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Original Article

The role of oxidative stress in acute subjective idiopathic tinnitus

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Abstract

Aim: The aim of this study was to investigate the role of oxidative stress (OS) in the etiopathogenesis of acute subjective idiopathic tinnitus (SIT). We investigated the plasma thiol-disulphide ratios which were markers of OS in SIT.

Materials and Methods: The thiol and disulphide levels and ratios of 65 patients who were admitted with acute SIT and 46 healthy volunteers were determined and compared by the method published recently. Native (NT) and total thiol (TT), disulphide (Ds), disulphide/native thiol (Ds/NT), disulphide/total thiol (Ds/TT), native/total thiol (NT/TT) were determined to investigate the thiol-disulphide balance. Audiologic tests including tinnitus frequency and severity, pure tone audiograms were also compared among the groups.

Results: In patient group, NT, TT levels and NT/TT ratio were significantly lower, Ds level and Ds/NT, Ds/TT ratios were significantly higher than the healthy group. NT was significantly related to acute SIT in binary logistic regression model ($p<0.001$). ROC-curve analysis showed that NT levels over 409 mmol/L predicted acute SIT with 66% sensitivity and 35% specificity.

Conclusion: Oxidative stress, as pointed in our research by thiol-disulphide measures, may have an important role in the etiology of acute SIT.

Keywords: Antioxidant; disulphide; hearing loss; thiol; tinnitus

INTRODUCTION

The occurrence of reactive oxygen species is an inevitable event that develops in aerobic species. In cases of increased ROS can lead to irreversible changes in lipids, DNA and proteins. Thiol has an important role in preventing intracellular oxidation in case of increased oxidative stress (OS) (1). Thiols are organic compounds containing sulphydryl group (-SH) and can be present in the form of protein or albumin thiols. Redox regulation, which is comprised of oxidation and reduction reactions, is critical in cellular homeostasis such as DNA synthesis and enzyme activation and controlled by free and protein incorporated thiols (2). Increased oxygen status can lead to elevated OS conditions and result in promoting disulphide (Ds) formation in molecular level. Under reduced oxygen status, the formed disulphide bonds can be converted to back to thiol groups, thus the balance is preserved (3).

Thiol-disulphide exchange reactions play a critical role in cellular function. Corruption of thiol-disulphide balance can lead to loss of cellular functions resulting in apoptosis, and cell death. For this reason, the determination of thiol-disulphide balance can give precious insights about the relationship between diseases and OS (2-4). Over the past decade, tinnitus has been one of the diseases investigated for its association with OS (5-7). In recent years, several researches have been published indicating the role of OS in the etiology of tinnitus (8-10). For this purpose, we investigated the plasma NT, TT, Ds levels and Ds/NT, Ds/TT, NT/TT ratios as markers of OS in acute subjective idiopathic tinnitus (SIT) patients in this study. To our best knowledge, this research is the first study to report an association between thiol-disulphide balance and acute SIT.

MATERIAL and METHODS

From January 2014 to September 2016, 65 patients presenting with unilateral or bilateral tinnitus complaint

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within 3 months of development were included. Local ethical committee approval and written informed consents of all patients were obtained.

All subjects met the following criteria; no history of past otologic disease or autoimmune disease and no history of ototoxic drug use. A treatable underlying cause of tinnitus couldn't find in all patients. Audiometric examinations were performed, and hearing loss and tinnitus severity and frequencies were recorded. Blood samples were withdrawn from the antecubital vein and NT, TT and Ds concentrations were determined by the novel and automated essay which was described recently by Erel O et al (2). In this method, thiol and disulphide ratios were measured in serum after centrifugation, thus thiol disulphide balance was determined. The ratios between the values were calculated. Patients with diseases of middle or inner ear, hypertension (blood pressure over 140/90), diabetes, cardiovascular diseases, thyroid disorders, metabolic and systemic inflammatory disease and malignancies were excluded. Sudden hearing loss that may be the cause of tinnitus were also excluded. Acute SIT is defined as acute idiopathic subjective tinnitus developed within 3 months as previously described (11).

Measurement of tinnitus

To quantify tinnitus in terms of its possible frequency, pitch matching test were used. Pure tone and narrow band noise were used to determine the frequencies between 125 and 16.000 Hz. Loudness of tinnitus had the range from 0 to 110 dB, which were adjustable at intervals of 1dB. Two tones were presented to the patients and the patients were asked to choose which one most closely matched the tinnitus that they hear until the match were made. As previously described, the test was repeated seven to nine times to ensure the correct match. When a pure tone similar to the tinnitus tone could not be found, narrowband noise was applied (12).

