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Correlation between Expression Level of Serum miRNAs and SSNHL

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Abstract: Objective: To study the correlation between the serum expression levels of miR-143 and miR-145 and the incidence of SSNHL. Methods: 20 cases of SSNHL were collected as the experimental group, and 20 cases of normal hearing were collected as the control group. The expression levels of miR-143 and miR-145 in serum were detected by real-time fluorescence quantitative PCR method, and the t-test and Logistic regression analysis were used for statistical analysis. Results: The expression levels of serum miR-143 and miR-145 in SSNHL group were lower than those in the control group, and the differences were statistically significant ($P < 0.05$). Conclusion: The expression levels of miR-143 and miR-145 in serum were highly correlated with the incidence of SSNHL.

1. Introduction

SSNHL known as sudden sensorineural hearing loss, refers to an unexplained sensorineural hearing loss within 72 hours with at least two consecutive hearing losses of frequency greater than or equal to 20dB [1]. In addition to hearing loss, the main symptoms of SSNHL are often accompanied by tinnitus, ear tightness, and sometimes dizziness, and nausea and vomiting [2]. SSNHL is mainly unilateral hearing loss, whereas bilateral hearing loss also occur in some patients. The onset age of SSNHL is mainly concentrated in an age range of 40~60 years, but the incidences of SSNHL in adolescents are increasing gradually. At present, the incidences of SSNHL ranges from 5 to 20/100,000 people. There is no specific epidemiological investigation on the incidences of SSNHL in China, but previous studies show that the incidences of SSNHL in China are increasing year by year. At present, the etiology of most patients with SSNHL is unknown, and the exact etiology can only be traced for only 10%-15% of patients with SSNHL [3].

MiRNAs known as microRNAs. MiRNAs are composed of 18-22 ribonucleotides that are transcribed in the nucleus by RNA polymerase II to form initial miRNAs (pri-miRNAs). Under the action of RNA polymerase III and DGCR8 protein, pri-miRNAs form precursor miRNAs with hairpin structure (pre-miRNAs), which are transported to cytoplasm under the action of exportin-5 (RNA binding protein). The mature miRNAs can form RNA-induced silencing complex (RISC) in the cytoplasm. RISC is complementary to the corresponding messenger RNA (mRNA), resulting in translation inhibition and mRNA degradation. Its role is to regulate the expression of target genes at

the post-transcriptional level [4]. At present, over 1000 human miRNAs have been discovered, and 30% of the genes are regulated by miRNAs [5]. MiRNAs are involved in the regulation of multiple signaling pathways such as cell development, proliferation, apoptosis, differentiation, fat metabolism, and organogenesis and hematopoiesis [6].

The mechanism of microcirculation disturbance of SSNHL is similar to that of cardiovascular disease. Many scholars have studied the mechanism of microcirculation disturbance in SSNHL, and a large number of studies on miRNA in the field of cardiovascular disease are also available, but few scholars have integrated the two studies. MiR-143 and miR-145 were proven to be closely related to the occurrence of cardiovascular diseases, therefore, we selected miR-143 and miR-145 to study the mechanism of microcirculation disorders in SSNHL.

2. Materials and methods

2.1. Subjects

Data of the inpatients with SSNHL in the Department of Otolaryngology of the First Affiliated Hospital of Kunming Medical University from October 2020 to March 2021 were collected. All the patients were collected with signed informed consent. The clinical data were collected by inquiring medical records, hearing testing items (pure tone electrical audiometry, acoustic conductance) and imaging examinations (ear CT and MRI). In total, 20 cases of SSNHL (experimental group) and 20 cases of normal hearing (control group) were studied. The age distribution of 40 samples ranged from 16 to 60 years. There were 21 males and 19 females.

