DNA Damages in Patients with Congenital Hearing Loss

ozge Caglar[1], Hayal Cobanoglu[2], Atilla Uslu[3], and Akin Cayir[2]

[1]18 March University Medical School
[2]Canakkale Onsekiz Mart Universitesi
[3]Istanbul Universitesi

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Abstract

Objective We aimed to investigate DNA damage level and new potential biomarkers that can assist the diagnosis and treatment of congenital hearing loss. Design A prospective, non-randomized study. Setting Canakkale Onsekiz Mart University, Canakkale, Turkey Participants We included a patient group consisting of 17 patients with congenital hearing loss and a control group consisting of 17 healthy individuals. Main outcome measures We applied the brainstem-evoked response audiometry (BERA) tests to determine the hearing loss. After taking blood samples, we applied cytokinesis-block micronucleus cytome (CBMN) assay. Methods After the demographic characteristics, family stories and Brainstem Evoked Response Audiometry results of both groups were obtained, their blood was taken. The cytokinesis-blocked micronucleus assay technique was applied to the blood samples to measure the frequency of micronucleus, nucleoplasmic bridge, and nuclear bud in both groups. Results We observed that the micronucleus, nucleoplasmic bridge, and nuclear bud frequencies were found to be significantly higher in hearing loss patients than the control group ($p<0.0001$). Also, we observed that the frequency of micronucleus in hearing loss patient was positively correlated with nuclear bud, which may indicate a common mechanism for these endpoints. Conclusion It was, for the first time, demonstrated that micronucleation, nucleoplasmic bridge, and nuclear bud formation were found to be higher, which is an indication of genomic instability in patients with congenital hearing loss. Since the markers we evaluated were linked with crucial diseases, our findings might suggest that patients are susceptible to many crucial diseases, including cancer.

Key points

- Congenital hearing loss patients have more DNA damages than the control,
- Higher frequencies of micronucleus which is an indication of different types of chromosomal anomalies indicated susceptibility of congenital hearing loss patients,
- Higher nucleoplasmic bridge frequencies indicated the distinct anomalies such as telomere end-fusion or dicentric chromosomes,
- Higher nuclear bud frequencies indicated higher gene amplification rate,
- MN, NPB, and NBUD frequencies might be biomarker of congenital hearing loss patients

Keywords : Sensorineural hearing loss, micronucleus, nucleoplasmic bridge, nuclear bud, DNA damage

1 INTRODUCTION

Sensorineural hearing loss (SNHL) is the most common disorder of the sensory system. It is estimated that 1-3 births per 1000 are diagnosed with a severe hearing loss. Approximately 50% of the cases with prelingual onset of hearing loss have a genetic basis as their medical history informs (1). Hereditary hearing loss includes almost all the categories of hearing impairments, including conduction, sensory, and neural type of hearing loss; syndromic and nonsyndromic hearing loss; congenital, progressive, and adult-onset types; high frequency, low frequency or mixed frequency hearing loss; light or deep hearing loss; and recessive,

dominant or gender-dependent types. Advances in genomics resulted in the identification of more than 6,000 variants in more than 150 genes causing hearing loss. The identification of genes causing hearing impairment provides more insight into the normal development and function of the cells of the auditory system. These defective genes are promising in becoming the major therapeutic targets in the future. However, the hearing system is still too complex for us to understand completely and our insight into this system still awaits improvement (2). The mutation in the gap junction beta-2 protein (GJB2) gene is the most common cause of genetic hearing loss (3). The GJB2 gene encodes connexin 26 (Cx26), a transmembrane protein involved in the functioning of the inner ear by forming pores to allow potassium transport. A mutation in the SLC26A4 gene is the second most common mutation, and it leads to Pendred syndrome (PS), which is manifested by enlarged vestibular aqueduct syndrome (EVAS). SLC26A4 mutations account for about 5-7% of the hereditary deafness cases. The mtDNA1494C> G mutation is common in 0.45% of the children with impaired hearing. The most common genetic cause of SNHL is congenital cytomegalovirus (CMV) infection. Nance et al. (3) reported that CMV infections accounted for approximately 21% of the congenital SNHL cases and 25% of the late-onset SNHL cases.

