J Chin Med Assoc

ORIGINAL ARTICLE

Elevated matrix metalloproteinase-3 level may affect hearing function in patients with rheumatoid arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is an autoimmune disease. Sensorineural and conductive hearing loss have been reported in RA, but the results of most studies are not in agreement. The pathogenesis of the hearing loss is not clearly understood. The presence of sensorineural hearing loss was related to matrix metalloproteinase-3 (MMP-3). The aim of this study was to assess hearing loss in RA patients and to examine the correlation between plasma MMP-3 levels and hearing loss in such patients.

Methods: This is a cross-sectional and analytic research. Subjects consisted of 21 RA patients with hearing loss as a study group and 21 RA patients without hearing loss as controls. All patients were evaluated by pure tone audiometry and tympanometry. The amounts of plasma MMP-3 were determined using enzyme-linked immunosorbent assay. Pearson Chi-square test was used to determine the correlation of gender, age, disease duration, erythrocyte sedimentation rate, and platelet count of both groups. Independent *t*-test was used to assess equality of mean values at 250 to 8000 Hz hearing thresholds, pure tone mean values, air-bone gaps, and MMP-3 plasma levels of both groups.

Results: This study found sensorineural (76.2%), conductive (14.3%), and mixed (9.5%) hearing loss. The most common degree of hearing loss was mild (66.7%). There was an increased incidence of As-type tympanogram in the study group (28.6%) and control group (47.6%). There were significant differences between both groups in mean hearing thresholds ($p < 0.001$), mean of air conduction thresholds at 1000 to 8000 Hz ($p < 0.05$), and mean of bone conduction thresholds in all frequencies ($p < 0.05$). The significant difference of mean MMP-3 levels was also found between the groups ($p < 0.001$).

Conclusion: Hearing loss is a common finding in RA. MMP-3 plasma contributed to degrade the incudomalleolar and incudostapedial joints and could damage the inner ear hair cells due to oxidative process in RA.

Keywords: Audiometry; Enzyme-linked immunosorbent assay; Hearing loss; Rheumatoid arthritis

1. INTRODUCTION

Rheumatoid arthritis (RA) severely reduces patient's quality of life because it is difficult to treat and is associated with a high morbidity rate.[1] In addition to articular and periarticular manifestations, RA may attack extra-articular organs such as liver, lung, skin, and eyes.[2] Similarly, the auditory system may be affected by the pathology of the disease.[3] Incudomalleolar and incudostapedial are true diarthroses; thus, they may also be affected by RA, like any other synovial joints in the body.[2]

Hearing loss was found to be 51.4% in RA patients, whereas the percentage of hearing loss was found to be only 14.3% in the control group ($p < 0.001$).[4] The prevalence of hearing loss in RA patients varied. Sensorineural hearing loss (SNHL) was the most common type of hearing loss in RA patients at 25% to 72%.[2] The prevalence of conductive hearing loss (CHL) and mixed hearing loss (MHL) was reported to be less than SNHL.[5]

Slight bilateral high-frequency hearing loss can be found in RA patients although the pathogenesis is not clearly known. However, the involvement of ossicular joints, vasculitis, neuritis, and medication ototoxicity has been reported.[6] It is important to keep matrix metalloproteinases (MMPs) in normal condition and in destruction acceleration in various diseases such as RA. The results of a previous research found that MMPs played a role to damage inner ear cells by an oxidative process.[2,7] On the basis of the research, SNHL was correlated with plasma matrix metalloproteinase-3 (MMP-3; $p < 0.001$).[7] Thus, the aim of this study was to assess hearing loss in RA patients and to examine the correlation between plasma MMP-3 levels and hearing loss in such patients.

2. METHODS

2.1. Subjects

This is an analytic cross-sectional research, which was conducted from July 2015 to June 2016 at Adam Malik General Hospital, Indonesia. The subjects consisted of 21 RA patients with hearing loss (study group). They were compared to 21 RA patients without hearing loss (control group). The diagnosis of RA was based on the criteria set by the American Rheumatism

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Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2019) 82: 272-276.

