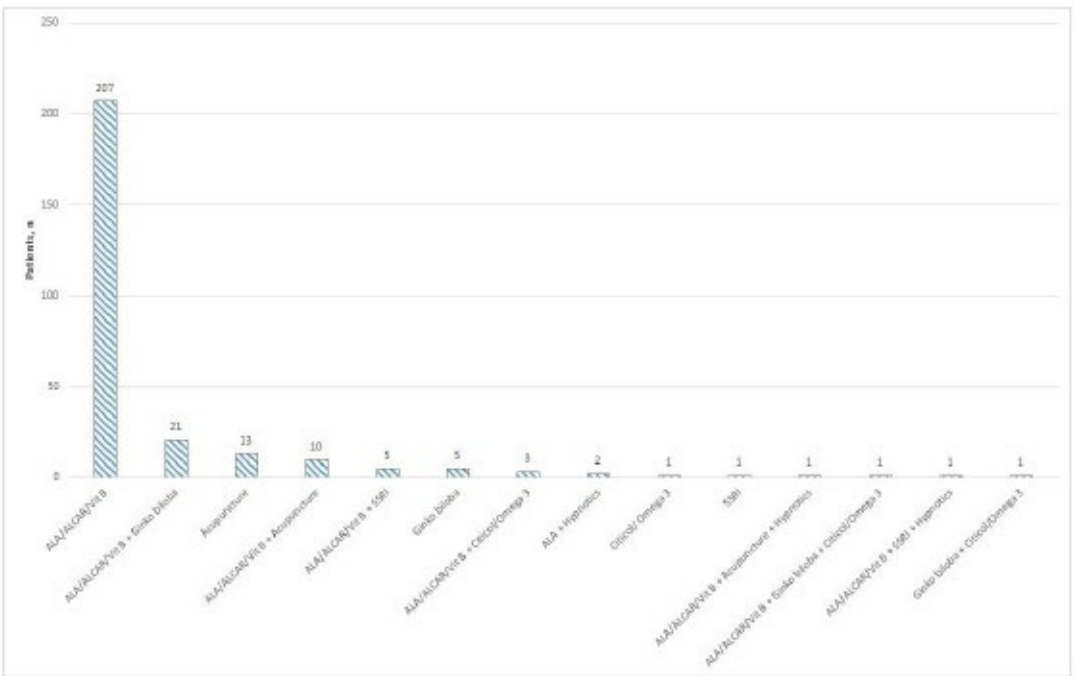

Fig. 3. *The histogram shows the treatments prescribed after observation.*

over time, from T0 to T3, based on the severity of the discomfort (moderate, severe or mild). Furthermore, Fig. 6 shows changes in the VAS scores at T0 and T3, comparing the most commonly adopted therapeutic strategy, represented by the association of alpha-lipoic acid (ALA), acetyl L-carnitine (ALCAR) and vitamin B complex (Tioneural Retard®), to other therapeutic alternatives. Notably, all therapeutic strategies

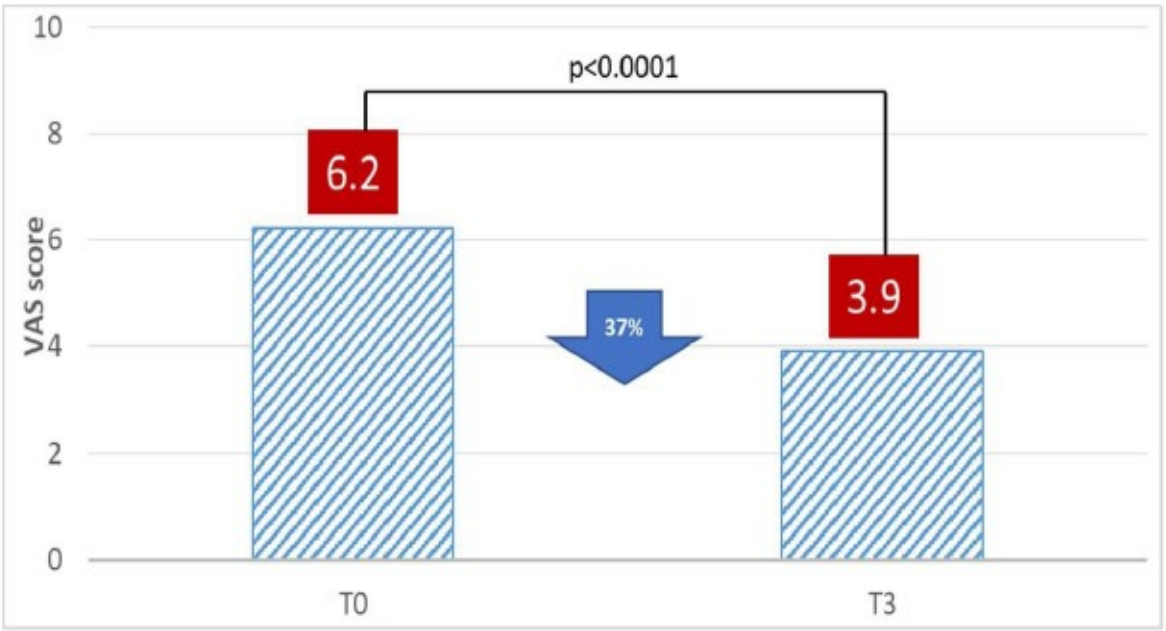

Fig. 4. *The bar chart shows how the average VAS score attributed by patients to tinnitus distress decreased from T0 to T3 (non-parametric Wilcoxon test, p<0.0001).*