Assays for oxidative stress

NT, TT, Ds levels and Ds/NT, Ds/TT, NT/TT ratios of the patients in the study and control groups were determined and statistically compared. Moreover, in the study group, these levels and ratios of the patients with hearing loss (pure tone average>25 dB) or normal hearing (pure tone average≤25 dB) were compared. Although the upper and lower limits of Ds, NT and TT levels were defined by the researchers, they are not absolute values, so we compared the values of patients with healthy subjects.

Statistical analysis

Kolmogrov-Smirnov test was used to determine whether the data were distributed normally. Descriptive results were expressed as mean ± SD. Independent student t test was used for normal distribution values. $p < 0.05$ was considered statistically significant. In the paired comparisons, binary logistic regression was used. The sensitivity, specificity and the optimal cut off value were determined by the ROC curve. SPSS statistical software (SPSS for MacOS, version 25.0; SPSS Inc., Chicago, IL) were used for statistical comparisons.

RESULTS

Table 1 shows the demographic distribution and Ds/NT ratios in study and control groups. Study group included 65 patients whose ages were between 18 and 78, with a mean age of 43.33 ± 15.9 (19 males, 27 females). 46 healthy volunteers were enrolled as the control group whose ages were between 19 and 83, with a mean age of 52.2 ± 15.6 (24 male, 41 female). Body mass index (BMI) of the groups were similar. Of 65 patients, 45 had unilateral and 20 had bilateral tinnitus. The patients' hearing loss levels were between 5 and 55 dB (mean 24.1 ± 13.9). Tinnitus frequencies were between 2 and 16 KHz (mean 10.16 ± 4.18) and tinnitus severities were between 10 and 90 dB (mean 59.23 ± 16.84).

Study group presented a statistically significant increase in Ds concentrations and Ds/NT, Ds/TT ratios ($p<0.05$). NT, TT concentrations and NT/TT ratio of study group were found lower compared to the control group ($p<0.05$) (Table 2). The levels and ratios of study and control groups are shown in Figure 1. The Ds/NT ratios of the patients who have hearing loss or normal hearing are shown in Table 3. Study arm did not reveal a statistically significant increase in any concentrations and ratios (p>005).

Table 1. Demographic and clinic characteristics of the patient and control group

	Patient Group, N=65	Control Group, N=46	P value
Age (years)	46.33 ± 15.9	49.2 ± 15.61	NS
Sex			
Male	24	19	NS
Female	41	27	NS
BMI (kg/m^2)	25.4 ± 3.2	25.7 ± 3.9	NS
Tinnitus severity (dB)	59.23 ± 16.84	-	-
Tinnitus frequency (kHz)	10.16 ± 4.18	-	-
Hearing Loss Levels - Pure tone average (dB)	24.1 ± 13.9	-	-

All parameters were expressed as mean ± standard deviation; $p<0.05$ value was accepted as significant level and the significant differences between the groups were shown in bold; NS: Not Significant; N: Number of patients