2.2. miRNA Primer Sequences

The sequence information of miRNA is shown in Table 1

Table 1: miRNA primer information

miRNAs	Primers	Primer sequence
miR-143	Forward Primer	5´-UGAGAUGAAGCACUGUAGCUC-3´
	Reverse primer	5´-CCTACGATCGAAAACGACGCGAACG-3´
miR-145	Forward Primer	5´-UGUGCAAAUCCAUGCAAAACUGA-3´
	Reverse primer	5´- CCTACGATCGAAAACGACGCGAACG-3´
Inside	Forward Primer	5´-CTCGCTTCGGCAGCACA-3´
Outside	Reverse primer	5´- CCTACGATCGAAAACGACGCGAACG-3
the cords		

2.3. Statistical Analysis

Statistical software SPSS23.0 was used for data analysis. The differences in the expression levels of serum miR-143 and miR-145 between the experimental group and the control group were analyzed by independent sample T-test. The correlation between the expression levels of serum miR-143 and miR-145 and the incidences of SSNHL were analyzed by binary Logistic regression. $P < 0.05$ was considered statistically significant.

3. Results

3.1. miR-143, miR-145 dissolution and amplification curves

The amplification curves of miR-143 and miR-145 demonstrated obvious logarithmic early phase, logarithmic amplification period and plateau phase of PCR, which represented high content of PCR products and sufficient sensitivity of primers. The curves of miR-143, miR-145 and dissolution were all single peak, indicating that the primer had good specificity and specific amplification was absent (Figure 1-4).