Biomarkers are cellular, biochemical or molecular alterations that can easily be measured in human tissues, cells or fluid (4). Biomarkers have the potential to extend knowledge regarding the underlying mechanism of diseases and also to identify individuals susceptible to disease (5). In this context, we aimed to search for new potential biomarkers for congenital hearing loss to assist in diagnosis and treatment and to evaluate the genetic damages of such individuals for the disease's susceptibility. Since we evaluated the DNA damages associated with different diseases including cancer, obtaining knowledge will help to determine individuals who will be susceptible to diseases. Thus, we aimed to measure different DNA damages measured by the cytokinesis-blocked micronucleus assay (CMBN) which was a comprehensive method to enable us to evaluate different markers. We measured the micronucleus (MN), nucleoplasmic bridges (NPB), and nuclear buds (NBUD) frequencies of healthy individuals and patients with congenital hearing loss.

MATERIALS & METHODS

Studied population

The study design is presented in figure 1. This study was conducted in conformity with the Helsinki Declaration and the ethical permission was granted. We included 17 individuals with congenital hearing loss from the School for the Deaf in Turkey. In the brainstem-evoked response audiometry (BERA) tests, all of them had a severe hearing loss. Mental controls were normal in all individuals. Sixty percent of the individuals had congenital hearing loss in their siblings. Two had a history of infectious. The participants had no additional disease. The patients were previously diagnosed. All the patients were male. The participants without hearing loss were selected in the same area with a similar age range and a similar number with the population with hearing loss. In total, 24 individuals including 13 males and 11 females were included to measure the frequencies of DNA damage markers. Both patients with hearing loss and without hearing loss stated that they had voluntarily participated in the study.

Cytokinesis-block micronucleus cytome (CBMN) assay

The CBMN assay was applied to the blood samples to determine the frequency of MN, NPB, and NBUD in both groups (Figure 2). The method was carried out as described by Fenech (6) and routinely used by our group (7, 8). Five ml of the blood sample was taken from each donor in a sterile heparinized tube for all the participants. 0.5 mL blood samples from 5 ml were cultured in a cell culture medium containing RPMI 1640 (4 mL), fetal calf serum (1 mL) and phytohemagglutinin (0.2 mL). Cytochalasin B (6 mg/mL) was added to each culture to block cytokinesis 44 h after phytohemagglutinin stimulation. Lymphocyte culture was maintained 72 hours at 37°C in a humidified condition containing 5% CO_2. We harvested the lymphocyte culture at the end of the 72h. In this stage, the cells were treated with a cold hypotonic solution (0.075 M KCl) and then fixed three times with cold methanol-acetic acid (7:1, v/v). Finally, the slides were stained with Giemsa (5%) in Sörenson buffer. The microscopic analysis of the slides was conducted according to Fenech's criteria with a light microscope at 1000 × magnification (9). In the study, 1000 binucleated cells

with well-preserved cytoplasm were evaluated for each individual in both groups. Thus, MN individual.

In the current study, reagents were purchased from the following suppliers: culture medium (RPMI 1640) and cytochalasin-B from Sigma (Germany), phytohaemagglutinin (PHA) from Biological Industries (Israel), Giemsa, methanol, glacial acetic acid, potassium chloride from Merck (Germany).

Statistical Analysis

We compared MN, BNMN, NPB, and NBUD frequencies for both groups by applying the *Mann-Whitney U* test. The correlation between MN, BNMN, NPB, and NBUD frequencies was conducted by applying the Spearmen Rho's test. IBM SPSS Statistics 19.0 and Prism-GraphPad were used for statistical analysis and for drawing the graphs.

RESULTS

The descriptive statistics of the patients with congenital hearing loss and without hearing loss are presented in Table 1. The mean age of the patients was 18.1. One of the patients was a smoker. All the individuals in the patient's group are non-alcoholic and all are the same gender. The mean age of the healthy individuals was 21.5 years. Four out of 24 individuals were a smoker.

We evaluated MN frequencies as two parameters: MNL is the frequencies of the total number of micronuclei in the lymphocytes. BNMN is the frequencies of the total number of bi-nucleated cells with micronuclei (BNMN). The MNL frequency of both groups is presented in Figure 3. We observed that the MNL frequencies of the patients were consistently significantly higher than those observed in the healthy individuals (p <0.0001, *Mann-Whitney U*). The BNMN frequencies of both groups are presented in Figure 3. The mean frequencies of the patients with hearing loss were higher than the healthy individuals. According to the results of *Mann Whitney U* , we observed statistically significant differences between the patients with hearing loss and the healthy individuals (p <0.0001,*Mann-Whitney U*). The NPB frequencies of both groups are presented in Figure 3. The NPB frequency of the hearing loss was in the range of 0-5, which is a large range compared with the control group. The mean frequencies of the patients with hearing loss were significantly higher than the control group (p =0.0001, *Mann-Whitney U*). The NBUD frequencies of both groups are presented in Figure 3. Like NPB, NBUD frequencies showed a wide range of distribution in hearing loss compared with the control. It was observed that the mean of the patients with hearing loss significantly higher than the control group (p <0.0001, *Mann-Whitney U*).