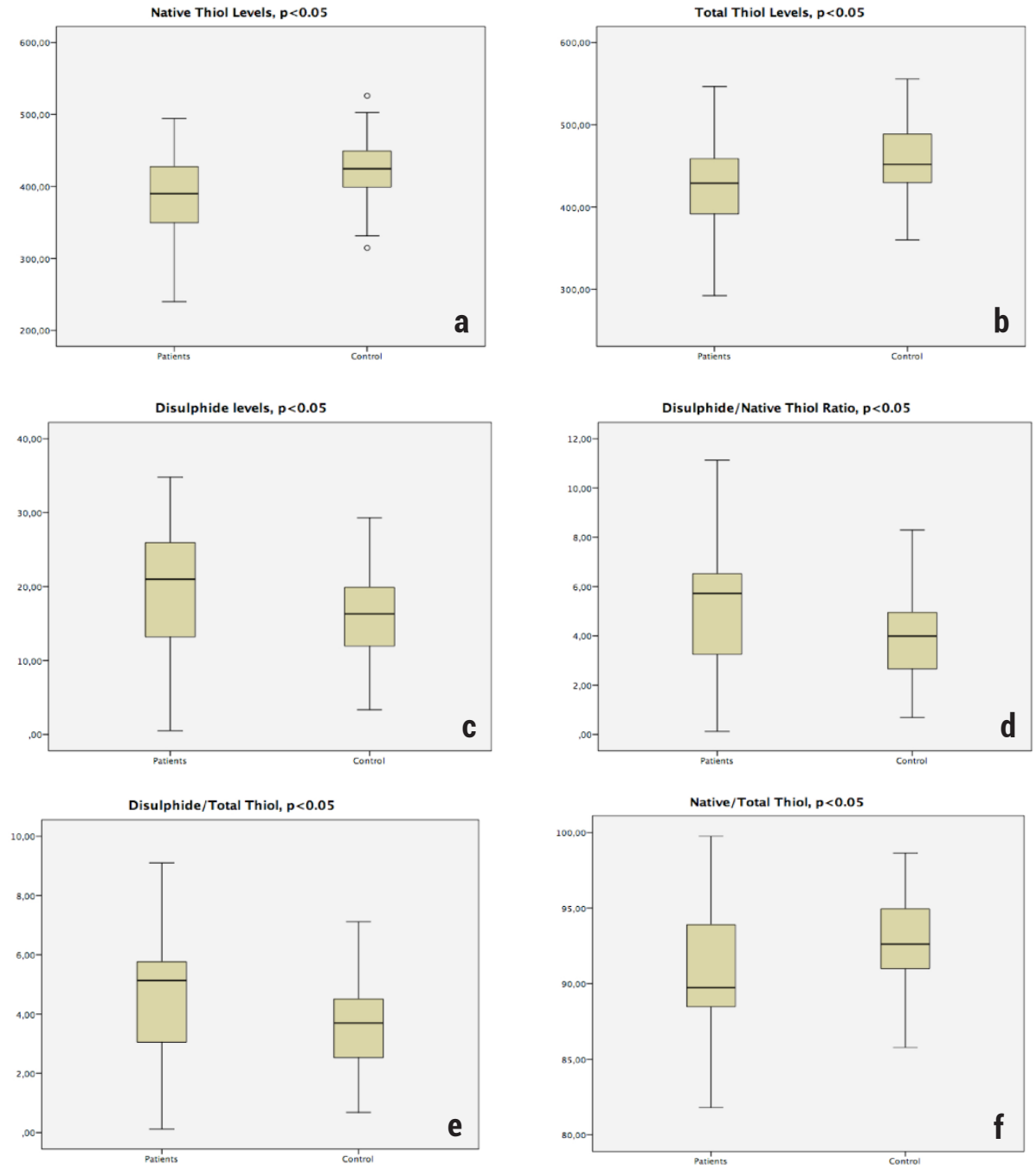

Figure 1. a-f Comparison of thiol and disulphide levels and ratios between the patient and the control group

Table 2. Thiol and disulphide datas as oxidative stress markers between the patient and control groups

	Patient Group, N=65	Control Group, N=46	P value
Native Thiol	388.34 ± 51.4	423.03 ± 47.5	**<.001**
Total Thiol	426.99 ± 53.48	455.43 ± 45.23	**.004**
Disulphide	19.32 ± 8.73	16.2 ± 6.17	**.04**
Disulphide/Native Thiol	5.09 ± 2.47	3.93 ± 1.68	**.005**
Disulphide/Total Thiol	4.53 ± 2.04	3.6 ± 1.44	**.005**
Native Thiol/Total Thiol	90.93 ± 4.08	92.79 ± 2.88	**.005**

All parameters were expressed as mean ± standard deviation; p<0.05 value was accepted as significant level and the significant differences between the groups were shown in bold; NS: not significant; N: Number of patients

Table 3. Association between oxidative stress and pure tone audiogram levels in normal hearing (<25 dB) and hearing loss (>25 dB)

	<25 dB, N=39	>25 dB, N=26	P value
Native Thiol	393.8 ± 52.28	380.14 ± 50.09	NS
Total Thiol	432.62 ± 54.34	418.53 ± 52.06	NS
Disulphide	19.41 ± 8.42	19.19 ± 9.34	NS
Disulphide/Native Thiol	5.03 ± 2.37	5.18 ± 2.65	NS
Disulphide/Total Thiol	4.49 ± 1.94	4.59 ± 2.21	NS
Native Thiol/Total Thiol	91.01 ± 3.89	90.81 ± 4.43	NS

All parameters were expressed as mean ± standard deviation; p<0.05 value was accepted as significant level and the significant differences between the groups were shown in bold; NS: not significant; N: Number of patients