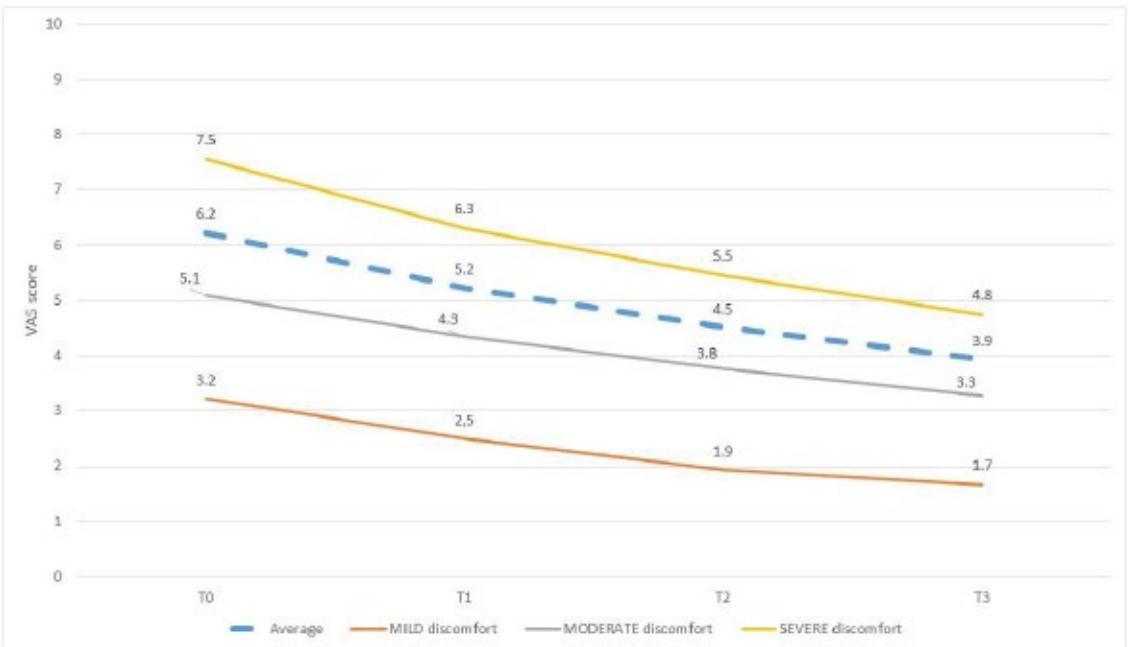

Fig. 5. *The bar chart shows how the average VAS score decreased over time, from T0 to T3, based on the severity of the discomfort (mild, moderate, or severe).*

resulted in a statistically significant reduction in VAS scores. However, statistical significance on the non-parametric Wilcoxon test was greater in patients treated with Tioneural Retard® alone ($p<0.001$) or in combination ($p <0.001$), compared to patients treated with other compounds ($p<0.01$).

DISCUSSION

Tinnitus is a major global burden with a wide spectrum of clinical manifestations and severity, reflecting the different underlying pathophysiological mechanisms not yet fully understood (10). Current controversies over the etiology hinder efforts to develop effective treatments that aim not only at reducing discomfort but also at cure tinnitus (11). Indeed, only a clear understanding of the etiology and pathophysiology of a disorder allows the development of a suitable treatment. However, in the case of subjective tinnitus, identifying a precise cause is a difficult challenge and treating it permanently (12).

Another factor that negatively influences the therapeutic choices is represented by the lack of objective parameters that allow establishing when tinnitus becomes pathological (13). As a matter of fact, prior to the observation, only 22.2% of patients underwent various treatments to relieve tinnitus; this reflects the current tendency to not consider tinnitus as a full-fledged disease, along with the tendency not to prescribe any treatment, given the partial therapeutic efficacy and the absence of standardised therapeutic protocols.

On the other hand, at the time of observation, almost all patients were prescribed treatment to relieve tinnitus, thanks to a greater consciousness of the Otolaryngologists who joined the project and were made aware of the importance of recognising tinnitus and treating it properly.

In this context, most ENT specialists freely chose therapies based on nutraceutical agents. As shown in the results, in most cases, dietary supplements based on ALA, ALCAR and vitamin B complex (Tioneural Retard®) were chosen as the therapeutic strategy. Recent studies have shown that antioxidant therapy

