International Journal of Pediatric Otorhinolaryngology 112 (2018) 188–192

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Decreased disulphide/thiol ratio in patients with autosomal recessive non-syndromic hearing loss

Burhan Balta[a,*], Ramazan Gundogdu[b], Murat Erdogan[a], Murat Alisik[c], Aslihan Kiraz[a], Ibrahim Ozcan[b], Ozcan Erel[c]

[a] Department of Medical Genetics, Kayseri Training and Research Hospital, Kayseri, Turkey
[b] Department of Otorhinolaryngology, Kayseri Training and Research Hospital, Kayseri, Turkey
[c] Department of Biochemistry, Yıldırım Beyazıd University, Ankara, Turkey

ARTICLE INFO

Keywords:
Hearing loss
Autosomal recessive non-syndromic hearing loss
Thiol
Disulphide
Oxidative stress

ABSTRACT

Introduction: Oxidative stress plays a key role in the formation of age-related, noise-induced and drug-induced hearing loss. Thiols are organic compounds which can react with free radicals to protect against tissue and cell damage caused by reactive oxygen. There are no studies in literature on the association between autosomal recessive non-syndromic hearing loss(ARNSHL) including GJB2 and non-GJB2 mutations and thiol-disulphide balance. In this study, we aim to assess whether thiol-disulphide balance is disrupted in patients with ARNSHL.
Methods: Thirty-one ARNSHL patients and thirty-one healthy controls were included in this study. Patients whose parents were first degree cousins and who had at least two congenital hearing loss in the same family were included in the study. Audiological tests included air - bone pure tone audiometry and auditory brain stem response. GJB2 gene analysis was performed using sanger sequence method. Tests of thiol/disulphide homeostasis were conducted using the automated spectrophotometric method. We first investigated whether there was a significant difference between ARNSHL patients and healthy controls. Then, in order to determine the differential effect of the GJB2 gene mutations and non-GJB2 gene mutations on the thiol-disulphide balance, subjects were divided into three groups: Group 1 included patients with GJB2 mutations; Group 2 included patients with non-GJB2 mutations; Group 3 included healthy subjects.
Results: Patients with ARNSHL had significantly higher native thiol (411.6 ± 54.3 μmol/l vs. 368.0 ± 64.3 μmol/l, p = 0.006), total thiol levels (440.3 ± 56.2 μmol/l vs. 402.4 ± 65.9 μmol/l, p = 0.018), and lower disulphide levels (14.3 ± 5.7 μmol/l) vs. (17.1 ± 4.9 μmol/l), (p = 0.043) compared to the control group. Moreover, disulphide /native thiol ($p < 0.001$) and disulphide/total thiol ($p < 0.001$) were also detected lower in the ARNSHL group compared to the control group. Thiol-disulphide hemostasis parameters between all three groups showed that the native thiol and total thiol were increased in the Group 1 and Group 2. The disulphide levels decreased in Group 1 and 2, although not statistically significant.
Conclusion: It was shown that thiol levels increased and disulphide levels decreased in patients with autosomal recessive non-syndromic hearing loss. It also may suggest that there is a reverse association between ARNSHL and oxidative stress. Further studies are needed on whether or not ARNSHL cause oxidative stress limited to the inner ear and cochlea.

1. Introduction

Hearing loss is one of the important health problems worldwide, and 1–3 out of each 1000 newborns are affected by this condition. It is estimated to affect approximately 600 million people in the world [1], with adverse effects on the quality of life. More than half of the cases occur in the congenital or pre-lingual period. Of these patients, 77% show autosomal recessive inheritance, and they typically develop pre-lingual hearing loss [2]. Autosomal dominant inheritance accounts for 22% of hearing loss cases, generally post-lingual hearing loss [2]. X-dependent and mitochondrial inheritance have also been reported [3,4].

Sensorineural hearing loss has a multifactorial etiology [5]. Genetic, and environmental factors such as exposure to loud noise, ototoxic

* Corresponding author.
E-mail addresses: burhan0629@gmail.com (B. Balta), gundogduramazan@yahoo.com (R. Gundogdu), drmerdogan40@gmail.com (M. Erdogan), muratalisik@gmail.com (M. Alisik), aslihankiraz@yahoo.com (A. Kiraz), eahkayseri@gmail.com (I. Ozcan), erelozcan@gmail.com (O. Erel).

https://doi.org/10.1016/j.ijporl.2018.07.014
Received 12 May 2018; Received in revised form 5 July 2018; Accepted 9 July 2018
Available online 10 July 2018

substances, and aging play a role in this case [6]. The primary reason behind age-related hearing loss is that the auditory cells that convert mechanical transmission in the inner ear into electrical signals lose their functional ability with the degeneration of the organ of Corti. Furthermore, exposure to loud noise damages the hair cells and causes hearing loss.

The cochlea is in the inner ear and is responsible for the mechanical transmission of sound. Interconnected cells in the scala media of the cochlea are indispensable for hearing. Mutations in the genes that influence the development, structure, and function of the cochlea cause hearing loss. Tight junctions, adherent junctions, gap junctions, and connexin play a key role in hearing. Mutations in *GJB1, GJB2, GJB3 GJB6*, and *GJA1* have been reported to cause non-syndromic hearing loss [5,7]. The organ of Corti consists of two types of hair cells, outer and inner. These cells are innerved by spiral ganglion neurons [8]. Irreversible loss of hair cells and/or neurons or failure in their functioning causes sensorial hearing loss.

Pathogenic mutations in the *GJB2* is caused DFNB1 that is characterized by congenital non-progressive mild-to-profound sensorineural hearing loss. DFNB1 is an autosomal recessive inherited disorder and account for almost 50% of congenital severe to profound autosomal recessive non-syndromic hearing loss in United States [9].