Table 4. Predictive value of Native Thiol, Disulphide and Disulphide/Native Thiol with binary logistic regression analysis

	B	Wald	P	Exp(B)	95% CI for Exp(B)	
					Lower	Upper
Native Thiol	0.22	5.139	0.023	1.023	1.003	1.043
Disulphide	-0.219	1.658	0.198	0.804	0.576	1.121
Disulphide/Native Thiol	0.712	1.137	0.286	2.039	0.550	7.550
			$R^2 = 0.264$			

p<0.05 value was accepted as significant level

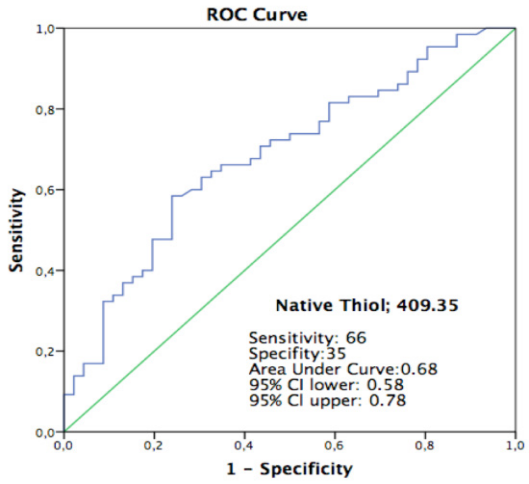

Figure 2. The receiver operating characteristic (ROC) curve of native thiol for the prediction of acute SIT

Binary logistic regression analysis was performed in order to find out the predictive values. In paired comparisons, NT variable revealed a statistically significant difference whereas Ds, Ds/NT variables did not reveal. Finally, the created model was explained 26.4% of the variation (Table 4).

Last but not least, the cut-off value of NT concentrations was determined by ROC analysis to predict acute SIT. The cut-off value of native thiol concentrations on admission to predict an acute SIT in all population was 409.35, with a sensitivity of 66% and a specificity of 35% (area under the curve 0.68, p < 0.001) (Figure 2).

DISCUSSION

OS is defined as an unbalanced situation between the antioxidant defense and oxidant production in the cells (13). Several response systems that provides a fast and sensible response to the alteration of oxidant levels have been described to prevent oxidative damage. ROS targets the protein thiols in vivo, and oxidative conversion of thiols into disulphides followed by their reduction mediates thiol-disulphide exchange. Indeed, intracellular redox homeostasis may be primarily ensured by thiol-disulphide pool, and these mechanisms are central for the arrangement of the antioxidant defense and regulation of redox signaling in the cell (14).

In many diseases, OS is considered to be accounted for the cell and tissue damage. The plasma Ds levels were reported higher in several degenerative diseases such as obesity, smoking, diabetes and pneumonia and lower in proliferative diseases including colon cancer, renal cancer, bladder cancer and multiple myeloma (2). Vittorio et al. (15) investigated the effects of OS in the etiology of Meniere disease and demonstrated that systemic OS affected the patients with Meniere disease. They also stated that weak endogenous antioxidant defenses might cause a neural damage in Meniere disease (15). Sergio et al. (10) searched for the role of OS in the etiology of idiopathic tinnitus and demonstrated an increased level of plasma oxidants in cerebral venous reflux flow, indicating the free radicals may be central in this disorder.

We investigated the plasma Ds/NT ratio as markers of OS which may have a key role in the etiology of tinnitus. Study group analyses revealed that plasma NT, TT, Ds levels and Ds/NT, Ds/TT, NT/TT ratios were significantly higher compared to healthy group. This result can be interpreted as an evidence of the role of OS in the etiology of tinnitus. On the other hand, there was no statistically significant difference in terms of thiol disulphide balance between patients with hearing loss and without hearing loss among acute SIT patients. The small sample of patients without hearing loss might have hampered the study's outcomes. Current study and other several studies investigating the OS markers in the etiology of tinnitus clearly indicates the role of OS in the etiology of tinnitus but in the literature, only limited number of trials demonstrated the effectiveness of antioxidants in the treatment of tinnitus. As the role of OS in the etiology of tinnitus will be better acknowledged, the role of the antioxidants in the treatment may become more significant, thus more studies are necessary in this regard.

CONCLUSION

We believe that OS as pointed in our research by Ds/NT ratios as OS markers may be central in the etiology of tinnitus.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: This study was approved by the Institutional Ethics Committee and conducted in compliance with the ethical principles according to the Declaration of Helsinki.

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