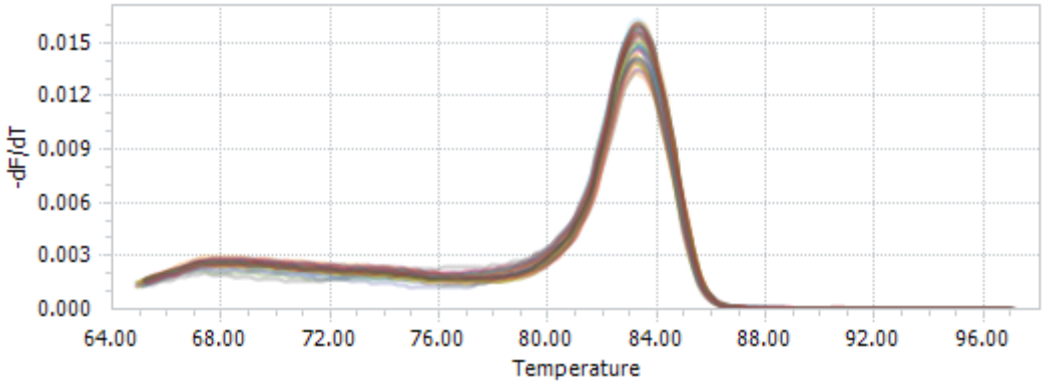

Figure 1: Dissolution curve of serum miR-143 in patients with SSNHL

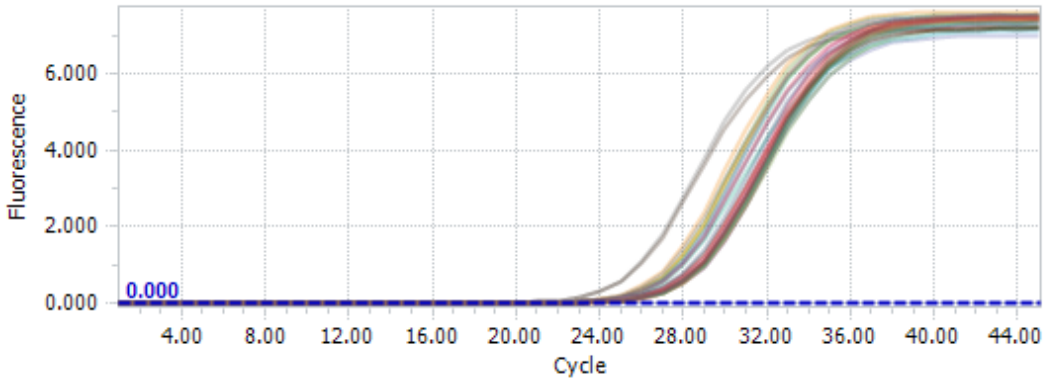

Figure 2: Serum miR-143 amplification curve of patients with SSNHL

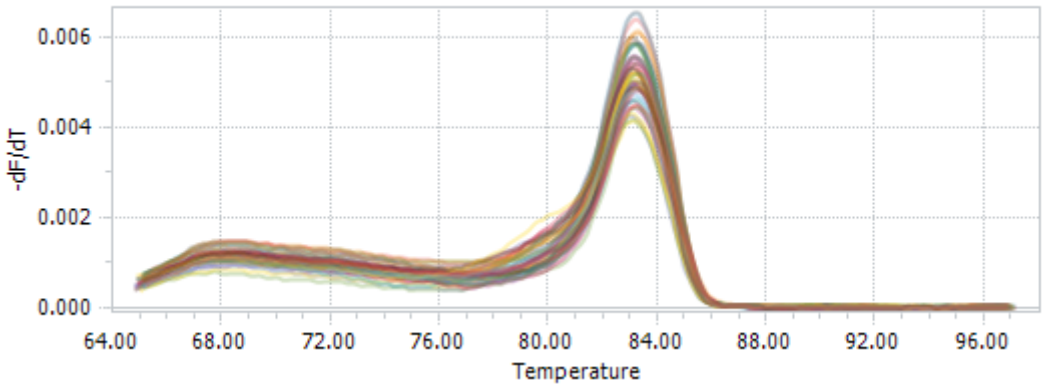

Figure 3: Dissolution curve of serum miR-145 in patients with SSNHL

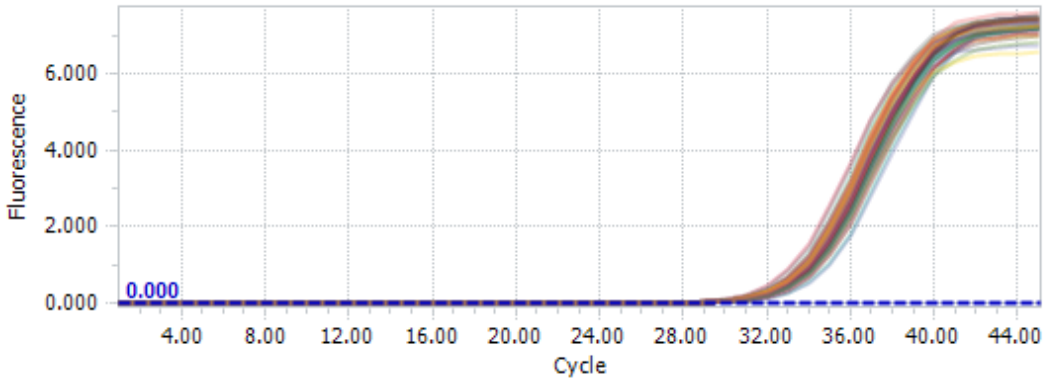

Fig. 4: Serum miR-145 amplification curve of patients with SSNHL

4. Results

4.1. Difference Test of CT Mean Value of Two Kinds of Serum miRNA Expression Levels between SSNHL Group and Control Group

The expression levels of miR-143 and miR-145 in the SSNHL group were lower than those in the control group, and the differences were statistically significant ($P < 0.05$)(Table 2).

Table 2: Independent sample T-test

miRNA	T	Df	P
miR-143	-4.135	38	＜0.001
miR-145	-4.033	38	＜0.001

4.2 Correlation Analysis of miR143 and miR145 Expression Levels

The pairwise correlation between the two serum miRNA expression levels was analyzed by Pearson linear correlation analysis, and the expression levels of serum miR-143 and miR-145 were significantly correlated (r=0.855, P < 0.05)(Table 3).

