Expression of AQP8 in serum of patients with Meniere's disease and its value in evaluating the degree of hydrolabyrinth and predicting prognosis

Rui Zhao[1], Tianhua Yi[2], Qinqin Wu[2], Xuemei Liu[2], Jianqiao He[2], and Yufang Tan[2]

[1]The First Affiliated Hospital of Shaoyang University,Shaoyang City
[2]The First Affiliated Hospital of Shaoyang University

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Abstract

Abstract Objective To explore the value of serum aquaporin 8 (AQP8) expression in evaluating the degree of hydrolabyrinth and predicting prognosis in patients with Meniere's disease. Methods 105 patients with Meniere's disease in our hospital were included in the Meniere's disease group.Another 102 healthy subjects in our hospital were included as the control group. The multivariate Logistic regression was used to analyze the influencing factors of poor prognosis in patients with Meniere's disease. Results The expression of serum AQP8 mRNA in the Meniere's disease group was greatly higher than that in the control group (P<0.05). Serum AQP8 mRNA expression in severe hydrops group was greatly higher than that in mild hydrops group and no endolymphatic hydrops group, and serum AQP8 mRNA expression in mild hydrops group was greatly higher than that in no endolymphatic hydrops group (P<0.05). The course of disease, the proportion of severe hydrops and the expression of serum AQP8 mRNA in the poor prognosis group were greatly higher than those in the good prognosis group (P<0.05). The area under the curve (AUC) of serum AQP8 mRNA for predicting poor prognosis in Meniere's disease patients was 0.812 (95%CI: 0.702-0.922), with a sensitivity of 81.0% and a specificity of 70.2%. AQP8 mRNA was an independent risk factor for poor prognosis in patients with Meniere's disease (P<0.05). Conclusion AQP8 mRNA is associated with the degree of hydrolabyrinth in patients with Meniere's disease, and has a high performance in predicting prognosis.

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0.812 (95%*CI:* 0.702-0.922), with a sensitivity of 81.0% and a specificity of 70.2%. AQP8 mRNA was an independent risk factor for poor prognosis in patients with Meniere's disease (P <0.05).

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Key words Meniere's disease; Aquaporin 8; Degree of hydrolabyrinth; Prognosis; Serum

key points

- Serum AQP8 is highly expressed in Meniere's disease patients
- The high expression of serum AQP8 in patients with Meniere's disease is closely related to the aggravation of endolymphatic hydrops
- AQP8 mRNA was an independent risk factor in poor prognosis of Meniere's patients
- AQP8 expression has important predictive value in poor prognosis of Meniere's disease, which features high predictive sensitivity and specificity
- AQP8 mRNA is associated with the degree of hydrolabyrinth in patients with Meniere's disease

1 Introduction

Meniere's disease is a clinical syndrome characterized by recurrent episodes of spontaneous vertigo, unilateral fluctuating sensorineural hearing loss, tinnitus, and sufficient hearing [1,2]. Endolymphatic hydrops is considered as the pathophysiological basis of the disease, which has been confirmed in anatomical pathology studies and recent magnetic resonance imaging (MRI) [3,4]. However, MRI is not appropriate for repeated detections, and there are certain risks in detection. Also, the image quality will be affected by the dose of contrast agent. In clinical practice, it is necessary to find relevant auxiliary detection indexes and then improve the accuracy in Meniere's disease assessment. Aquaporin (AQP) is a transmembrane protein originally identified as a water channel with the ability to regulate the transport of water, glycerin, urea and other small molecules [5]. Recent studies have shown that, AQP plays an important role in the treatment of liver cirrhosis, heart failure, Meniere's disease, cancer, bullous pemphigoid, eczema, and Sjogren syndrome [5]. Considering the lack of laboratory indexes for Meniere's disease, this study detects the expression of serum AQP8 mRNA in Meniere's disease patients, preliminarily discusses the value of serum AQP8 mRNA in evaluating the degree of endolymphatic hydrops in Meniere's disease and predicting its prognosis.

2 Research objects and methods

2.1 Research objects

From February 2016 to April 2022, 105 Meniere's disease patients treated in our hospital were included in the Meniere's disease group. The patients were 40˜73 years old, with an average age of (60.32±8.34) years old, including 40 males and 65 females. The gender, age, body mass index, smoking history, drinking history, hypertension, hyperlipidemia, diabetes, disease site and disease course were collected for Meniere's disease patients. A total of 102 healthy subjects were included in the control group, ranging in age from 40 to 75 years old, with an average age of (60.45±7.96) years old and including 36 males and 66 females.

