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The Association of Glutathion Peroxydase-1 Serum and Sensorineural Hearing Lossin MDR TB Patients with Kanamycin Therapy

Ratna Anggraeni[1], Arif Darmawan[1], Febri Wisudawan F.[1]

[1]Department of Otorhinolaryngology-Head and Neck, Faculty of Medicine, Universitas Padjajaran

Abstract

Introduction: Kanamycin therapy in Multi-Drug Resistance Tuberculosis (MDR-TB) patients increases the possibility of sensorineural hearing loss through increasing the level of Reactive Oxygen Species (ROS) production in cochlea, particularly in hair cells. In normal state, ROS is detoxicated by numerous antioxidant enzymes, including glutathione peroksidase-1 (GPx-1). Imbalance of antioxidant enzymes and ROS production leads to death of hair cells and eventually sensorineural hearing loss.

Objective: This study aimed to observe the association of GPx-1 level and sensorineural hearing loss in MDR-TB patients with Kanamycin therapy.

Method: This study was a prospective observational study conducted at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, between February to April 2017. 17 patients were included into the study with pre- and post-kanamycin therapy examination within 3 weeks duration using pure tone audiometry and serum level of GPx-1. Statistic analysis was done using Man Whitney test with significant level of $p < 0.05$.

Result: A significant reduction of GPx-1 level in 3 weeks period after the initial Kanamycin administration was found in the study; $p < 0.001$. Furthermore, there was a significant alteration in the hearing threshold on frequency of 500-800 Hz after Kanamycin administration; $p < 0.05$. There was a significant association between GPx-1 level and sensorineural hearing loss in Kanamycin therapy; $p < 0.05$.

Conclusion: Sensorineural hearing loss in patient with history of Kanamycin therapy was associated with level of GPx-1 degradation.

Keywords: *GPx-1, Kanamycin, MDR TB, Sensorineural hearing loss.*

Introduction

Tuberculosis (TB) remains a concern among global health problem with a high mortality and morbidity rate.[1] WHO also stated that Indonesia had approximately 6,800 new Multi Drug Resistance TB (MDR TB) annually with approximately 2% of new cases and 12% of re-treatment TB were MDR TB.[2,3,4] Among all injection drugs for MDR TB, Kanamycin is widely used as stated in WHO's recent MDR TB guideline due to its wide distribution area and affordable price.[5,6,7,8]

In general, three types of antioxidant present in human body.[14] Among all GPx, Glutathione peroxide-1 (GPx-1) is the main glutathione peroxide enzyme family; mainly found in erythrocyte, liver, lungs, kidney, and almost in all cells' organ (cytosol, mitochondria, and peroxisome).[15,16] Furthermore, in cochlea, GPx-1 has a higher enzymatic activity in organ of corti, spiral ganglion, stria vascularis, spiral ligament, and another supporting cell.[17,18,19] Higher activity of SOD and CAT are also found in stria vascularis and organ of corti. GSH and GPx are the main antioxidants in those areas.[14]

Study conducted by Alli, et al. in 2014 on 83 MDR TB patients showed that there was a significant degradation of antioxidant enzyme activity, including glutathione transferase and glutathione peroxide.[20] It was strengthened by Madebo et al who also showed a significant decrease in glutathione peroxide level in TB MDR patients.[20,22]

Rakhmawati in her study found sensorineural hearing loss, particularly in high frequencies, 4000-8000 Hz, in MDR TB patient treated using Kanamycin within the 19th to 22nd day of therapy, affecting high frequency hearing ability to lower frequency.[8] Study conducted by Jiang et al in 2006 found that there was a shift in auditory brain stem response (ABR) by 45-50 dB in the 14th day that was remained for 5 weeks also showed that the death of hair cells happened in the 11th day and 30% of the superficial hair cells died after 14 days.[24] It is proposed that hearing loss due to Kanamycin's toxicity mostly started on higher frequency tone as it is located on the basal of cochlea; this progressivity happens due to difference of survival ability among the hair cells on basal and apex cochlea; as explained by the lower level of GPx-1 in basal hair cell in comparison with apex of cochlea.[25]

This study aims to observe the association between GPx-1 level and SNHL in MDR TB patients treated using Kanamycin.