Table 3: Pearson linear correlation analysis

		miR-126	miR-143	miR-145	miR-155
miR-143	r	0.524	1	0.855	0.047
	P	0.001	-	＜0.001	0.772
miR-145	r	0.553	0.855	1	0.208
	P	＜0.001	＜0.001	-	0.199

5. Discussion

As the blood supply vessels of the inner ear, the labyrinthine artery (internal auditory artery) originates from the vertebrobasilar artery, anterior inferior cerebellar artery, and posterior inferior cerebellar artery. When the vertebrobasilar artery and posterior inferior cerebellar artery occlusion and stenosis occur, it is easy to cause insufficient blood supply to the inner ear. Moreover, due to the lack of collateral circulation of blood supply to the inner ear, in the event of thromboembolism, a single factor and/or the combination of various multiple factors, such as vasospasm, high fibrinogen hematic disease, atherosclerosis, hyperlipidemia, and hemodynamic change may lead to the inner ear tissue ischemia, hypoxia, edema, metabolic disorders, and damages to the inner ear hair cells and cochlear nerve receptor, and consequently hearing loss, tinnitus, dizziness [7].

To date, considerable research efforts have addressed the mechanism of microcirculation disturbance in SSNHL, including mainly thrombosis, hyperlipidemia, hemodynamics and other aspects. Through the clinical study on cochlear ischemia, MOM.T et al. [8] found that the formation of microthrombosis in the inner ear is related to the occurrence of SSNHL. However, it is not possible to detect cochlear ischemia and microthrombosis in a non-surgical manner. Studies have found that in patients with SSNHL, the increased fibrinogen level detected within 7 days after onset is correlated with poor hearing recovery effect, and it is speculated that this is caused by inner ear ischemia [9]. Guo et al [10] conducted high-fat feeding on the mice with the knockout apolipoprotein E gene, and found that the experimental mice developed hyperlipidemia, internal auditory artery atherosclerosis, artery wall thickened, lumen narrowed, and cochlear helicoid damage and hearing loss. This experiment showed that the degree of hearing loss in mice was positively correlated with the severity of atherosclerosis and the content of serum cholesterol.

Atherosclerosis plays an important role in the pathogenesis of microcirculation in SSNHL. The formation mechanism of atherosclerosis is complex, mainly due to vascular remodeling caused by the inflammatory response of vascular wall. In pathophysiology, it is attributable to the activation of vascular endothelial cells induced by external stimulation, and the release of various inflammatory factors by vascular endothelial cells. Under the action of inflammatory factors, vascular smooth muscle cells, fibroblasts, adipocytes, platelets, and macrophages and their evolved foam cells are also activated and begin to proliferate, aggregate and deposit on the vessel wall, eventually forming atherosclerotic plaques [11]. MiR-143/145 is widely present in vascular smooth muscle cells and plays an important role in maintaining vascular stability by regulating the proliferation, differentiation, contraction, migration and other physiological functions of vascular smooth muscle cells [12].

Cordes KR et al. [13] found that miR-143/145 was specifically expressed in smooth muscle cells, and could promote the differentiation of smooth muscle cells of mice, but had an inhibitory effect on the proliferation of smooth muscle cells. MiR-143/145 is a necessary miRNA for the contraction of vascular smooth muscle cells of mice.

Lovren F et al. [14] gave high fat feeding to ApoE (-/-) mice, and the results showed that the size of atherosclerotic plaques in the aortic sinus, ascending aorta and brachiocephalic artery of mice targeted with miR-143/145 decreased significantly, the area of fibrous cap increased significantly, whereas the area of necrotic core decreased, and the collagen content of plaques increased. The content of macrophages in atherosclerotic plaques decreased, and the stability of atherosclerotic plaques increased. This study suggested that miR-143/145 inhibited the occurrence and development of atherosclerotic plaques, and that miR-143/145 was a new therapeutic target for atherosclerosis.