Inclusion criteria: The Meniere's disease patients were diagnosed based on the relevant diagnostic criteria formulated by the European Society of Otoneurology and the American Society of Otolaryngology Head and Neck Surgery[6]; The study complies with ethical standards, the patient follow-up data is complete, the patients and their families can cooperate with the treatment and follow-up; The control group has no abnormal vestibular function in various hearing tests, no Meniere's disease and other vertigo diseases. Exclusion criteria: Patients with vestibular neuritis and other vertigo diseases; Patients previously with chronic diseases such as otitis media; Patients with cerebrovascular disease, acoustic neuroma, and sudden deafness. The study follows transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): The TRIPOD statement

2.2 Methods

2.2.1 Determination of AQP8 mRNA expression level in serum

10 mL peripheral venous blood samples were collected from Meniere's disease patients before treatment, placed in a dry centrifuge tube, left standing at room temperature for 30 min, and centrifuged at 3000 r/min for 15 min at room temperature to collect the upper serum, and stored at -80 degC. After freeze-thaw on ice, RNA extraction kit (Beijing Yita Biotech Co., Ltd., article number: YT9197) was used to isolate and extract total serum RNA. After testing its concentration, purity and integrity, the first-strand cDNA synthesis kit (Norgen Biotek, Canada, article number: NGB-54420) was used for reverse transcription into cDNA, and the operation was carried out in strict accordance with the instructions. Serum AQP8 mRNA expression was determined by V115896 quantitative real-time PCR (qRT-PCR) instrument produced by Applied Biosystems, the United States, and the internal reference was GAPDH. Loading system: cDNA (50 ng/μL) 1 μL, 2×SYBR Green qPCR Master Mix (Beijing Bairuiji Biotechnology Co., Ltd., article number: BN12014-20μL×100T) 10 μL, upstream and downstream primers 0.5 μL each, ddH_2O 8 μL. Reaction procedure: pre-denaturation at 95 °C for 1 min; denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s, a total of 45 cycles. For the reaction results, the relative expression of AQP8 was statistically calculated by the $2^{-[?][?]CT}$ method. The primer sequences are shown in Table 1.

2.2.2 The degree of endolymphatic hydrops in Meniere's disease, evaluation and grouping of poor prognosis

All Meniere's disease patients underwent inner ear scans using MRI technology (the instrument was a 3.0T Magnetom Verio scanner produced by Siemens, Germany) to evaluate the degree of endolymphatic hydrops. The degree of endolymphatic hydrops was divided into grade 0, without vestibular membrane displacement and endolymphatic hydrops (non-endolymphatic hydrops group, 29 cases); grade 1, with mild endolymphatic hydrops and vestibular membrane displacement, but spatium endolymphaticum area is smaller than the vestibular canal area (mild hydrops group, 48 cases); grade 2, with severe endolymphatic hydrops, vestibular membrane displacement and spatium endolymphaticum area is greater than the vestibular canal area (severe hydrops group, 28 cases). The Meniere's disease patients were evaluated after 3 months of treatment. If the symptoms tinnitus and dizziness were not significantly improved, the speech recognition rate increased by <5%, and the pure tone audiometry improved by <15 dB, the prognosis was poor (poor prognosis group, 21 cases). Otherwise, the prognosis was good (good prognosis group, 84 cases).

2.3 Statistical processing

All data were processed using SPSS 25.0. The enumeration data was expressed by n (%), and tested by; the measurement data obeyed the normal distribution, and was expressed by (+-). The two groups were subjected to independent sample t test, the three groups were subjected to one-way analysis of variance, and SNK-q test was used for further comparison between the two groups. Receiver operator characteristic (ROC) curve analysis was used to analyze the predictive value of serum AQP8 mRNA expression for poor prognosis in Meniere's disease patients; Multivariate Logistic regression was used to analyze the factors influencing poor prognosis in Meniere's disease patients. $P< 0.05$ indicates that the difference is statistically significant.

3 Results

3.1 Comparison of serum AQP8 mRNA expression in control group and Meniere's disease group The serum AQP8 mRNA expression was significantly higher in the Meniere's disease group than in the control group (P <0.05). See Table 2.

3.2 Comparison of serum AQP8 mRNA expression in Meniere's disease patients with different degrees of endolymphatic hydrops Serum AQP8 mRNA expression was significantly higher in severe hydrops group than in mild hydrops group and non-endolymphatic hydrops group. See Table 3.

3.3 Comparison of general data and serum AQP8 mRNA expression between the good prognosis group and the poor prognosis group Differences in gender, age, body mass index, smoking history, drinking history, hypertension, hyperlipidemia, diabetes, and disease site between the two groups were not statistically significant (P >0.05). The disease course, the proportion of severe hydrops and the expression of serum AQP8

mRNA were significantly higher in the poor prognosis group than in the good prognosis group (P <0.05). See Table 4.