Received September 9, 2017; accepted October 9, 2018.

doi: 10.1097/JCMA.0000000000000036.

Association.[8] The subjects aged 16–55 years; had no history of hearing loss, ear infections, ear trauma, etc., which affected hearing function; and had no other systemic disease included in the research. All subjects had normal tympanic membranes on otoscopic examination. This research obtained the approval from the Health Research Ethical Committee of our institution and informed consent from each subject.

2.2. Audiology examination

Pure-tone audiometry (PTA) and tympanometry were examined in both groups. The examination was performed by the same examiner. The PTA examinations were conducted with circumaural headphones in a sound-proof chamber using an AD-28 interacoustics clinical audiometer (Interacoustics, Assens, Denmark). The assessment was made at the frequencies of 250 to 8000 Hz for the air conduction (AC) thresholds, while the assessment at the frequencies of 250 to 4000 Hz was done for bone conduction (BC) thresholds using a bone vibrator placed on the appropriate mastoid process. The calculation found the hearing thresholds from the average of the hearing thresholds at 500, 1000, 2000, and 4000 Hz. Audiogram was classified according to normal (≤25 dBHL), mild (26-40 dBHL), moderate (41-60 dBHL), severe (61-80 dBHL), and very severe (≥81 dBHL) hearing loss.[9] Hearing loss was classified by SNHL when AC and BC was >25 dBHL, CHL when air-bone gap (ABG) obtained was ≥10 dBHL with at least two successive frequencies, or MHL when AC and BC were >25 dBHL with ABG ≥10 dBHL for at least two successive frequencies.[10] A subject was classified to suffer from hearing loss when one or both of his or her ears experienced hearing loss. To assess the difference in the mean of AC, BC, ABG, and hearing thresholds, the highest threshold value of both ears was taken.

The tympanometry examination was performed with an interacoustics AA222 impedance audiometer (Interacoustics) at a probe tone frequency of 226 Hz. The tympanogram was classified according to Jerger[11] as types A, As, Ad, B, and C.

2.3. Enzyme-linked immunosorbent assay

Blood plasma of the patients was collected using citrate anticoagulant. Particles were removed by centrifugation and refrigerated at 23°C. The amount of MMP-3 was examined with Quantikine® enzyme-linked immunosorbent assay kits (R&D System, Minneapolis, MN, USA). The absorbance at 450 nm was measured using a Chemwell® 2910 automated enzyme-linked immunosorbent assay and chemistry analyzer (Awareness Technology Inc., Palm City, FL, USA).

2.4. Statistics

The type and degree of hearing loss and the type of tympanogram were presented in descriptive statistics. The Pearson Chi-square test was used to determine the correlation of gender, age, disease duration, erythrocyte sedimentation rate, and platelet count of both groups. The independent t-test was used to assess equality of means at 250 to 8000 Hz PTA hearing thresholds, pure tone means, ABGs, and MMP-3 plasma levels of both groups. The Statistical package for the social sciences (SPSS) (SPSS Inc., Chicago, IL, USA) version 17.0 was used for statistical analysis. A p-value <0.05 was considered as statistically significant.

3. RESULTS

Table 1 shows the proportion of gender, age, disease duration, erythrocyte sedimentation rate, and platelet count of the subjects with the mean age of study group i.e. 35.38 ± 2.00 years and that of control group i.e. 35.19 ± 2.25 years. These patients were undergoing treatment with both steroidal and nonsteroidal anti-inflammatory agents. Nonsteroidal anti-inflammatory agents used were diclofenac sodium, ibuprofen, meloxicam, or acetaminophen. The corticosteroid agents were methylprednisolone or prednisone. Other drugs consumed were the disease-modifying antirheumatic drug group, i.e., methotrexate or chloroquine. These patients took either single or combination drugs.

PTA examinations were conducted to find out the hearing loss types and degrees of the study group. The mean of hearing thresholds was 38.39 ± 3.37 dBHL in the study group and 20.48 ± 0.72 dBHL in the control group. The independent t-test results showed that there was a significant difference in the hearing threshold of both groups (p < 0.001).

