Volume-1, Issue-2, March-April -2019: 43-49
International Journal of Recent Innovations in Academic Research ISSN: 2659-1561

Increased Apoptosis Index in Cochlear Fibroblasts Diabetic Rat Model (A Pilot Study)

Tengku Siti Hajar Haryuna[1], Muhammad Budi CaecarianLubis[1], Delfitri Munir [1], Dyah Fauziah[2] and Gusbakti Rusip[3]

[1]Department of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia
[2]Department of Pathology Anatomy, Faculty of Medicine, Universitas Airlangga, Surabaya, 60131, Indonesia
[3]Department of Physiology, Faculty of Medicine, University of Muhammadiyah Sumatera Utara, Medan, 20238, Indonesia
E-mail: tengkusitihajarharyuna@gmail.com

Abstract: DiabetesMellitus (DM) is a metabolic disease characterized by an increase in blood sugarlevel due to a disruption in insulin secretion, function, or both. This study aimed toevaluatethe Apoptotic Index on the cochlealateral wall of the diabetic rat modelsThis experimental study used 32 male Rattus norvegicusdivided into a control groupand aninterventiongroup which received streptozotocin (STZ) injection. Rats were terminatedthe fifth day and the Apoptotic Index was measured by the TUNNEL test. Rats in the intervention group showed a significant increase in the Apoptotic Index (P <0.05) compared to the control group.
Keywords: Apoptosis, diabetes, streptozotocin, cochlea.

1. Background

The association between Diabetes Mellitus (DM) and sensorineural hearing loss has been studied for more than a century. Cochlear histopathology study in diabetic animals has proven the thickening of the capillary basement membrane, the loss of outer and inner hair cells, the atrophy of spiral ganglion cell, and the atrophy of vascular striae (Fukushima et al., 2005).

Poor neurodegenerative damage in DM patients can include increased apoptosis, oxidative damage, and intracellular calcium excitotoxicity (Frisina et al., 2006).

Apoptosis is a defence mechanism against damage, loss of function, and cell aging. Some of its mechanisms can be induced in cells by extrinsic signals and intrinsic signals due to cellular stress. The sensitivity varies depending on several

factors, such as pro- and anti-apoptotic proteins, the stage of cell cycle, and the degree of stimulus. Proapoptotic protein consists of Bad and bcl-XL proteins, while antiapoptotic proteins consist of Bad, Bax, and Bid. In regard to cytosolic action, Bcl-2 protein is often found as a cellular sensor or stress prevention (Oever et al., 2010).

This study is a pilot study to evaluate the effect of hyperglycaemia on the incidence of apoptosis in the fibroblast cells of the lateral cochlear wall. Prior study has found that there is an increase in Apoptosis Index in fibroblast cells of the lateral cochlear wall in ototoxic rat models injected with intratymphanic gentamycin (Haryuna et al., 2018), decreased SOD expression in fibroblast of lateral cochlear wall in diabetic rat models observed in other study (Haryuna et al., 2017).

From study conducted by Xueqin et al., was stated that pathological changes in the auditory system in chronic diabetes may predict the occurrence of apoptosis and autophagy in spiral ganglion cell and nucleus neurons toxicity in the cochlea (Xueqin et al., 2017).

Study on the lateral cochlear wall is still scarce and this motivated us to conduct a study on the Apoptosis Index in the lateral cochlear fibroblasts cells of diabetic rat models which subsequently became the basis for further research in efforts to prevent and treat DM-related hearing loss.

2. Materials and Methods

This study used a randomized post test only control group laboratory experimental design. The rat used were male Rattus norvegicus with a mean weight of 150-250 grams. There was a total of 32 rats included in this study equally distributed between the control group and the intervention group.

The taken samples were rats in the same strain, homogeneous, and bred in the Biochemistry Laboratory, Faculty of Medicine, Airlangga University with adequate lighting was served, food was given ad libitum, relative humidity between 55%±15% and room temperature between 20-26^{0}C.

The control group was given a single injection of Citrate Buffer. The intervention group was injected with streptozotocin (Streptozotocin Bioworld, USA) 12 mg intraperitoneal single dose. DM was defined as a blood sugar level of more than 200 mg/dl (ADA, 2016). All rats were terminated on day 5. Rats were terminated and temporal bone necropsy was taken from the rat's head. Samples were collected and fixed with 10% formalin buffer solution and decalcified with EDTA

for 4 weeks. Samples were tested in the laboratory of Pathology Anatomy to assess the Apoptosis Index by TUNNEL assay in lateral wall of cochlear fibroblasts.

TUNNEL Assay

Samples were examined with TUNNEL assay to assess Apoptosis Index using TREVIGEN (TACS 2 TdT-DAB In Situ Apoptosis Detection Kit). Apoptosis was characterized by a brown cell nucleus measured under 40x magnification using the Olympus CX21 microscope with Panasonic Lumix G3. Calculations were carried out by a pathologist and a researcher in a double blind method.

