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ORIGINAL ARTICLE

Increased platelet activity in tinnitus patients

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Abstract

OBJECTIVE: The aim of our study was to establish whether or not tinnitus patients have higher platelet activity, as measured by plasma 11-dehydro-thromboxane B2 levels, compared with individuals without tinnitus.

METHODS: The study group included patients without documented organic causes of tinnitus or a cause of non-vascular hearing impairment. Laboratory tests included complete blood count, biochemistry, coagulation activity, and thromboxane levels. To exclude a pathology in the cerebellopontine angle, CT and MRI were performed together with an X-ray scan of cervical vertebrae. For the purpose of this study, blood samples were screened for 11-dehydro-thromboxane B2 levels using commercial kits.

RESULTS: A comparison of the main marker of increased platelet activity i.e., thromboxane levels of tinnitus patients with those of a control group, showed increased thromboxane levels in the former. The average plasma concentrations of 11-dehydro-thromboxane B2 were 2.0234±1.80 ng/ml in the group of tinnitus patients and 1.3247±1.33 ng/ml in the control group. Our results showed that patients with tinnitus have significantly higher values of 11-dehydro-thromboxane B2.

CONCLUSION: Tinnitus patients showed higher levels of increased platelet activity, a marker that may play an important role in the pathogenesis of tinnitus.

INTRODUCTION

Tinnitus is a nonspecific symptom developing due to dysfunction of the auditory system. The underlying causes of tinnitus may differ; an important role in its pathogenesis is presumably played by a microcirculation disorder of the inner ear and other parts of the central nervous system (Arndt *et al.* 2020; Anders *et al.* 2010; Seidman *et al.* 2011; Nakashima *et al.* 2003; Mahrmoudian *et al.* 2013). Proper function of the ear is dependent on its adequate microcirculation with its regulation controlled by a variety of factors (Arndt *et al.* 2020; Xiaorui 2011; Eikelboom *et al.* 2002). An important role in the process of hemostasis is played by platelet aggregation. Platelet activation results in the formation of unstable thromboxane A2 (with significant prothrombotic properties)

and its derivatives thromboxane B2 and 11-dehydro-thromboxane B2. While thromboxane B2 is unstable with a half-life of about 36 seconds, 11-dehydro-thromboxane is relatively stable with a half-life of 45 minutes. Thromboxanes produced by activated platelets cause vasoconstriction and play a key role in platelet aggregation. In patients with poorly suppressed thromboxane levels, the risk of cardiovascular events is higher (Cheng *et al.* 2020; Xiaorui 2011). In animal experiments, thromboxane synthase inhibitors and thromboxane A2 antagonist receptors significantly prolonged the time needed to complete suppression of cochlear action potential in comparison with a control group (Umemura *et al.* 1993).

The reported incidence of subjective tinnitus varies being in the range of 8 to 15%. Population-based studies designed to assess hearing impairment in adult patients aged 48 to 92 years have reported a prevalence of 8.2% (start of study) with an incidence of 5.7% during 5-year follow-up (Wells *et al.* 2020; Zhu *et al.* 2015; Coelho *et al.* 2007; Semaan, Megerian 2010; Shargorodsky *et al.* 2010). Tinnitus prevalence has been shown to increase with age. Cardiovascular risk factors associated with a higher incidence of tinnitus include increased body mass index (BMI), hypertension, and dyslipidemia (average incidence 21–24% of the general tinnitus population as against 28–32% in at-risk patients) (Rodriguez-Ayala *et al.* 2020; Wójcik *et al.* 2018; Daniell, Fulton-Kehoe *et al.* 2002; Daniell, Swan *et al.* 2002; Deggouj *et al.* 2009; Han *et al.* 2009).

Thromboxanes are very potent platelet agonists and vasoconstrictors thought to be of functional importance in the precipitation of certain vascular occlusive diseases (Oravec *et al.* 2011; Hamberg *et al.* 1975). Thromboxane A2 (TxA2) is a labile derivative of prostaglandin (PG) endoperoxide metabolism that amplifies platelet response to a variety of pro-aggregation stimuli (Eikelboom *et al.* 2008). Because of chemical instability of the oxane ring, TxA2 is rapidly converted into the chemically stable and relatively biologically inactive hydration product thromboxane B2 (TxB2). Thromboxane B2 undergoes two major pathways of metabolism, one involving β-oxidation, resulting in the formation of 2,3-dinor-TxB2 and the other involving dehydrogenation of the hemiacetal alcohol group at C-11, resulting in the formation of a series of metabolites with a δ-lactone ring structure. One metabolite is 11-dehydro-thromboxane B2 (11dTxB2) which is advantageous to determine, because its levels are not artificially affected during venipuncture (Ciabattoni *et al.* 1989).