As shown in Fig. 1A, the most common type of hearing loss, SNHL, was found in 16 patients (76.2%), of which eight were bilateral and the other eight were unilateral. CHL was found

Table 1

Gender, age, disease duration, erythrocyte sedimentation rate, and platelet count of the subjects

		Study group, n (%)	Control group, n (%)	p
Gender	Male	5 (23.8)	5 (23.8)	1.000
	Female	16 (76.2)	16 (76.2)	
Age, y	≤20	1 (4.8)	1 (4.8)	1.000
	21-30	7 (33.3)	7 (33.3)	
	31-40	5 (23.8)	5 (23.8)	
	≥41	8 (38.1)	8 (38.1)	
Disease duration, y	≤5	14 (66.7)	17 (81.0)	0.236
	6-10	7 (33.3)	3 (14.3)	
	≥11	0	1 (4.8)	
Erythrocyte sedimentation rate, mm/h	Increased	19 (45.2)	10 (23.8)	0.003*
	Normal	2 (4.8)	11 (26.2)	
Platelet count, $\times 10^3/mm^3$	Thrombocytosis	17 (40.5)	21 (50)	0.110
	Thrombocytopenia	3 (7.1)	0	
	Normal	1 (2.4)	0	

Values are expressed as n (%). The p-values were tested using Pearson Chi-square test.
*Statistically significant.

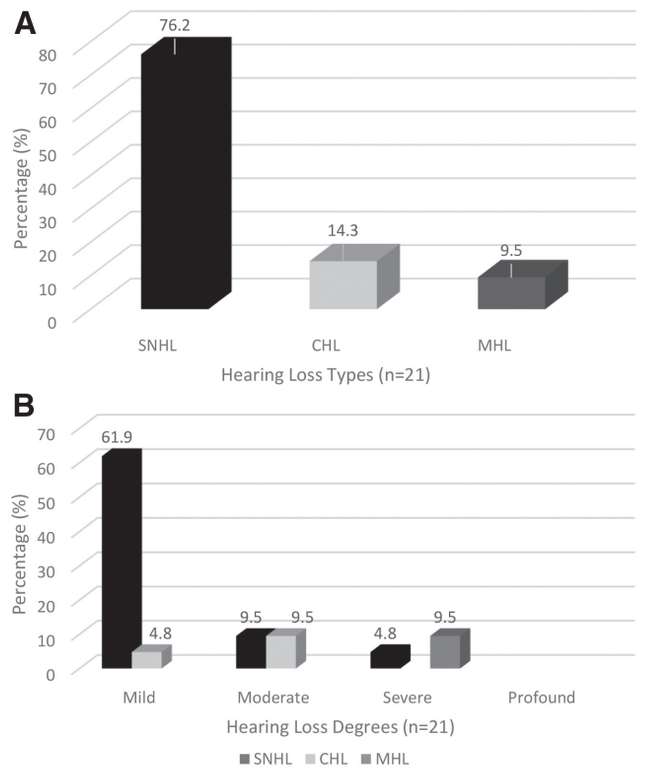

Fig. 1 Hearing loss (A) types and (B) degrees within the study group. Values are expressed as %. Abbreviations: CHL, conductive hearing loss; MHL, mixed hearing loss; SNHL, sensorineural hearing loss

in three patients (14.3%); one of them was bilateral, and the other two were unilateral. MHL was only found in two patients (9.5%), both of which were bilateral. As shown in Fig. 1B, the most common hearing loss degree within the study group was mild, which was found in 14 patients (66.7%) consisting of SNHL in 13 patients (61.9%) and CHL in one patient (4.8%). It was followed by severe and moderate hearing loss.

As shown in Fig. 2A, mean of AC thresholds increased in all frequencies in the study group. The independent *t*-test revealed a significant difference in the mean of AC thresholds at 1000 to 8000 Hz between both groups ($p < 0.05$). As shown in Fig. 2B, the mean of BC thresholds increased in all frequencies in the study group. The independent *t*-test revealed significant differences in the mean of BC thresholds between both groups in all frequencies ($p < 0.05$). As shown in Fig. 2C, the ABG value increased at 1000 to 4000 Hz in the study group. The Student's *t*-test revealed a significant difference in the mean of ABG value at 4000 Hz ($p < 0.05$).