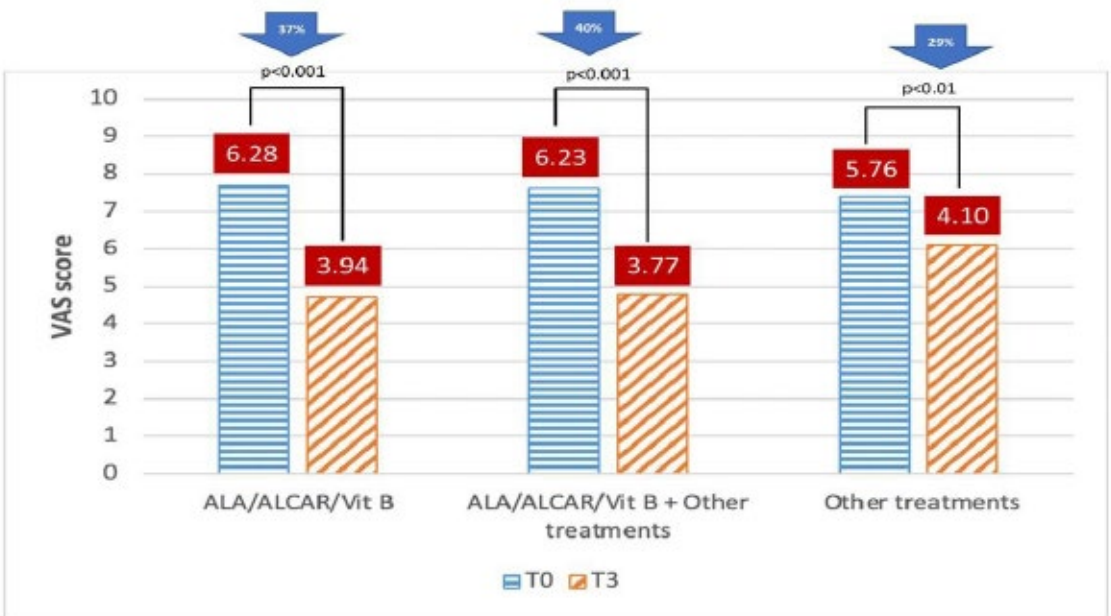

Fig. 6. *Comparison of changings in the VAS score from T0 to T3 as a function of the chosen treatment (Association of ALA, ALCAR and Vitamin B complex versus association of ALA, ALCAR and Vitamin B combined with other treatments versus other treatments). Nonparametric Wilcoxon test.*

reduces subjective discomfort and the intensity of tinnitus since total oxidant status and oxidative stress index are higher in patients with tinnitus (9, 14).

Although no data on the efficacy of the association of ALA, ALCAR and vitamin B complex are currently available in the literature, each component has previously been shown to reduce tinnitus discomfort. Notably, a randomised, double-blind placebo-controlled trial has significantly reduced tinnitus loudness and minimum masking level (MML) following antioxidant treatment based on vitamins, minerals, and phytochemicals combined with ALA. This natural antioxidant represents a cofactor for pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase complexes (9, 15). Furthermore, studies *in vivo* and *in vitro* have suggested that carnitine intake may be a valuable pharmacological option in tinnitus treatment due to its safety profile and antioxidant properties (16, 17).

The intake of vitamin B complex has also been supposed to positively influence the severity of tinnitus, established by changes in the amplitude of Distortion Product Otoacoustic Emissions (DPOAEs), especially in patients without sensorineural hearing loss (SNHL) (18). This hypothesis is supported by recent altered ABR findings in patients with tinnitus and Vit B12 deficiency, which suggest the modulatory effect of vitamin B complex on cochlear function (19). Moreover, a pilot study has highlighted the improvement in tinnitus severity scores and Visual Analog Scale (VAS) in cobalamin-deficient patients receiving intramuscular Vit 12 weekly for 6 weeks (20). Although some studies debunk the effectiveness of dietary supplements and antioxidants in treating tinnitus, due to possible side effects and lack of complete recovery, the result of this survey is promising (21-23); prior to observation, most of the patients had not been treated and complained of considerable discomforts, such as the worsening of their *QoL*.

We believe that an impairment of the patient's *QoL* should induce the ENT specialist to prescribe one of today's treatments. Regardless of the therapeutic strategy, we found a reduction in the VAS score attributed by patients to the discomfort caused by tinnitus, from T0 to T3, reflecting their overall satisfaction, and even patients who complained of severe tinnitus reported a moderate discomfort at the end of the therapy. Furthermore, we compared the VAS scores at T0 and T3 according to the patients' treatment. Interestingly, the best results were obtained precisely from the association of ALA, ALCAR and Vitamin B complex, the most prescribed treatment, compared to combining this association with other treatments and other treatments that did not include ALA. Thus, the association of ALA, ALCAR and vitamin B complex alone seems to reduce tinnitus without the need to associate further treatments.

Alternative treatments included acupuncture and Ginko Biloba, traditionally recognised as effective in reducing tinnitus severity. In particular, acupuncture has been shown to offer subjective benefits to tinnitus patients (24, 25). Similarly, several studies highlight the effectiveness of Ginko Biloba in contributing to the improvement of tinnitus (26, 27). Albeit the sample of patients who had undergone these latter treatments was much smaller than that treated with ALA-based supplements, the results obtained were less satisfactory. Given the lack of data on the efficacy of the specific association of ALA, ALCAR and vitamin B complex, unlike the numerous studies conducted on alternative therapies, studies on this therapeutic strategy on a larger scale would be useful.

These results underline the importance of not underestimating tinnitus, it should be considered a full-fledged disease and treated as such. Further epidemiological studies are needed to standardise the therapeutic approaches and develop a possibly definitive cure.

In this context, the growing attention paid to oxidative stress as a cause of tinnitus and, therefore, as a possible therapeutic target seems to represent a promising treatment direction.

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Disclosure of potential conflicts of interest

The authors declare no conflicts of interest

Informed consent

All patients included in the study signed informed consent

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Alpha-lipoic acid and acetyl l-carnitine: molecular structures, mechanism of action and therapeutic role in otolaryngology

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During physiological processes and stressful conditions (pollution, chemical agents, radiation, sports), the body releases free radicals, especially reactive oxygen species (ROS), unstable and particularly reactive molecules. Although the body has developed several defence mechanisms to neutralise free radicals, ROS production can increase in numerous conditions, triggering an imbalance redox condition known as oxidative stress, which affects proteins, lipids and nucleic acids, damaging their structure and their normal functionality. Mitochondria are the major producers of ROS and organelles that undergo the most impacting structural and functional alterations in case of oxidative stress. Mitochondrial dysfunctions caused by ROS accumulation typically result in a decrease in cellular energy production, accelerating the ageing processes and inducing important tissue alterations. The involvement of oxidative stress in the development of various pathological processes, including inner ear disorders and oral-facial pain, is now recognized. In this context, various molecules with antioxidant functions, such as alpha-lipoic acid (ALA) and acetyl L-carnitine (ALCAR), could be useful in inactivating free radicals and triggering chemical reactions to hinder the perpetuation of the inflammatory state.