In the group with hearing loss, we observed that the MNL frequency was positively correlated with the NBUD frequencies (Table 2). Similarly, we found a positive correlation between the BNMN and NBUD frequencies. We did not observe any other correlation between other parameters.

Since the patients with hearing loss consisted of only males, we compared the results in terms of gender with the control groups (Figure 4). We found that there were statistically significant differences between the male patients with hearing loss and the males in the control group in terms of MNL, BNM, NPB, and NBUD frequencies (p <0.0001, *Mann-Whitney U*). Similarly, we found similar results between the male patients with hearing loss and the females in the control group (p <0.0001,*Mann-Whitney U*).

DISCUSSION

In the present study, we investigated genetic damage measured by means of the comprehensive CBMN method in patients with congenital hearing loss and healthy individuals without hearing loss. We observed that the MNL, BNMN, NPB, and NBUD frequencies of the patients with hearing loss were significantly higher than the matched healthy group. Concordantly, we found that MN and NBUD were significantly correlated in the group with hearing loss. Together, our findings, for the first time, indicated that congenital hearing loss in younger patients had significantly higher DNA damages.

Micronucleus are defined as structures originating from complete chromosomes or acentric chromosome fragments that are not involved in the core nucleus during the mitosis of the cell (10). Therefore, after MN formation, cells may gain or lose the DNA sequence (11), which may be associated with diseases. Until now,

it has been known that the CBMN assay end-points including MN, NPB, and NBUD have been evaluated as a risk prediction of diseases. As a general consequence, micronucleus formation has been associated with a wide range of disorders and/or diseases including fertility, diabetes, obesity, and cardiovascular diseases, chronic kidney diseases, neurodegenerative diseases (11). Furthermore, the increase of MN frequencies has been associated with cancer including cervical cancer, bladder cancer, oral, head and neck cancer, and lung cancer (11). NPB occurs during the anaphase phase of mitosis during the withdrawal of the dicentric chromosome centromeres on the opposite poles which originate from the false repairing of chromosome fractures or from the fusion of telomeres (6). NBUD has been associated with chromosomal instability and observed in cultures with increased gene amplification (moderate folic acid deficiency) grown under very selective conditions (6). NPB and NBUD have also been associated with diseases. It has been reported that NPB and NBUD frequencies in different types of cells were higher than the controls in patients with urothelial cell carcinoma (12), in neurological diseases (13, 14).

As a general approach from previous studies, it was reported that there was an association between congenital anomalies and cancer in children. It was reported that children with genomic instability had a predisposition to both cancer and congenital anomalies. In a study, the association between childhood cancer and congenital anomalies was proved in children with cancer (Congenital Anomalies and Childhood Cancer in Great Britain). In a case-control study, children with malformation were found to have a fourfold risk of cancer (15-17). In our study, we found that the young patients with hearing loss had significantly higher MN, NPB, and NBUD frequencies. From this point of view, we could suggest that significant increased MN, NPB, and NBUD frequencies might be an indication of the susceptibility of patients with hearing loss to crucial diseases. Therefore, MN frequencies in patients with hearing loss may be a useful biomarker to predict the disease which may develop in the future in patients with hearing loss.

We found a positive correlation between the MN and NBUD formation in the patients. A similar correlation was observed in previous studies (7, 8). We could suggest that there might be a common mechanism for the formation of MN and NBUD in the patients with hearing loss. For example, DNA lagging in the cytoplasm can be entrapped by a nuclear membrane, or excess DNA which may be a result of gene amplification can form NBUD extruded from the nucleus to form MN (18)

We acknowledge a few limitations of our study. The number of individuals in populations is low since we could not add more patients with congenital hearing loss. In addition, we could not find female patients with congenital hearing loss to evaluate the two gender in terms of gender.

CONCLUSIONS

In summary, our study has for the first time revealed that micronucleation, nucleoplasmic bridge, and nuclear bud formation are crucial genomic alterations which might be the indications of genomic instability in patients with congenital hearing loss. Our findings indicate the susceptibility of the patients to several important diseases including cancer. Furthermore, MN, NPB, and NBUD frequencies might be used to predict several crucial diseases for the further development of the disease in patients with congenital hearing loss. Further work is required in large population studies to validate our observations.

Financial and competing interest disclosure

The authors report no conflicts of interest.

Availability of data

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table 1. Characteristics of patients with congenital hearing loss and healthy individuals.