Material and Method

This study was an analytic prospective observational study with pre- and post- intervention examination for association between variables, which had been ethically legalized before. **Participants.** Patients with MDR TB at MDR TB Policlinic of Internal Medicine Department of Dr. Hasan Sadikin General Hospital, Bandung between February to April 2017. The inclusion criteria for the study were patients with MDR TB with plan for Kanamycin therapy, aged 20-50 years old, had intact tympanic membrane in both ears, had type A result on tympanometry examination, normal hearing threshold on DPOAE examination and audiometry. Exclusion criteria for the study were patient with history of treatment using ototoxic drugs except TB-MDR treatment, had a history of another diseases, including renal failure, diabetes mellitus, liver diseases, systemic lupus erythematosus (SLE), and cardiovascular diseases. **Intervention**. Data was collected from physical examination and laboratories data of already diagnosed MDR TB patients and planned for kanamycin therapy. Data before and 3 weeks after treatment consist of personal data collection, physical exam of ENT, tympanometry, pure tone audiometry, DPOAE, and blood sample collection for glutation peroksidase-1 (GPx-1) serum. **Outcome.**The data then analyzed for comparison of subject group characteristic using paired t-test if the data is normally distribute, and using Wilcoxon if the data is abnormally distribute. The data is also analyzed for GPx-1 level correlation with SNHL using Mann-Whitney test. The result is statistically significant if p≤0,05.

Findings: This study was held from February 2017 to April 2017, using 17 subject that fulfill the inclusion criteria. All subject received same test for pre and post Kanamycin therapy, which includes tympanometry, pure tone audiometry, DPOAE, and GPx-1 level.

Table 1: Subject Characteristic

Characteristics	n=17
Gender, n (%)	
Male	7 (41,2)
Female	10 (58,8)
Age (Years)	
Mean ± SD	36 ± 8
Range	23 – 46

Table 2. GPx-1 Level Before and After Kanamycin Therapy

	Measurement		Decendants (%)	p-value
	Before therapy (u/l)	After 3 weeks therapy (u/l)		
GPx-1				
Mean ± SD	4,49 ± 3,12	1,2 ± 1,0	70.42 ± 20,94	<0,001*
Range	1,01 – 14,01	0,07 – 5,15	18.81 – 98,54	

Analysis using paired-t test. *significant if p ≤ 0.05

Based on these table GPx-1 level before therapy with range 1,01-14,01 (4,49 ± 3,12) and GPx-1 level after therapy (1,2 ± 1,0) with range 0,07 – 5,15.

Table 3. Correlation between GPx-1 level and DPOAE value

	DPOAE test (Dp-NF)		p-value
	Pass n=4 (23,5%)	Refer n=13 (76,5%)	
GPx-1			
Mean ± SD	66,60 ± 21,02	84,59 ± 14,86	0,062*
Range	18,81 – 92,36	64,40 – 98,54	

Analysis using paired-t test. *significant if p ≤ 0.05

From the analysis above, GPx-1 median value at ear that having DPOAE test a "refer" value is higher (84,59 ± 14,86) compared to those who have DPOAE test a "pass" value (66,60 ±21,02), but it's not significant statistically (p=0,062).

Table 4. Audiometry examination before and after Kanamycin therapy

Frequency	Ear	Threshold (dB)		p-value
		Before Therapy Mean ± SD	After Therapy Mean ± SD	
500 Hz	AD	20,6 ± 5,6	23,7 ± 5,3	0,002
	AS	21,2 ± 4,5	24,7 ± 4,1	0,020
1.000 Hz	AD	18,8 ± 3,3	19,4 ± 5,0	0,041
	AS	18,2 ± 5,0	21,8 ± 3,5	0,048
2.000 Hz	AD	14,4 ± 3,6	17,7 ± 3,3	0,045
	AS	14,4 ± 3,9	18,5 ± 5,8	0,016
4.000 Hz	AD	16,8 ± 5,6	20,9 ± 4,4	0,050
	AS	15,3 ± 6,0	21,8 ± 6,4	0,020
8.000 Hz	AD	19,1 ± 7,3	24,4 ± 6,6	0,003
	AS	18,0 ± 7,7	25,9 ± 10,3	0,004

Analysis using paired-t test. *significant if p ≤ 0.05

There's a significant increase in hearing threshold on both ear from pre to post Kanamycin therapy using audiometry each frequency.

Table 5. Correlation between GPx-1 Level and Sensorineural Hearing Loss

	SNHL		P value
	Yes n=13 (76,5%)	No n=4 (23,5%)	
GPx-1 Pre Therapy			
Mean ± SD	3,68 ± 1,99	7,13 ± 4,88	
Median	3,73	5,88	0,049
Range	1,01 – 6,32	2,75 – 14,01	
GPx-1 Post Therapy			
Mean ± SD	0,95 ± 0,71	1,99 ± 2,17	
Median	0,82	1,30	0,013
Range	0,07 – 2,59	0,21 – 5,15	

Analysis using Mann-Whitney test. *significant if p ≤ 0.05

There's a significant correlation between GPx-1 level and SNHL condition.