Oxidative stress is thought to be involved in developing many otologic and neuropathic disorders, including hearing loss, otitis media, tinnitus, drug ototoxicity, trigeminal neuralgia and burning mouth syndrome (1-3). Reactive oxygen species (ROS) and free radicals damage cellular structures due to the production of cytokines and chemokines responsible for chronic inflammatory phenomena and to the direct damage of macromolecules such as lipids, proteins and nucleic acids. These events not only generate a vicious circle that leads to the increased production of free radicals but also justify the development of several pathologies (Fig. 1).

Recent studies confirm the implication of oxidative stress and mitochondrial damage in the onset and progression of hearing loss and tinnitus. Indeed, the overproduction of ROS, the reduction of antioxidant defences and the accumulation of mitochondrial alterations would accelerate cochlear senescence. In addition, mitochondria would play a crucial role in the apoptosis of cochlear hair cells (4). However, cochlear cellular components would not respond equally to ROS-induced injury. The outer hair cells, especially those at the base of the cochlea, would seem more susceptible to ROS than the supporting cells, which instead have a greater survival capacity (5, 6). In this context, antioxidant compounds, such as alpha-lipoic acid (ALA) and

Keywords: oxidative stress, ROS, antioxidant, alpha-lipoic acid, acetyl l-carnitine, ototoxicity, tinnitus, hypoacusis, trigeminal neuralgia, burning mouth syndrome

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Acetyl-L-carnitine (ALCAR), could be useful in the prevention and treatment of inner ear pathologies and neuropathic disease of the orofacial district.

Alpha-lipoic acid

ALA is one of the most powerful antioxidants found in nature (7). ALA, also known as thioctic acid, is a small molecule consisting of two sulfur and eight carbon atoms. Due to the presence of a chiral carbon, ALA has two enantiomers, R and S. The R form, biologically more active, is the only one present in nature. Endogenous ALA is synthesized *de novo* by the liver, starting from a fatty acid with eight carbon atoms (octanoic acid) and cysteine (as a source of sulfur). The highest concentrations of ALA are found in the liver, heart and skeletal muscle (8). In addition to endogenous synthesis, ALA can be integrated from the diet.

Excellent sources of ALA are red meat and offal (heart, liver and kidney) and vegetables such as spinach, broccoli, tomatoes, peas, Brussels sprouts. Taken orally, ALA is absorbed by the small intestine, transported to the liver, and then distributed in the various tissues of the body, especially those rich in mitochondria. Thanks to its structure, ALA is involved in redox reactions and the biological transport of electrons and acetyl groups. In addition, it acts as a cofactor of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase, having a crucial role in the Krebs cycle, necessary for the conversion of glucose and fatty acids into adenosine triphosphate (ATP). Therefore, the availability of ALA is considered fundamental for the efficiency of the entire Krebs cycle (9). Moreover, ALA is involved in acetyl-coenzyme A (CoA) production through the oxidative decarboxylation of pyruvate (10).

A peculiar feature of ALA is its ability to be active both in the oxidized and reduced form, named dihydrolipoic acid (DHLA), in the aqueous phase (cytoplasmic) and the lipid phase (typical of cell membranes). It also crosses the blood-brain barrier without causing adverse effects (Fig. 2).

DHLA donates an electron to the oxidized (inactive) forms of glutathione and vitamin C, converting them into active forms. Vitamin C, in turn, can reactivate the oxidized form of vitamin E. After having donated the electron, DHLA returns into its oxidized form, which continues to maintain its antioxidant properties and regenerate further antioxidants, thus amplifying the organism's defence systems (Fig. 3) (11).

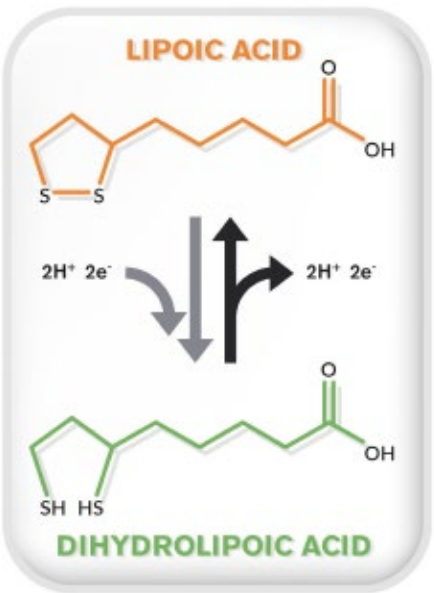

Fig. 2. *Oxidation and reduction of ALA.*

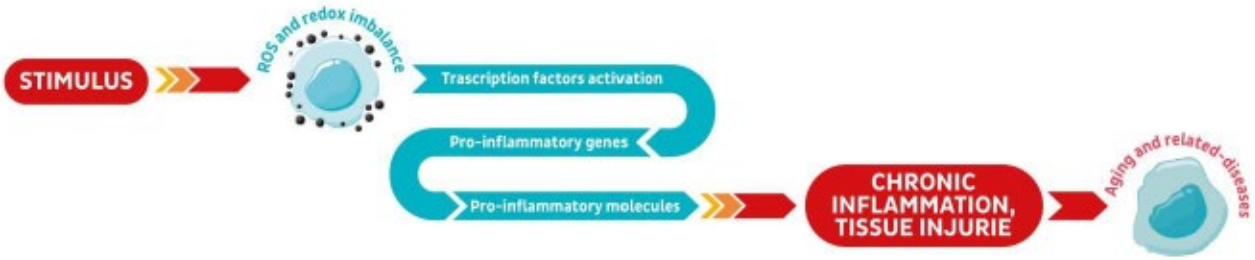

Fig. 1. *ROS activate transcription factors involved in the synthesis of pro-inflammatory molecules, such as cytokines and chemokines, responsible for the production of additional ROS and the triggering of a chronic inflammatory state.*