MATERIALS AND METHODS

The study was designed as a longitudinal prospective cohort one, with the study group including patients without documented organic causes of tinnitus and any cause of non-vascular hearing impairment (Meniere´s disease, acoustic trauma, barotrauma, vestibular schwannoma, sclerosis multiplex, Lyme disease, ototoxic factors). Eligible patients could not have serious internal comorbidities potentially affecting study results. Patients were free of inflammation, cardiovascular disease, diabetes mellitus, tumors, renal dysfunction, and were not taking ototoxic medication or any medication that affects blood clotting. Patients could not have hearing loss of more than 40 dB.

All patients were examined by an internist and an otorhinolaryngologist performing otomicroscopy, epipharyngoscopy, audiometry with tinnitus masking, tympanometry, and completing questionnaires specifying duration of the problem and tinnitus characteristics (high- or low-frequency sound). Laboratory tests included complete blood count, biochemistry (ALT, AST, urea, creatinine, fibrinogen, GMT, CRP), coagulation activity and, for the purpose of this study, blood samples were screened for 11-dehydro-thromboxane B2 levels using commercial kits (Cayman Chemicals, Tallin, Estonia and Neogen, Lexington, Ky, USA). To exclude a pathology in the cerebellopontine angle, CT and MRI were performed together with an X-ray scan of cervical vertebrae.

Blood samples were collected by venipuncture from the antecubital vein into plastic evacuated Vacutainer tubes containing heparin. All samples were incubated at 37°C for 1 hour, then centrifuged for seven minutes at 878 g to separate plasma. Plasma 11-dehydro-thromboxane B2 (11-dTxB2) levels were measured using commercially available kits (Cayman Chemicals and Neogen). The measurement was performed according to recommendations provided by each manufacturer.

Our study group included 40 tinnitus patients and 40 age- and sex-matched individuals in a control group (total 80 individuals). The control group comprised individuals without tinnitus or any comorbidities, and taking no medication.

The statistical methods used in this study included both qualitative and quantitative parameters. We used the Wilcoxon and Kruskal-Wallis tests, chi-square approximation, parametric and nonparametric tests in all cases where the distribution of values was not consistent with Gaussian distribution (medians), also were used multidimensional regression and OPLS model. For variables to which Gaussian distribution is not applicable (e.g. CRP, 11-dTxB2), logarithmic transformation was performed. The results were considered significant if $p<0.05$.

RESULTS

A total of 80 study participants were divided into tinnitus patients (n=40) and individuals in a control group (n=40), with a mean age of 50.86 years (median, 53 years). The men vs. women ratio was 70% (n = 56) to 30% (n = 24).

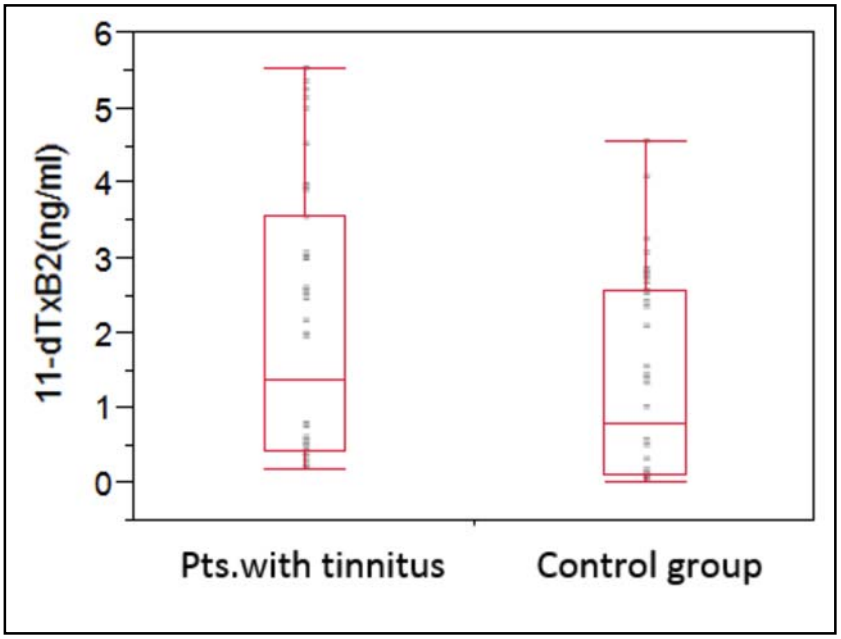

Fig. 1. Differences in 11-dTxB2 levels between tinnitus patients and controls

The average 11-dehydro-thromboxane B2 levels were 2.023±1.80 ng/ml in tinnitus patients and 1.325±1.33 ng/ml in the control group. Statistical results are shown in Figure 1. The above data show that tinnitus patients have significantly higher platelet activity as measured by 11-dehydro-thromboxane B2 levels. The above results show that elevated 11-dehydrotromboxane B2 concentration in patients with tinnitus is statistically significant at p=0.0145. Results are shown in Tables 1–2.