	Healthy Individuals	Healthy Individuals	Patients with Congenital Hearing Loss	Patients with Cong
	n (%)	Mean ± SD	n (%)	Mean ± SD

	Healthy Individuals	Healthy Individuals	Patients with Congenital Hearing Loss	Patients with Cong
Age (years)				
Total	24	21.5 ± 1.84	17	18.1 ± 1.58
Male	13 (54)	23 ± 0.00	17 (100)	18.1 ± 1.58
Female	11 (46)	20 ± 1.50	0 (0)	-
Smoking Habits				
Smokers	4 (17)	-	1 (6)	-
Non-smokers	20 (83)	-	16 (94)	-

SD: Standard deviation

Table 2. Correlation coefficients obtained for genetic endpoints in patients with congenital hearing loss.

	MNL	BNMN	NPB	NBUD
MNL	1.00			
BNMN	0.99**	1.00		
NPB	0.24	0.29	1.00	
NBUD	0.62**	0.58*	0.24	1.00

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

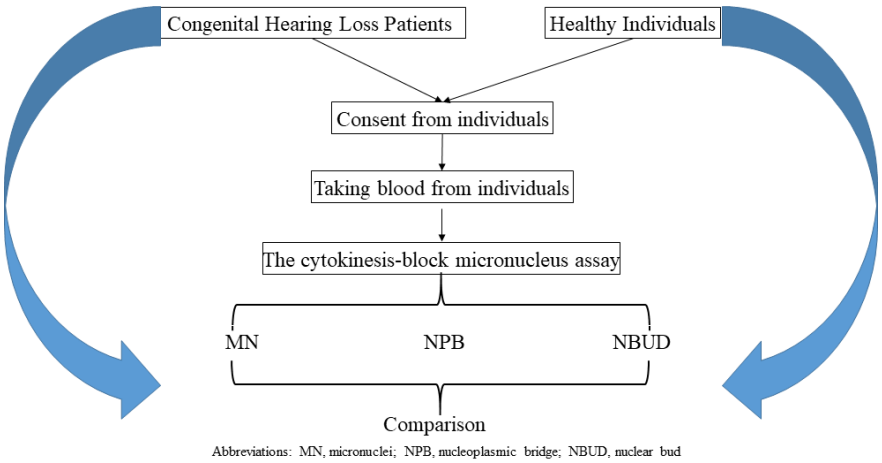
Figure captions

Figure 1. Flowchart of the study design

Figure 2. Cells with DNA damages evaluated in CBMN assay. a) Binucleated cell with nuclear bud, b) Binucleated cell with micronucleus, c) Binucleated cell with nucleoplasmic bridge

Figure 3. **A:** MNL frequency, **B:** BNMN frequency,**C:** NPB frequency, **D:** NBUD frequency of patients with congenital hearing loss and healthy individuals.

Figure 4. **A:** MNL frequency in terms of gender, **B:** BNMN frequency in terms of gender, **C:** NPB frequency in terms of gender, **D:** NBUD frequency in terms of gender in patients with congenital hearing loss and healthy individuals.



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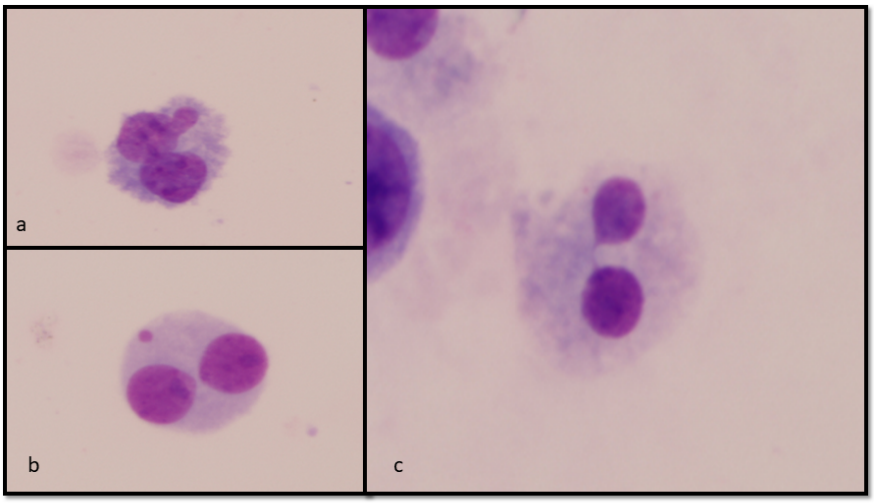