Interestingly, the effects of ALA are also referable to other mechanisms as well. In bacterial infections, ALA may act as an anti-inflammatory by inhibiting the release of lipopolysaccharide (LPS)-induced pro-inflammatory cytokines due to the inhibition of the phosphorylation of inhibitor of NF-κB (IκB) proteins and the translocation of NF-κB into the nucleus (10several factors significantly limit its effectiveness. Among them, the initial inflammatory graft reaction due to ischemia-reperfusion injury (IRI). ALA also has a chelating action. The ALA / DHLA pair has demonstrated the ability to chelate different metals such as copper, zinc, excess iron, mercury and arsenic, favoring their elimination by the body (12). ALA also contributes to controlling insulin levels in the blood due to its ability to increase insulin efficiency and improve insulin-independent pathways (13). Therefore, the beneficial effect of ALA and its DHLA form is mediated through different mechanisms of action (Fig. 4).

ALA therapeutic applications

In light of this evidence, ALA can find application in various therapeutic areas. For example, in the ENT field, ALA could be effective in treating hearing loss. Indeed, ALA has shown otoprotective effects by preserving outer hair cells, spiral ganglion neurons, and *stria vascularis* (14). Moreover, a recent study has shown that ALA, with its antioxidant, anti-inflammatory and tissue-protective properties, may decrease the clinical sequelae and morbidity associated with acute otitis media, acting on both tissue inflammation and damage and enhancing with the antibiotic effect (15).

ALA has also shown efficacy in drug ototoxicity, the toxic property shown by drugs and toxins towards the structures of the inner ear, especially cochlear and vestibular cells, or the acoustic nerve. Toxic damage is usually accompanied by tinnitus, vertigo, hearing loss, hyperacusis. These symptoms can occur separately or in combination, develop suddenly or gradually, and be reversible or irreversible. The ototoxic action can lead to marked hearing loss up to complete deafness in severe cases. The potential toxicity also depends on subjective susceptibility, genetic predisposition and the possible presence of pathological conditions, such as renal failure (16).

Therefore, the administration of these drugs should be followed by careful monitoring of the cochlea-vestibular function to minimize the risk of permanent damage. In this context, ALA has been shown to rescue ototoxic hearing loss caused by ototoxic drugs (17), like cisplatin, a small platinum-containing molecule.

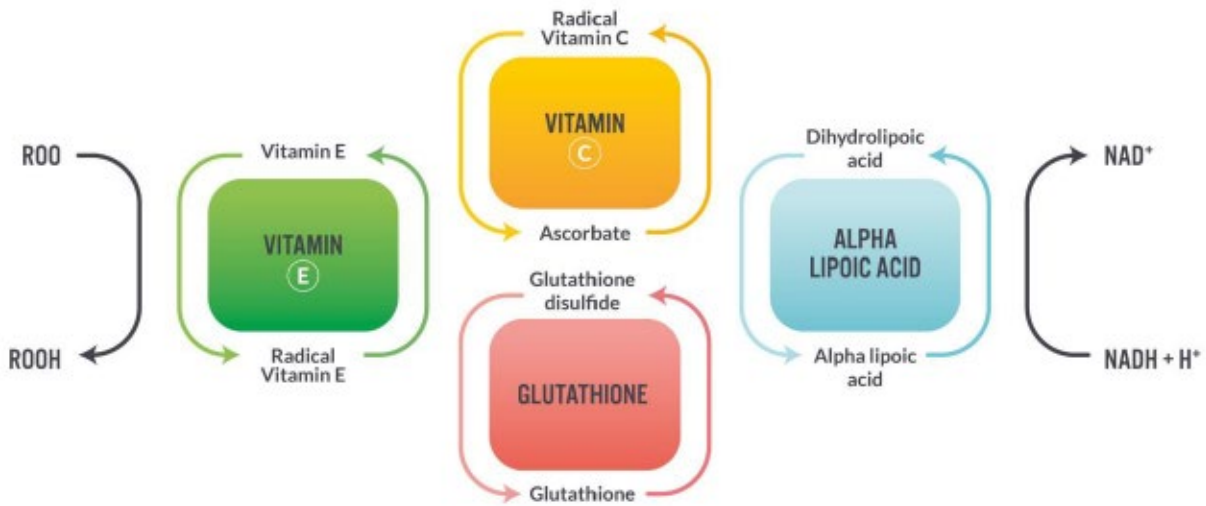

Fig. 3. *Activation of Glutathione and Vitamin C by DHLA. Vitamin C, in turn, activates the oxidized form of vitamin E. After donating the electron, DHLA returns to its oxidized form.*

However, severe side effects have been found in cancer patients treated with cisplatin, including nephrotoxicity, neurotoxicity, and ototoxicity. These cisplatin-induced side effects can have a major impact on patient quality of life, including social development problems in pediatric patients that develop hearing loss. Previous studies have suggested that the major cause of cisplatin-induced ototoxicity is abnormal accumulation of reactive oxygen species (ROS).

In addition, ALA could represent a promising alternative in treating trigeminal neuralgia and burning mouth syndrome. Trigeminal neuralgia is caused by the irritation of the trigeminal nerve that innervates the face, being responsible for facial sensitivity and jaw motility. Trigeminal neuralgia causes acute and stabbing pain compared to electric shocks and is usually limited to one side of the face. Due to the correlation between oxidative stress and neuralgias, ALA would improve nerve conduction and reduce pain (18). Similarly, ALA could be considered a therapeutic option for burning mouth syndrome, a chronic condition characterized by oral burning in the absence of clinical abnormalities. The main sites involved are the tongue and lips, followed in order of frequency by the hard palate, ridges, cheeks and mouth.