Additional data (biochemistry, coagulation activity) in our study did not reveal statistically significant differences. Results are shown in Tables 3-4.

DISCUSSION

Our study documented a correlation between the presence of tinnitus and increased plasma 11-dehydro-thromboxane B2, supporting our hypothesis that impaired microcirculation plays a major role in the pathogenesis of tinnitus. All tinnitus patients in the present study showed elevated levels confirming increased platelet activity compared with the control group. It is clear that changes in the perfusion of sensory organs – that is inner ear microcirculation –are closely linked to the development of hearing impairment and tinnitus. In the guinea pig, transient occlusion of the internal auditory artery resulted in complete cessation of auditory function within several minutes and irreversible cochlear degeneration. While the apical end of the cochlea is particularly vulnerable (reflected as low-frequency hearing loss), the vestibular end of the organ is somewhat more resistant (Kim, Hyung 2009). Data obtained from our patients are not biased by secondary factors as all patients with comorbidities and other factors that could have possibly affected the results had been excluded. In general, those ineligible included patients with diabetes, cardiovascular disease, tumors, infection or patients taking medications that can affect increased platelet activity.

It is generally recognized that the incidence of tinnitus increases with age, old age is also likely to be

Tab. 1. Calculated 11-dTxB2 levels at significant level p=0.0145 (ng/ml).

Level	Minimum	25%	Median	75%	Maximum
Tinnitus patients	0,189	0,431	1,365	3,553	5,521
Control group	0,013	0,117	0,808	2,556	4,547

Tab. 2. Statistical data of 11-dTxB2 with means and standard deviation (SD)

Level	Number	Mean	SD	Std Err Mean	Lower 95%	Upper 95%
Tinnitus patients	40	1.73776	1.79457	0.29112	1.1479	2.3276
Control group	40	1.28213	1.3±3711	0.21691	0.8426	1.7216

Tab. 3. Biochemistry

	Tinnitus patients (average)	Control group (average)
Creatinin (μmol/l)	78,5±13,64	86,68±12,15
Urea (mmol/l)	5,19±1,40	5,828±1,87
AST (μkat/l)	0,44±0,1	0,47±0,16
ALT (μkat/l)	0,47±0,25	0,50±0,19
GMT (μkat/l)	0,83±1,01	0,76±1,12
CRP (mg/l)	4,56±1,43	4,74±2,32

Tab. 4. Coagulation activity

	Tinnitus patients (average)	Control group (average)
PT (s)	12,95±0,72	12,62±0,94
INR	0,9978±0,07	1,021±0,12
Fibrinogen (g/l)	3,337±1,06	3,012±1,16

associated with increased coagulation activity while the activity of anticoagulation mechanisms remains unaltered; in addition, increased serum fibrinogen levels are also frequently seen in the elderly (Zaldua *et al.* 2020; Drenos *et al.* 2007, Fu *et al.* 1998). All of these relationships give the impression that the increased prothrombogenic platelet activity may be associated with impaired hearing and tinnitus. There was no statistically significant age difference between the control group and the tinnitus patient group.

This potential relationship between higher platelet activity and tinnitus supports the possibility of a beneficial pharmacological effect on blood clotting and, consequently, improved outcome. Several authors have suggested protective effects of non-steroidal anti-inflammatory drugs (NSAIDs) on cochlear injury (Boettcher, Salvi 1991). A variety of protective effects of NSAIDs on cochlear injury have been reported in animal studies. Several NSAIDs allegedly exhibit protective effects on the inner ear against acoustic injury in rodents (Brien 1993; Cazals 2000; Lamm, Arnold 1998; Kopke et al 2000). In addition to their anti-inflammatory action, NSAIDs may also exert a beneficial anti-aggregation effect due to reversible COX-1 inhibition in platelets. Although quinacrine, a general PLA2 (phospholipase A2) inhibitor, ameliorated cochlear ischemia-reperfusion and acoustic injury, the exact protective mechanisms are largely unknown (Tabuchi *et al.* 2000). As described above, the protective effects of inhibitors of COX-1 (cyclooxygenase-1) and LOX (lipoxygenase) as well as PLA2 have been documented in various types of cochlear injury (Hirose *et al.* 2007). Further studies on the arachidonic cascade in the cochlea and its inhibitors (or modulators) will shed light on the understanding of the generation mechanisms of various types of cochlear injury (Hoshino *et al.* 2010).

CONCLUSIONS

In our study, the platelet activity of tinnitus patients was higher compared with controls. Our results provide another rationale for the use of drugs affecting hemostasis in the treatment of tinnitus.

CONFLICT OF INTEREST STATEMENT

All authors do not have any financial and personal relationships with any organization or entity with any financial interest that could inappropriately influence their work.

The studies were approved by the appropriate ethics committee and were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Declaration of Helsinki 1975, as revised in 2008.

Informed consent was obtained from all patients included in the study.

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