As shown in Fig. 3, A-type tympanogram was the most common finding in either the study group with 15 patients (71.4%) or the control group with 10 patients (47.6%). It was followed by As-type tympanogram in six patients (28.6%) in the study group and 10 patients (47.6%) in the control group. There was only one patient with CHL of all patients with As-type tympanogram in the control group.

The highest and the lowest MMP-3 plasma levels in the study group were 9.47 and 2.06 ng/mL, respectively. The highest and the lowest MMP-3 plasma levels in the control group were 2.00 and 0.02 ng/mL, respectively. The mean MMP-3 plasma level in female and male RA patients with hearing loss were 3.77 and 6.69 ng/mL, respectively. The mean MMP-3 plasma level of the study group with the disease duration of ≤5 years was 4.15 ng/

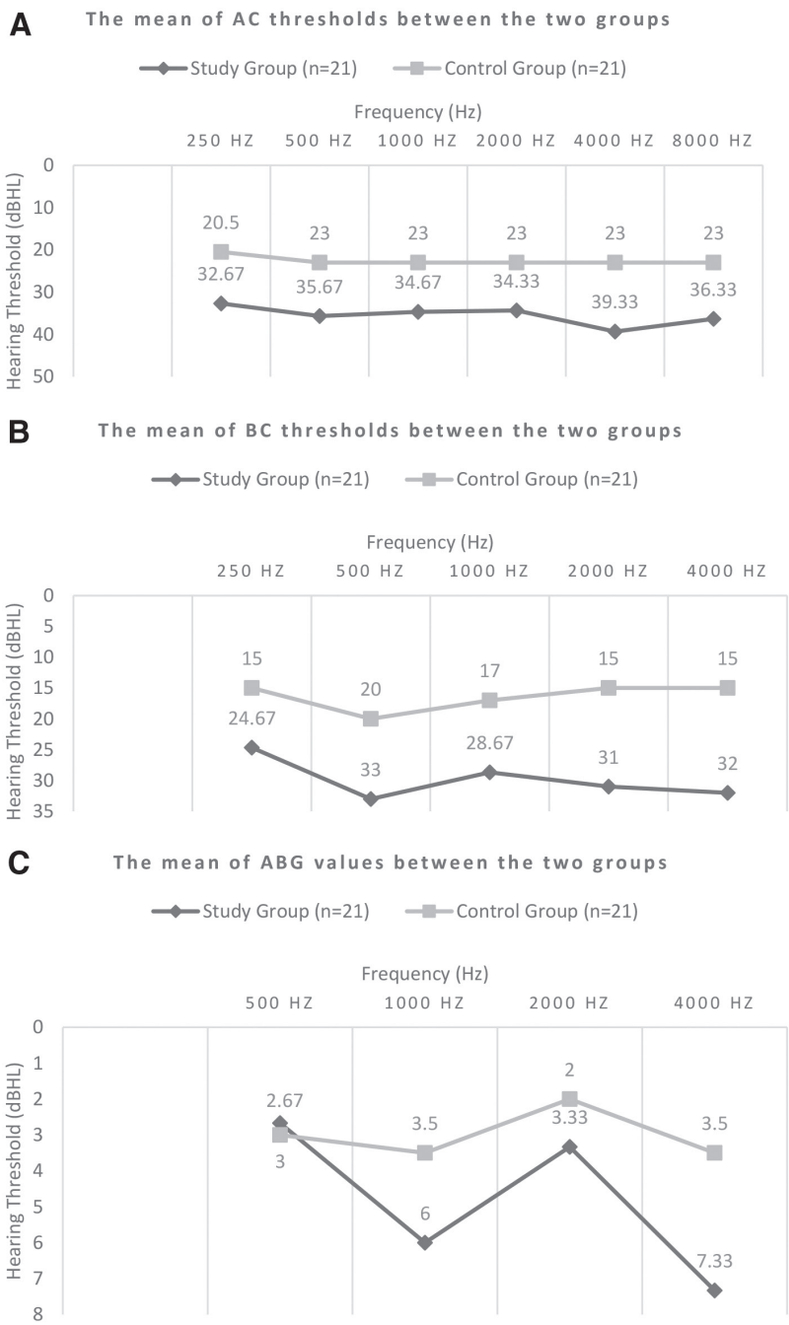

Fig. 2 The mean of (A) AC thresholds, (B) BC thresholds, and (C) ABG values of both groups. Values are expressed as dBHL. Abbreviations: ABG, air-bone gap; AC, air conduction; BC, bone conduction