Patients suffering from burning mouth syndrome frequently report dysgeusia or ageusia and dysosmia or, rarely, anosmia. The etiology of burning mouth syndrome remains unclear. However, several studies have shown the involvement of the peripheral nervous system and, in particular, an increase in the sensitivity of the trigeminal nerve and axonal degeneration of the epithelial and sub-papillary fibres (19). In addition, in a double-blind, randomized placebo-controlled study conducted on 34 women and 4 men, the intake of ALA 600 mg per day for 30 days has resulted in a significant improvement in symptoms related to burning mouth syndrome compared to placebo (20).

Available ALA-based drugs and supplements

Ageing and pathological condition can cause a drastic reduction in ALA, which is not always

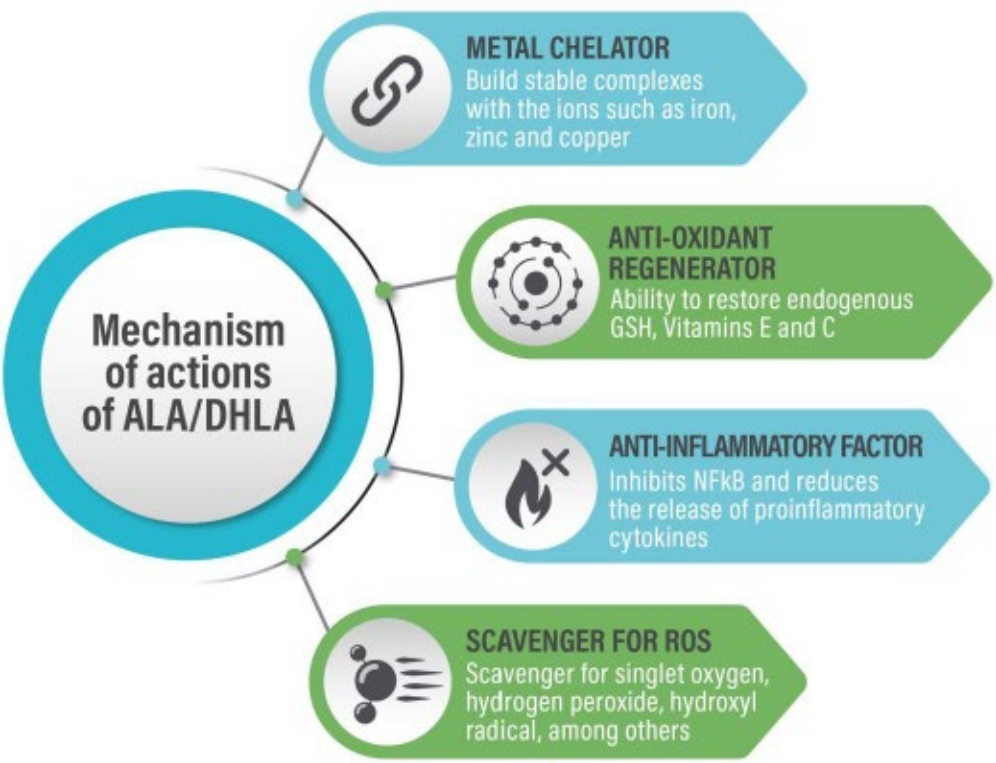

Fig. 4. *Mechanisms of action of ALA/DHLA.*

compensable with the diet. In this context, nutraceuticals can provide up to 1000 times more ALA than food sources, ensuring greater bioavailability, especially in delayed and prolonged-release formulations. ALA has an optimal safety profile, with no known contraindication in pregnant women and children. International guidelines have recommended daily dosage of 600 mg (21). However, the adult intake of up to 2400 mg has not been shown to cause harmful side effects. High doses of ALA are not recommended as they do not provide any additional benefit (22, 23).

In Italy, ALA is only available as a dietary supplement at a dosage of 600 mg. However, in several countries, such as Germany, Austria, Hungary, Czech and Slovak Republic, Romania, Bulgaria, South Korea and the USA, ALA is, by all accounts, a prescription drug.

Acetyl-l-carnitine

ALCAR, a small endogenous molecule, is the acetylated derivative of L-carnitine, an amino acid whose function is related to mitochondrial energy metabolism. The total carnitine content in the human body is around 300 mg/kg, with the highest tissue concentration in the skeletal muscles and myocardium, followed by liver, kidneys and plasma. Circulating carnitine comes mainly from foods of animal origin and, to a lesser extent, from endogenous production in the liver and kidney. Carnitine not derived from the diet is synthesized endogenously from two essential amino acids, lysine and methionine. The half-life of ALCAR after oral administration is approximately 4.2 hours (24). The bioavailability of dietary carnitine is between 54% to 87% (25).

ALCAR is involved in various anabolic and catabolic pathways of cellular metabolism responsible for the production and transport of energy. For example, ALCAR intervenes in the transport of long-chain fatty acids from the cytosol to the mitochondria, where they undergo β-oxidation, and in the transport of the ATP produced by the mitochondrion to the cytosol (26, 27). ALCAR also has antioxidant properties, modulates neurotransmitters in the brain such as acetylcholine, serotonin and dopamine, and acts on neurotrophic factors such as nerve growth factor (NGF) and metabotropic glutamate receptors (mGlu). More recently, its activity as a donor of acetyl groups has also extended to epigenetic mechanisms, including analgesia (24).