Cells are exposed to reactive oxygen radicals during the oxidative process. The antioxidant system neutralizes these oxidants and protects the cells [10]. Thiols are organic compounds that can react with free radicals to protect against tissue and cell damage caused by reactive oxygen products and they contain the sulfhydryl (-SH) group. Thiol groups are oxidized by oxidizing molecules and the resultant disulphide bonds are reduced to thiol groups, so the thiol-disulphide balance is maintained. The loss of thiol groups in proteins is one of the main molecular mechanisms that cause structural and functional changes in proteins. The thiol-disulphide ratio has a critical significance for detoxification, signal transmission, regulation of enzymatic activities, apoptosis, and cellular signal transmission [11,12]. When the thiol-disulphide balance is disrupted by oxidative stress, the aforementioned cell activities are also disrupted.

The formation of reactive oxygen radicals in the inner ear has been found to play a key role in the development of age-related, noise-induced and drug-induced hearing loss [13]. In addition, increased oxidative stress and inflammation have been found to play important roles in hearing loss induced by ototoxic agents [14–16]. The chronic accumulation of reactive oxygen radicals disrupts the cochlear antioxidant system (superoxide dismutase, catalase, glutathione reductase, and peroxidase). Highly oxidized proteins lose their biological functions. Proteins with a high amount of sulfur-containing amino acids, cysteine, and methionine are the ones most sensitive to oxidation through reactive oxygen radicals [17]. While oxidized cysteine can be converted in enzymatic and non-enzymatic ways, oxidized methionine requires the action of methionine sulfoxide reductase (MSR), MSRA, and MSRB family. In a study conducted in 2011, mutations in the *MSRB3* gene were found to cause autosomal recessive hearing loss [18]. On the other hand, antioxidants reduce oxidative stress and decrease the risk of developing hearing loss [19–21].

To the best of our knowledge, no study has explored the association between autosomal recessive non-syndromic hearing loss (ARNSHL) including *GJB2* and non-GJB2 mutations and the thiol-disulphide balance. In this study, we aim to determine whether the thiol-disulphide balance is disrupted in patients with ARNSHL.

2. Materials and methods

2.1. Study population

Thirty-one ARNSHL patients and thirty-one healthy controls were included in this study. The study was performed between July 2016 and December 2016 at the Kayseri Training and Research Hospital Medical Genetics Clinic and the Yıldırım Beyazıd University Clinical Biochemistry laboratory. The study was approved by the Local Ethics Research Committee of Erciyes University with protocol number 2018/170 and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided written informed consent prior to participation in the study.

The patients with ARNSHL were recruited from the Department of Otorhinolaryngology of the Kayseri Training and Research Hospital. Patients whose parents were first degree cousins and who had at least two congenital hearing loss in the same family were included in the study. Thirty-one healthy volunteers had no history of chronic illness, regular drug use, or other health problems. Patients who were active smokers; had an infectious disease, cardiovascular disease, and/or other chronic diseases; and took vitamin supplements were excluded from the study.

We first investigated whether there was a significant difference between ARNSHL patients and healthy controls. *GJB2* gene analysis was performed using sanger sequence method. Then, in order to determine the differential effect of the GJB2 gene mutations and non-GJB2 gene mutations on the thiol-disulphide balance, subjects were divided into three groups: Group 1 included patients with GJB2 mutations; Group 2 included patients with non-GJB2 mutations; Group 3 included healthy subjects.

2.2. Thiol-disulphide homeostasis parameters measurement

In order to measure the thiol-disulphide homeostasis parameters, blood samples were taken from the subjects. Then, the blood samples were centrifuged at 1500 rpm for 10 min, plasma and serum samples were separated, and serum samples were stored at −80 °C until they were needed for study. Tests of thiol/disulphide homeostasis were conducted using the automated spectrophotometric method described by Erel and Neselioglu [22]. Briefly, disulphide bonds were first reduced by sodium borohydride to form free functional thiol groups. Unused reductant sodium borohydride was swept with formaldehyde to prevent reduction of DTNB (5,5′-dithiobis-(2-nitrobenzoic) acid), and all of the thiol groups, including the reduced and native thiol groups were determined using DTNB. Half of the difference between the total thiols and native thiols provides the dynamic disulphide amount. After determining the native and total thiols, the disulphide amounts, disulphide/total thiol percent ratios (SS/SH + SS), disulphide/native thiol percent ratios (SS/SH) and native thiol/total thiol percent ratios (SH/SH + SS) were calculated.

2.3. Sanger sequence analysis

Genomic DNA was extracted from peripheral blood samples using the DNA isolation kit according to the manufacturer's instructions (Zinexts Life Science Corp., Taiwan). All exons and exon-intron junctions of the *GJB2* (NM_004004) were sequenced using appropriate primers by using the Sanger sequence method. PCR conditions were as follows: Initial denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 30 s and 58 °C for 45 s; 72 °C for 1 min; and a final extension at 72 °C for 5 min. The PCR products were observed with 2% agarose gel electrophoresis. PCR products with enzyme transition were purified using the Exo-SAP kit (Exo SAP PCR purification kit, UAB Corporation, Cleveland, Ohio, USA). Cycle sequence was amplified using Big Dye Terminator, and extension products were purified using the Sephadex. The product was sequenced in both strands initiating from the forward and the reverse primers used in the initial PCR and analyzed on an ABI 3500 Genetic Analyzer (Applied Biosystems, Hitachi, Japan). Bioinformatic analysis was conducted using the SeqScape v2.6 program.