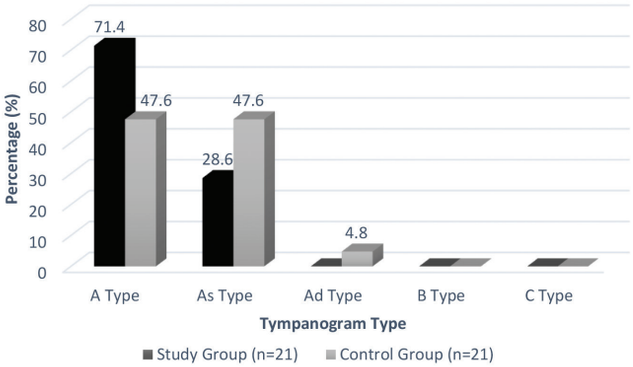

Fig. 3 The proportion of tympanogram types within the groups. Values are expressed as %.

Table 2

The difference in the mean of MMP-3 plasma level of both groups

	Study group (Mean ± SEM)	Control group (Mean ± SEM)	*p*
Plasma MMP-3 level	4.47 ± 0.50	1.08 ± 0.12	<0.001*

Values are expressed as ng/mL. The *p*-value was tested using independent t-test.
MMP-3 = matrix metalloproteinase-3.
*Statistically significant.

mL, while that of in the duration of disease >6 years was 5.09 ng/mL. As shown in Table 2, the difference in the mean of MMP-3 plasma levels in both groups was 3.38 ± 0.52 ng/mL. A significant difference in the mean of MMP-3 plasma levels was found between RA patients with hearing loss and RA patients without hearing loss ($p < 0.001$).

4. DISCUSSION

The prevalence of RA is known to be two to three times more common among women than among men and can start at any age, with a peak incidence between the fourth and fifth decades of life.[12] That the highest female predilection in RA patients that was found in the younger age group suggests that hormonal factors may play a role.[13] The research proves that the earlier the RA patient is treated, the better the prognosis will be. However, early diagnosis of RA is usually difficult to make, and there has been irreversible damage at the time of diagnosis.[14]

The high rate of SNHL found in this research was because RA caused much damage to the inner ear associated with vasculitis, neuritis, and medication ototoxicity.[6,7] Dikici et al.[6] stated that in patients with longer disease duration, both RA pathogenesis and the use of ototoxic medications could cause damage in the inner ear. Ototoxic medication is one of the causes of oxidative stress in cochlea. RA is one of the autoimmune diseases[1,6,7,12,15] that can produce a perceptive hearing loss.[6] The damage in the cochlea includes the damage to the organ of Corti, retrograde neural degeneration to the level of the spiral ganglion, endolymphatic hydrops, stria vascularis dystrophy, neo-fibroosteogenesis to the cochlear basal turn, as well as endolymphatic sac fibrosis and lymphocytes to the membrane compartment of the labyrinth.[16] The presence of autoantibodies counteracting the antigenic epitopes of the inner ear may cause SNHL. Hyperactivity of humoral and cellular immune systems against other autoantigens may lead to SNHL.[7] The autoimmunity of the inner ear usually affects patients in the third to sixth decades of life and produces progressive unilateral or bilateral SNHL within weeks to months.[16]