ALCAR therapeutic applications

The involvement in carbohydrate, lipid and protein metabolism makes ALCAR an ideal candidate molecule for treating otologic diseases. Indeed, recent studies have shown the protective role of ALCAR in cochlear damage induced by noise-induced hearing loss and the efficacy of ALCAR in reducing tinnitus (28, 29). The protective action of ALCAR would be linked to its ability to restore cardiolipin levels, a phospholipid of the inner mitochondrial membrane, and carnitine and improve the production of mitochondrial energy. In fact, ALCAR would improve the functioning of some mitochondrial respiratory enzymes, restore the correct transport of mitochondrial metabolites, and maintain the integrity of the mitochondrial membrane. Moreover, ALCAR would also play a scavenger role, increasing the concentration of glutathione, another molecule with an antioxidant action (26).

In addition, as regards disorders of neuropathic origin, ALCAR would exert its action at two levels: firstly, at the dorsal ganglia and the posterior horns of the spinal cord, carrying out an analgesic activity; secondly, at the nerve fibre, for its neuroprotective effect (30). For these reasons, ALCAR has shown to be a simple, economical and safe adjuvant option for treating neurological disorders, including neuralgias (31, 32).

CONCLUSIONS

The involvement of oxidative stress in the onset and progression of pathologies of the inner ear and oral-facial pain syndromes is nowadays clearly recognized. The increase in intracellular ROS levels is responsible for direct damage to lipids, proteins and DNA, triggering processes of apoptosis or necrosis. Experimental studies conducted in animals have demonstrated the ability of ROS to induce the death of hair cells in the inner ear with irreversible hearing damage, as neurons and hair cells are unable to regenerate. Therefore, molecules with antioxidant and anti-inflammatory action could be useful in

these degenerative processes. ALA and ALCAR, in particular, have been shown to restore cellular homeostasis thanks to their antioxidant and metabolic energy pathways optimization action, limiting hearing damage and reducing pain, even in the context of disorders orofacial, such as trigeminal neuralgia and burning mouth syndrome. In light of today's clinical and experimental data, ALA and acetyl L-carnitine could represent a valid therapeutic opportunity in various ENT diseases.

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Oxidative stress in ENT Disease: evidence for treatment of Craniofacial Neuralgia and Tinnitus

Recentemente, la ricerca si è concentrata sul possibile beneficio dei **trattamenti antiossidanti** per patologie **ORL** legate allo **stress ossidativo.**

Lo stress ossidativo è caratterizzato dallo squilibrio tra produzione e accumulo di sostanze ossidanti e dalla capacità di eliminare questi prodotti reattivi. É stato ampiamente dimostrato come le specie reattive dell'ossigeno (ROS) e i radicali liberi abbiano un effetto dannoso diretto sulle funzioni cellulari e siano coinvolti nella patogenesi di diverse malattie, tra cui l'aterosclerosi, la broncopneumopatia cronica ostruttiva (BPCO), il diabete, la malattia di Alzheimer e il cancro. Recentemente, la ricerca si è concentrata sul possibile beneficio dei trattamenti antiossidanti per altre patologie legate anch'esse allo stress ossidativo.

La misura in cui lo squilibrio redox partecipa allo sviluppo o alla progressione delle malattie legate allo stress ossidativo non è ancora del tutto chiaro.

Lo stress ossidativo sembra giocare un ruolo cruciale nella patogenesi delle **nevralgie craniofacciali** e degli **acufeni.** A questo proposito sono state condotte due indagini nazionali che hanno coinvolto **33 Centri.**

Pertanto, determinare se svolga effettivamente un ruolo primario o secondario rimane difficile. Ciò potrebbe avere importanti implicazioni terapeutiche, poiché la comprensione dei meccanismi alla base dell'insorgenza di patologie correlate allo stress ossidativo potrebbe consentire di definire correttamente i target terapeutici e indirizzare i trattamenti.

I contenuti nel supplemento mirano a chiarire meglio il coinvolgimento dello stress ossidativo nell'insorgenza e nella progressione delle patologie ORL, evidenziando l'efficacia della somministrazione di integratori, formulati con molecole antiossidanti, nella gestione delle nevralgie craniofacciali e degli acufeni.

The effectiveness of dietary supplements based on alpha-lipoic acid, acetyl L-carnitine and vitamin B complex in the treatment of craniofacial neuralgia

264 pazienti sono stati coinvolti nell'indagine sulle **nevralgie craniofacciali.**

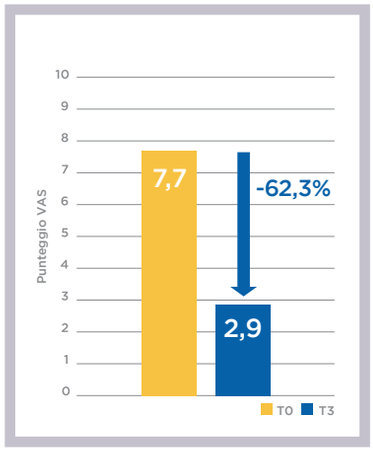
Le nevralgie craniofacciali influiscono negativamente sulla qualità della vita (QoL) dei pazienti, causando disturbi del sonno, riduzione delle prestazioni lavorative e impedimento di pasti regolari.

PREMESSA: il trattamento delle nevralgie craniofacciali rappresenta un'area di insoddisfazione per gli Specialisti a causa della difficile comprensione della fisiopatologia del dolore neuropatico. Recenti evidenze hanno dimostrato che lo stress ossidativo gioca un ruolo cruciale nella patogenesi delle nevralgie. Pertanto, i nutraceutici formulati con composti antiossidanti sono stati proposti come possibili trattamenti. Per analizzare gli attuali trattamenti del dolore neuropatico craniofacciale e per valutarne l'efficacia è stata condotta un'indagine nazionale, coinvolgendo 33 centri ORL italiani.