Statistical Analysis

Data were analyzed using T-test with IBM SPSS Statistics with a significance level of 5%.

3. Results

Apoptotic Index

In figure 1, group B was given Streptozotocin injection and showed a higher Apoptotic Index compared to group A (control); the TUNEL method assay showed fragmentation of DNA in brown cell nuclei.

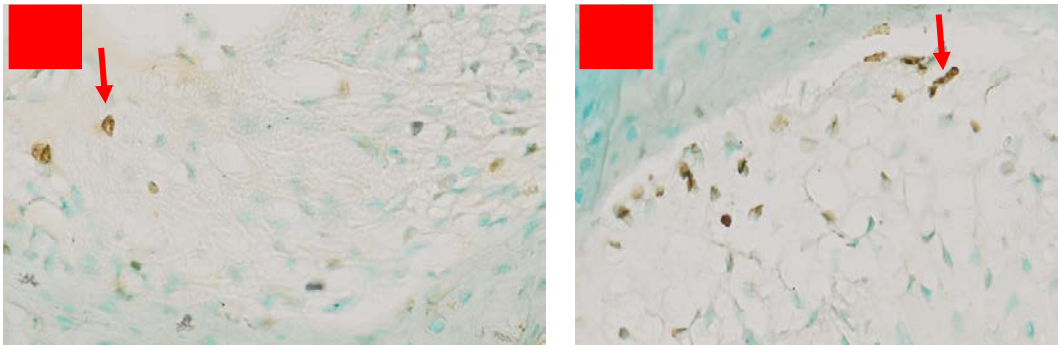

Figure 1. Apoptotic Index (40x magnification): (A) Group A; (B) Group B

The red arrows show apoptosis in the nucleus of cochlear fibroblasts characterized by brown colour.

As shown in figure 2, the mean value of the Apoptotic Index in the cochlear lateral fibroblasts of the diabetic rat models appears higher in group B.

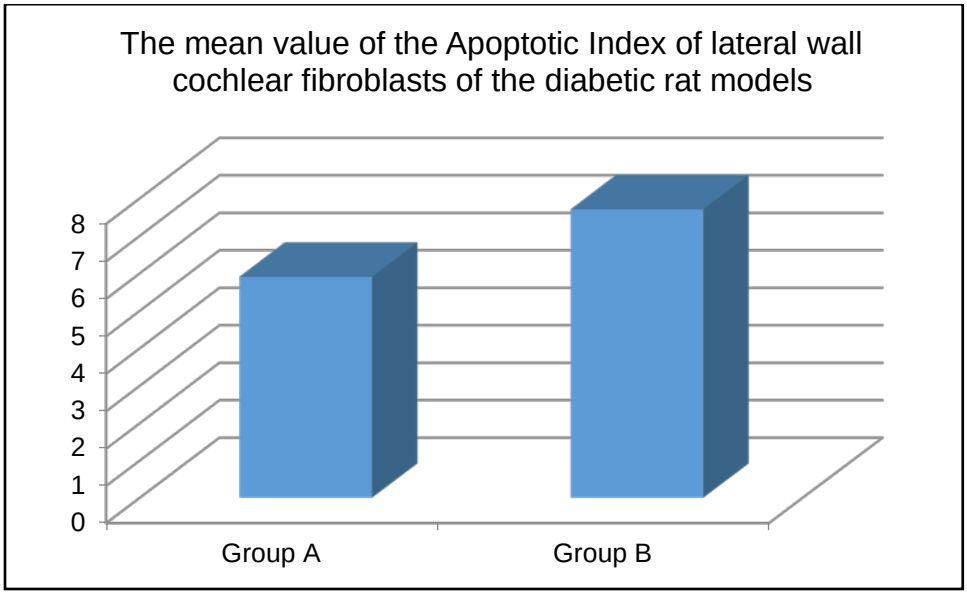

Figure 2. The mean value of Apoptotic Index of cochlear lateral fibroblasts of the diabetic rat models

Table 1 shows a significant difference ($P < 0.05$) between the control group compared to the diabetic rat model group.

Table 1. Results of the T-test for Apoptotic Index

Group	Mean difference ± Standard deviation	P value
A	7,69±1,302	0,001*
B	5,94±1,340	0,001*
*Statistically significant		

4. Discussion

Intraperitonial streptozotocin injection doses were given based on previous studies stating that single streptozotocin doses of 40 to 60 mg/kg can be given intraperitoneally to cause toxic effects and trigger DM after 6 hours of administration (Szkudelski, 2001). In this study, we gave intraperitoneal streptozotocin doses of 60 mg/kgbw with rat body weight ranging from 250-350 mg. In recent years, the number of in vitro studies has provided evidence that hyperglycaemia can induce endothelial cell apoptosis and extensively increase. These mechanisms include oxidative stress, increased intracellular Ca^{2+}, mitochondrial dysfunction known as the mitochondrial apoptotic pathway, alteration in intracellular fatty acid metabolism, mitogen-activated protein kinase (MAPK) signaling pathway, and impaired phosphorylation activation of protein kinase Akt (Oeveret al., 2010).