The result found on CHL corresponded to what had been reported in the literature i.e. 0% to 24.3%.[17] CHL in RA could be correlated with stiffness and discontinuity of the ossicles,[6,17] and stiffness of the ossicular joints in the middle ear.[2,6,18] Disk material dissolution along with synovial-type elements proliferation of the disk and articular surfaces, with pannus-like tissue formation, was observed in the ossicular joint of RA patients.[19] Reiter et al.[20] also expressed an indication of increased middle ear stiffness with or without decreased ligament stability. Inflammation in the active stage of the disease and the consequent fibrosis in the ossicular resulted in reduced elasticity and the emergence of CHL.[6]

The result found concerning MHL was consistent with the previous study i.e. up to 10.8%.[4] It showed a multifocal involvement of the auditory system in RA patients. In the beginning, SNHL occurred in RA patients.[2,4,5] In addition to this, the conductive component of the hearing loss was also evolving.[6] Due to the difference in the mean of ABG values, it was believed that the conductive component was developed and that it could develop along with the sensorineural component causing the MHL.

This research showed that hearing loss in RA patients could be found at middle and high frequencies. This result was consistent with several previous studies, which found that prevalence of the hearing loss in RA patients was found in all frequencies including low,[21] middle,[22] high,[5,23] and very high.[23] Colletti et al.[18] explained that when rheumatoid ossicular joint fixation occurred, inner ear protective mechanism was impaired and hair cells of the inner ear could be injured for being exposed to intrinsic and extrinsic traumas in the long term. This theory proposes that SNHL will occur at higher frequencies.[24]

The result found concerning the increased prevalence of As-type tympanogram was in accordance with other studies that also found subclinical CHL.[7] It was correlated with increased stiffness[4,7] of the middle ear ossicular system, but the damage was not large enough to be detected by the PTA. Ankyloses of the incudomalleolar and incudostapedial joints did not cause any change to the sound conduction in cochlea because they were functionally fixed during sound transmission.[6,7] This theory explained the normal hearing in spite of the increased stiffness in the middle ear. Another explanation was that the effect of RA on the ossicular joints ligaments and capsules increased laxity or decreased stiffness without disturbing the conduction of sound.[7] The result found on Ad-type tympanogram could be caused by vasculitis that resulted in inadequate perfusion of the ossicles, especially in the long process of the incus. Necrosis of this structure could lead to discontinuation of the ossicular.[17,18]

The result found concerning MMP-3 plasma level indicated that it had a role in degrading the matrix components of the incudomalleolar and incudostapedial joints. An increased expression of MMP-3 plasma had been observed in isolated synovium and cartilage of RA patients. MMP-3 serum levels had been reported to be associated with the development of joint structural damage.[25] In RA, MMP-3 was overproduced. MMP-3 and other MMPs were secreted into the synovium and attacked the cartilage lubricated with synovial fluid. The MMP-3 level in the rheumatoid synovial fluid was higher than that of other MMPs and proved to predict joint destruction.[14]

Takatsu et al.[7] found that the concentration of MMP-3, MMP-9, interleukin-6, and tumor necrosis factor alpha in plasma increased in RA patients with SNHL. They concluded that increasing SNHL in RA patients could be caused by systemic inflammation and tissue damage. That the molecular mechanism causes damages to the inner ear tissue is not fully known. MMP-3 plasma concentrations were significantly higher in RA patients who developed SNHL. MMP-3 worked in the initial phase and could activate other MMPs such as pro-MMP-1, pro-MMP-8, pro-MMP-9, and pro-MMP-13. Therefore, MMP-3 was considered as an important pathology mediator in RA.[25] Increased MMPs in RA patients accelerated inner ear damage resulting in SNHL.[7] However, there were limitations in this research. This research was designed as a real-world cross-sectional study. All patients were recruited from one center and treated with different medications. A further multicenter research was required

to investigate disease activity, MMP-3 plasma levels, or other MMPs given the same medication.

In conclusion, hearing loss is a common finding in RA. On the basis of this research, MMP-3 plasma contributed to degrade the incudomalleolar and incudostapedial joints and could damage the inner ear hair cells due to oxidative process in RA. Examination of MMP-3 plasma levels in the early stage of disease progression was necessary for early diagnosis and intervention of hearing loss. Periodic evaluation of audiometry and tympanometry examinations was also recommended to monitor the hearing loss.

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