Wei Y et al. [15] studied the correlation between miR-143/145 gene polymorphism and ischemic cerebral infarction. The study indicated that the expression level of miR-143/145 decreased in ischemic cerebral infarction, indicating that the expression level of miR-143/145 may be a protective factor in ischemic cerebral infarction. This is related to the involvement of miR-143/145 in the pathogenesis of atherosclerosis.MiR-143/145 co-regulates vascular endothelial cells with serum reactivity factors, and the serum reactivity factors of miR-143/145 form a mutual regulatory network. Serum reactivity factors can regulate the expression of miR-143/145, and miR-143/145 can reciprocally regulate the function of serum reactivity factors. Moreover, in mice lacking miR-143/145 expression, vascular endothelial repair ability decreased after injury. The process of vascular endothelial repair is similar to the process of atherosclerotic plaque formation, so it is indirectly proved that miR-143/145 is involved in the occurrence and development of atherosclerosis. MiR-143/145 regulate the transcription of adenosine triphosphate binding transporter genes, which reversely transcribes cholesterol deposited in peripheral arterial walls to the liver, thereby reducing atherosclerosis.

Liu K et al. [16] studied the correlation between miR-143/145, homocysteine and carotid atherosclerosis, and found that the expression level of miR-143/145 decreased in carotid atherosclerosis, and the expression level of miR-143/145 was associated with hyperhomocysteinemia. Hyperhomocysteinemia has been identified as a risk factor for atherosclerosis.

Serena M et al. [17] conducted a blood sampling study on 118 patients with SSNHL and 415 healthy volunteers, including coagulation factors I~VIII, homocysteine, and fibrinogen and antithrombin. The results showed that homocysteine, the increase of clotting factor VIII, protein, protein C and S of antithrombin is reduced, and cardiovascular risk factors (diabetes, high cholesterol, high blood pressure), and other uncertain factors increase the risk of SSNHL, which increased homocysteine and cardiovascular risk factors associated with poor prognosis of SSNHL. The study of Liu K and Serena M further confirmed that the pathogenesis of SSNHL is similar to that of cardiovascular diseases, and both are related to hyperhomocysteinemia and atherosclerosis.

Fichtlscherer S et al. [18] detected plasma miR-143/145 expression in 36 patients with coronary atherosclerosis and 17 healthy controls, and the results showed that plasma miR-143/145 expression decreased significantly in the coronary atherosclerosis group. This is consistent with the results in experiments related to carotid atherosclerosis. It can be speculated that circulating miR-143/145 is involved in the formation of atherosclerosis in systemic blood vessels. If atherosclerosis occurs in the inner ear supplying artery, it may lead to SSNHL.

In conclusion, miR-143/145 is closely related to the occurrence of atherosclerosis, and meanwhile, atherosclerosis is also closely related to the occurrence of SSNHL. In this study, miR-143/145 were extracted from human serum, and the expression levels of both were detected by

real-time fluorescence PCR. T-test and Logistic regression analysis showed that the expression level of miR-143/145 in the SSNHL group differed from that in the healthy control group ($P < 0.05$), and the mean expression level of miR-143/145 in the SSNHL group was lower than that in the healthy control group. It is speculated that the reason may be that the decreased expression level of miR-143/145 is related to the formation of atherosclerosis in the supplying artery of the inner ear, but the specific mechanism of action needs to be verified in animal experiments. The atherosclerosis model of the internal auditory artery can be established by high-fat feeding mice with the knockout apolipoprotein E gene (ApoE (-/-)). At the same time, the expression level of serum miR-143/145 was compared between the control group and the experimental group to determine whether the expression level of serum miR-143/145 was correlated with internal auditory atherosclerosis, and the expression level of miR-143/145 in atherosclerotic plaques could also be detected to further clarify the specific role of miR-143/145 in the process of atherosclerosis in the internal auditory artery.

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