RISULTATI: Dall'indagine è emerso che le nevralgie craniofacciali influiscono negativamente sulla qualità della vita (QoL) dei pazienti, causando disturbi del sonno, riduzione delle prestazioni lavorative e impedimento di pasti regolari. I pazienti trattati con integratori alimentari a base di acido alfa-lipoico,

acetil L-carnitina e complesso vitaminico B (Tioneural Retard®), che agiscono sinergicamente sulle infiammazioni del sistema nervoso periferico, hanno mostrato una riduzione del punteggio VAS attribuito al disagio nevralgico.

CONCLUSIONI: I composti antiossidanti, grazie ai loro effetti neuroprotettivi, sono efficaci nel ridurre il dolore neuropatico. La riduzione dello stress ossidativo può essere quindi considerata una direttrice promettente del trattamento del dolore neuropatico e dà speranza ai pazienti attualmente costretti a convivere con un dolore cronico, spesso invalidante.



Riduzione del punteggio VAS determinato nei pazienti con dolore severo (VAS 7-10) dopo tre mesi dall'inizio del trattamento rispetto al basale.
*** p<0.001 vs T0, paired t-Test.

La riduzione dello **stress ossidativo** può essere considerata una direttrice promettente nel controllo del **dolore neuropatico.**

I pazienti in trattamento con Tioneural Retard®, dopo 3 mesi di terapia, hanno indicato una riduzione statisticamente significativa del **dolore nevralgico** misurato con la scala **VAS (-62,3%).**

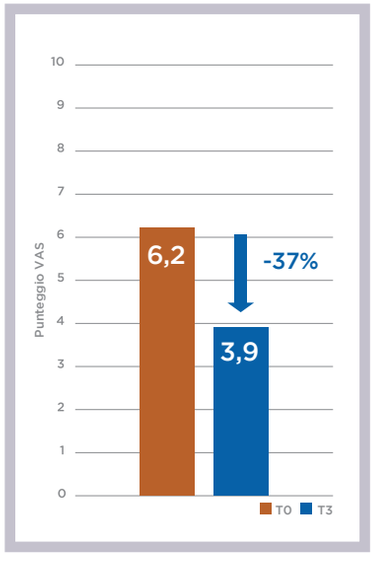
The effect of dietary supplements based on alpha-lipoic acid, acetyl L-carnitine and vitamin B complex in patients with tinnitus

PREMESSA: L'acufene colpisce più del 10% della popolazione, con un elevato onere sociale ed economico. Sebbene siano state proposte diverse ipotesi per spiegare lo sviluppo dell'acufene, l'eziologia rimane poco chiara, ostacolando lo sviluppo di terapie efficaci. Recenti evidenze hanno dimostrato che lo stress ossidativo gioca un ruolo cruciale nella patogenesi dell'acufene, suscitando un crescente interesse per il ruolo dei nutraceutici come utile approccio terapeutico. Per analizzare le strategie di gestione dell'acufene e per valutarne l'efficacia è stata condotta una survey nazionale che ha coinvolto 33 Otorinolaringologi presenti sul territorio nazionale.

306 pazienti sono stati coinvolti nell'indagine sugli **acufeni.**

RISULTATI: I risultati hanno mostrato che l'acufene non è ancora considerato una patologia a tutti gli effetti e ciò comporta che non sia adeguatamente trattato. Tra i trattamenti, gli integratori alimentari a base di acido alfa-lipoico, acetil L-carnitina e complesso vitaminico B (Tioneural Retard®) si sono rivelati efficaci nel ridurre il disagio indotto dall'acufene.

CONCLUSIONI: La crescente attenzione allo stress ossidativo come causa di acufene e come possibile bersaglio terapeutico rappresenta una promettente indicazione all'impiego di nutraceutici con attività antiossidante nel trattamento di questa patologia.



Riduzione del punteggio VAS nei pazienti con acufene tre mesi dopo l'inizio del trattamento rispetto al basale.
p<0.0001 non parametric Wilcoxon test.

La crescente evidenza relativa allo **stress ossidativo** come causa di **acufene,** rappresenta una promettente indicazione all'utilizzo di antiossidanti nel trattamento di questa patologia.

I pazienti trattati con Tioneural Retard®, dopo 3 mesi, hanno mostrato una riduzione del **disagio** indotto dall'**acufene** misurato con il punteggio **VAS.**

NOTE

TIONEURAL® retard

CONTENUTI MEDI PER UNA COMPRESSA

	Q.tà	VNR*
Acido alfa-lipoico	600 mg	
Acetil L-carnitina cloridrato	300 mg	
Vitamina B5	6 mg	100
Vitamina B12	2,5 mcg	100
Vitamina B6	1,4 mg	100
Vitamina B1	1,1 mg	100

***VNR:** *Valori Nutritivi di Riferimento - Reg. 1169/2011*

Acido alfa-lipoico al dosaggio raccomandato dalle linee guida internazionali, **Acetil-L-carnitina e Complesso delle vitamine B** agiscono sinergicamente sulle infiammazioni del SISTEMA NERVOSO PERIFERICO.

Con **tecnologia Physio release®** e **Compreezer** che preservano la qualità e la stabilità dei nutrienti garantendone il massimo assorbimento.

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NATURALMENTE PRIVO DI LATTOSIO

CONFEZIONE:
30 compresse

MODALITÀ D'USO:
1 compressa/die

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