Discussions

This study conclude MDR TB is mostly suffered by female (58.2%) compared with male (41,8%). Liu et.al hypothesized that female mostly spend their day taking care of their family who has MDR TB, compared to male, so the risk of bacterial infection transmission is higher in female.[28] This result also found in Pelaquin et.al study and WHO data survey on 2015.[2,29] Pelaquin study stated that gender does not affect the ototoxic effect of Kanamycin in MDR TB therapy and there is no direct correlation between MDR TB incidence and gender.[29]

Based on age group, this study conclude that MDR TB cases occur mostly on productive age (23 – 46 years old). This result was supported by Rakhmawati and Reviono et.al study that also found that MDR TB cases most likely occur on age 20 – 50 years.[7,8] Medical record data at Dr. Hasan Sadikin General Hospital, Bandung, Indonesia on 2016 stated that MDR TB mostly happen in age group 25-54 years.[4] Productive age have higher working time than other age group, which may affect the obedience for taking medicine, which then lead to drug resistance. Productive age also has more contact to different people in work, school, or other activity, so the risk of bacterial transmission is higher and could influence the incidence of MDR TB.[28]

Kanamycin is known for its side effect damaging outer hair cell of cochlea. This study used DPOAE on frequency ranged 1,500 Hz to 8,000 Hz which was tested prior and 3 weeks after the therapy begin. The result was most of the study subject exhibit "refer" value, which indicates damage at cochlear cell hair. Other study by Mustikaningtyas also exhibit the same result.[25,30] DPOAE test could provide initial data of hearing condition and early detection of ototoxicity. Reavis et.al stated that DPOAE could detect around 78% of hearing problem cases, which then confirmed using HFA. Other study also stated that DPOAE test is sensitive in monitoring of ototoxicity caused by drugs.[28]

MDR TB infection is a chronic infection, marked by a decrease in one of antioxidant enzyme. Study of Alli et.al and Madebo stated that the antioxidant enzyme known to be decreased by chronic infection is GPx-1.[16,17] This study found that GPx-1 level is significantly decrease after 3 weeks therapy of Kanamycin. ROS production happens continuously inside the cell, together with a decrease in antioxidant production, which results an imbalance level of antioxidant and ROS. This imbalance leads to DNA, cell membrane, cell protein and kinase protein damage. DNA damage can be repaired by Base Excision Repair (BER) mechanism, but if the damage exceeded BER capability, the cell will activate protein P53 and result in apoptosis.[27] GPx-1 level is determined by many factor, such as inflammation process, inadequate nutritional intake, and low social economy condition.[20]

Pure tone audiometry testing is used to monitor the change of hearing threshold due to Kanamycin therapy. A study conducted by Rakhmawati also shows a decrease on sensorineural hearing function from frequency 4.000 Hz to 8.000 Hz.[7] Other study conducted by Mustikaningtyas shows that SNHL after Kanamycin therapy happens in several level (48% mild, 24% moderate, 4% moderate-severe, 1% severe and 15% very severe).[30]

Baseline data, consist of HFA, tympanometry, speech audiometry, and OAE, should be recorded before administration of ototoxic therapy to determine the hearing threshold. Pure tone audiometry is the only exam that still used before administering ototoxic therapy.[26]

Early stage of Kanamycin therapy does not exhibit hearing problem on speech frequency (500 – 4.000 Hz), so not many patient realized that hearing problem is already happened. HFA exam can be useful for early detection of hearing problem, so further and more severe condition can be prevented.[14]

Table 3 showed a tendency of diminishing level of GPx-1 level after Kanamycin therapy, although it is not statistically significant. This may result from a minimal number of samples. On this study, decreasing level of GPx-1 level is more likely to be lower on "refer" value ear compared to "pass" value ear after therapy, whereas GPx-1 level is higher on "refer" value ear compared to "pass" value ear before therapy. This may result from higher exposure of ROS on "refer" value ear cochlea as an effect of intracellular defense, which then lead to an increase in GPx-1 level at the beginning to balance ROS level. This mechanism will end at some point due to maximal compensatory effect of GPx-1 enzyme, so the imbalance of ROS and antioxidant enzyme is no more tolerable, which lead to the damage of cochlear hair cell.[14,23]

Table 5 shows that GPx-1 level is significantly related to SNHL. Low GPx-1 level decreasing the capability of this enzyme to eliminate ROS, especially in basal area of cochlea.[31] This phenomenon is because GPx-1 level in basal area of cochlea is lower than in

apex area, causing basal area to be more vulnerable.[15] Study conducted by Sharma et.al showed that 18 MDR TB patient that is given Kanamycin therapy for 6 weeks, develop sensorineural hearing problem, 2% on the first week and 12% after 6th week. Mostly having bilateral hearing problem.[25]

The limitations of this study were the fact that GPx-1 examination performed with ELISA which only saw serum levels or amount of the enzyme, but not the activity of the enzyme.

Conclusion

There is a significant correlation between GPx-1 level and SNHL condition proceeding Kanamycin therapy on MDR TB patient, characterized by a decrease in GPx-1 level and an increase in hearing threshold on subjects after administration of Kanamycin therapy.

Conflict of Interest: There was no conflict of interest in this study.

Ethical Clearance: The ethical clearance is granted from KEPK, Dr Hasan Sadikin General Hospital, Bandung no.LB.04.01/A05/EC/033/II/2017.

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