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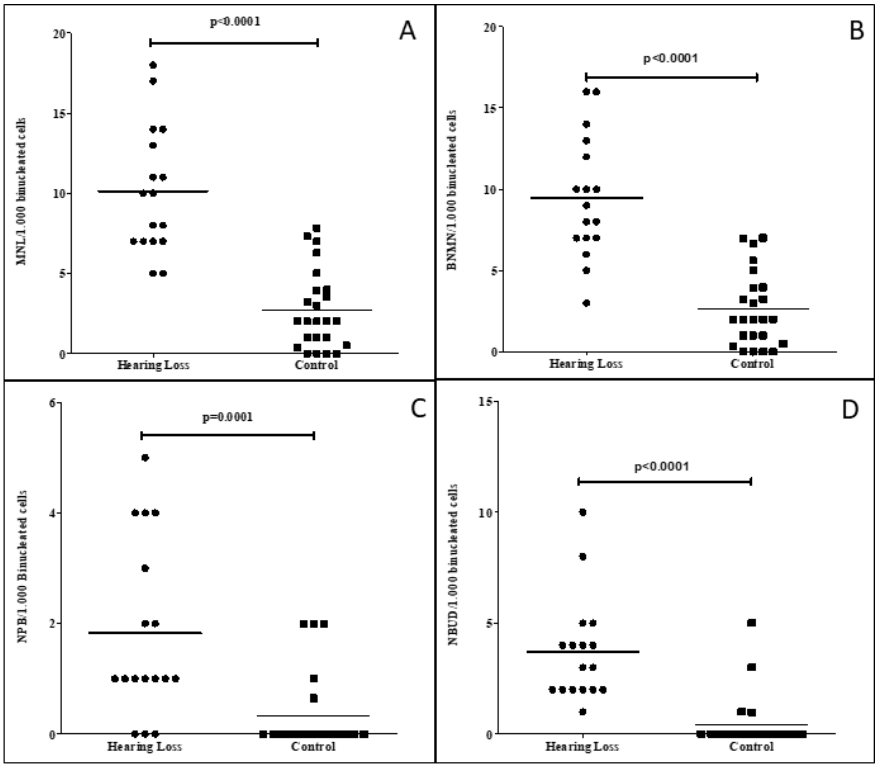

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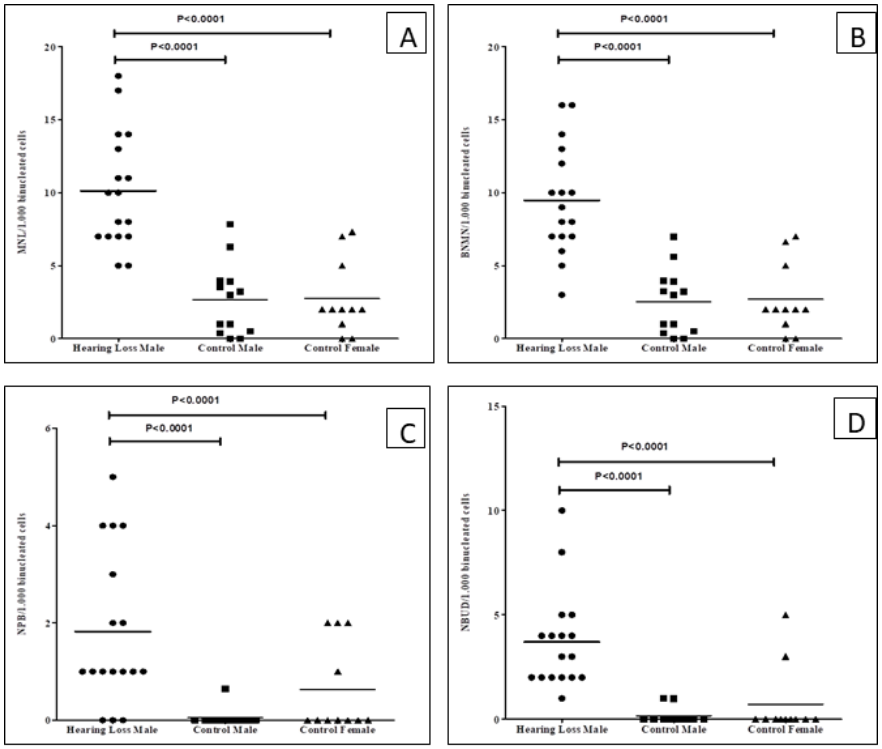

Figure 4. A: MNL frequency in terms of gender, **B:** BNMN frequency in terms of gender, **C:** NPB frequency in terms of gender, **D:** NBUD frequency in terms of gender in patients with congenital hearing loss and healthy individuals.