There are two main pathways that cause apoptosis, 'extrinsic pathways' (death induction receptors) and 'intrinsic pathways' (Bcl-2 regulation or mitochondria). The extrinsic pathway is mediated by cell death receptors such as Fas or TNFR, which are a series of signals that cause activation of the caspase and apoptosis. Intrinsic pathways are often activated by stress cells such as growth factors, chemotherapy drugs, and exposure to perforin and granzyme cytotoxic granule constituents. The intrinsic pathway is regulated by an equilibrium between pro- and anti-apoptotic members of the Bcl-2 family proteins (Thomas, et al., 2009).

The initial cellular response to high glucose is ROS which induces rapid apoptotic cell death. It is known that high diacylglycerol induces endothelial apoptosis through activation of the Bax-caspase protease pathway. The apoptosis effector is known as the family of intracellular cysteine proteases namely caspase. The characteristics of apoptosis in regard to caspase are alteration in mitochondrial function characterized by a decrease in electrochemical gradients throughout mitochondrial membrane and the release of cytochrome c mitochondria into the cytoplasm, and are inhibited by the presence of Bcl-XL in these organelles. Translocation of pro-apoptosis Bax protein into the mitochondrial membrane is accompanied by a significant increase in caspase-3 and caspase-9 activity (Frances et al., 2013).

Functional defects and decreased pancreatic beta cells contribute to failure of pancreatic beta cells in type 2 DM. Apoptosis detected by TUNNEL staining is the most common thing in pancreatic beta cell death in type 2 DM. In some experimental animals, glucose can also induce beta cell apoptosis. High glucose exposure over a long period of time results in an increase in Bid regulation from BH3, Bad, and a decrease in Bcl-XL (Thomaset al., 2009). In this pilot study, we have demonstrated an increase in the Apoptotic Index in the lateral wall of cochlear fibroblasts, where the comparison between the control group and intervention group showed a significant result ($P < 0.05$). This result is consistent with Zheng et al., 2018 which showed a significant increase in the Apoptotic Index in cardiomyocytes in streptozotocin-induced diabetic rat models compared to the control group (Zheng et al., 2018).

5. Conclusion

This study showed a significant increase in the Apoptosis Index in the cochlear lateral wall fibroblasts of diabetic rat models compared with controls.

Conflict of Interest

The authors declare that there is no conflict of interest in regard to the publication of this paper.

References

1. Frances, D.E., Ingaramo, P.I., Ronco, M.T. and Carnovale, C.E. 2013. Diabetes, an inflammatory process: oxidative stress and TNF-alpha involved in hepatic complication. Journal of Biomedical Science and Engineering, 6: 645-653.

2. Frisina, S.T., Mapes, F., Kim, S., Frisina, D.R. and Frisina, R.D. 2006. Characterization of hearing loss in aged type II diabetics. Hearing Research, 211(1-2): 103-113.

3. Fukushima, H., Nishiike, S., Masuda, K., Morita, N., Fukutsuji, K., Yoda, S. and Harada, T. 2005. Impairment in the sensorineural elements of the cochlea and vasculature in diabetes mellitus: a human temporal bone study. Kawasaki Medical Journal, 31(34): 56-62.

4. Haryuna, T.S.S., Munir, D., Maria, A. and Bashiruddin, J. 2017. The Antioxidant Effect of Curcumin on Cochlear Fibroblast in Rat Models of Diabetes Mellitus. Iranian Journal of Otorhinolaryngology, 29(93): 191-197.

5. Haryuna, T.S.S., Purba, A.H.W., Farhat, F. and Alviandi, W. 2018. The Antiapoptotic Effect of Curcumin in the Fibroblast of the Cochlea in an Ototoxic Rat Model. Iranian Journal of Otorhinolaryngology, 30(100): 247-253.

6. Szkudelski, T. 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological Research, 50(6): 537-546.

7. Thomas, H.E., McKenzie, M.D., Angstetta, E., Campbell, P.D. and Kay, T.W. 2009. Beta cell apoptosis in diabetes. Apoptosis, 14(12): 1389-1404.

8. van den Oever, I.A.M., Raterman, H.G., Nurmohamed, M.T. and Simsek, S. 2010. Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. Mediators of Inflammation, 2010: 1-10.

9. Xueqin, H., Wei, Z., Yixin, Y., Xiaolong, L., Chen, Y., Qi, L., Jincheng, Z., Keyuan, Z. and Yong, L. 2017. Implication of autophagy and apoptosis in spiral ganglion cells and cochlear nucleus neurons in diabetes-induced hearing impairment in rats. Biomedical Research, 28(5): 2164-2169.

10. Zheng, J., Cheng, J., Zheng, S., Feng, Q. and Xiao, X. 2018. Curcumin, A Polyphenolic Curcuminoid with Its Protective Effects and Molecular

Mechanisms in Diabetes and Diabetic Cardiomyopathy. Frontiers in Pharmacology, 9: 472.