3.4 Predictive value of serum AQP8 mRNA expression for poor prognosis in Meniere's disease patients

Using serum AQP8 mRNA as the detection variable, and using poor prognosis as the state variable, the ROC curve was plotted to analyze the predictive value of serum AQP8 mRNA expression in the poor prognosis of Meniere's disease patients. The results showed that, in prediction of poor prognosis of Meniere's disease patients based on serum AQP8 mRNA, the area under the curve (AUC) was 0.812 (95%CI: 0.702-0.922), the critical value was 2.52, the sensitivity was 81.0%, and the specificity was 70.2%. See Figure 1.

3.5 Multivariate Logistic regression analysis of poor prognosis in Meniere's disease patients Using poor prognosis of Meniere's disease patients after 3 months of treatment as the dependent variable, and using AQP8 mRNA, disease course, and endolymphatic hydrops degree as independent variables, a multivariate Logistic regression analysis was performed. The results showed that, AQP8 mRNA was an independent risk factor in poor prognosis of Meniere's patients (P < 0.05). See Table 5.

4 Discussion

In 2015, European Society of Otoneurology developed guidelines for the diagnosis of Meniere's disease. Meniere's disease patients should have the following symptoms: two or more spontaneous vertigo episodes (lasting 20 min to 12 h); mid-low frequency sensorineural hearing loss in one ear in audiometry records; fluctuating auditory symptoms (tinnitus or fullness) in the ear. The standardized definition marks an important research milestone for clinicians and researchers[6,7]. However, the above criteria are subjective or based on subjective hearing tests. Due to the lack of objective criteria for Meniere's disease, its diagnosis and disease assessment is sometimes controversial or unclear.

Traditionally, endolymphatic hydrops has been viewed as an objective histopathological evidence of Meniere's disease, but histopathology can only be performed after death and cannot be used to evaluate patients with episodes of vertigo. The development of active Meniere's disease and its time gap from autopsy assessments limit our understanding of disease progression[8,9]. Many otologic biomarkers are currently used to differentiate Meniere's disease from vestibular migraine and assess the progression of Meniere's disease[10,11]. AQP is transmembrane water channel that affects electrolyte balance and thus plays a central role in the regulation of transcellular water flux [12,13]. Previous studies have shown that, the underlying pathological mechanisms leading to Meniere's disease may include genetics, blood vessel, immunity, abnormal AQP expression, or a combination of multiple factors[10]. Mom et al.[14]showed that dexamethasone can affect inner ear water flux in Meniere's disease patients by targeting AQP2. Asmar et al. [15]showed that the expression of AQP2 was up-regulated in the endolymphatic sac of Meniere's disease patients and involved in the regulation of endolymph volume. It is speculated that AQP8 is also closely related to Meniere's disease in this study, which may be involved in the occurrence and development of Meniere's disease.

The results of this study showed that, serum AQP8 was highly expressed in Meniere's disease patients, and its expression increased with the aggravation of endolymphatic hydrops. It suggests that high expression of AQP8 may promote the occurrence and development of Meniere's disease. According to the AQP regulation of transcellular water flux, it is speculated that the up-regulation of AQP8 expression may disrupt the electrolyte balance, affect the permeability of endolymphatic sac epithelium against water, which harms absorption of endolymph, results in retention of endolymph, aggravates the degree of endolymphatic hydrops. The ROC curve analysis results showed that, AQP8 has a high predictive value in the prognosis of Meniere's disease, indicating that AQP8 may be used as a potential auxiliary biomarker for poor prognosis of Meniere's disease. In clinical practice, prognosis of Meniere's disease can be preliminarily predicted via serum AQP8 detection level, thus making it possible to better identify high-risk groups with poor prognosis. Close monitoring and special care should be given to patients with AQP8 expression above 2.52, so that the clinical treatment plan can be adjusted in a timely manner. The multi-factor results showed that, compared with factors such as disease course, degree of endolymphatic hydrops, etc. the elevated AQP8 expression was more closely related to the poor prognosis of Meniere's disease, suggesting that AQP8 could be used as

a biological therapy target for Meniere's disease for further in-depth research in clinical practice.

To conclude, serum AQP8 is highly expressed in Meniere's disease patients, and the increased expression is closely related to the aggravation of endolymphatic hydrops. AQP8 expression has important predictive value in poor prognosis of Meniere's disease, which features high predictive sensitivity and specificity. This study provides a theoretical basis for the targeted therapy of Meniere's disease. There will be more in-depth and flexible application of AQP8 in research of Meniere's disease and related diseases in the future. However, this study has the following limitations: endolymphatic hydrops has not been confirmed by pathological results, and no histopathological evidence has been found; the sample size is small, and the optimal critical value of serum AQP8 still needs to be further optimized for prognosis prediction.

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Figure 1 ROC curve of serum AQP8 mRNA expression predicting poor prognosis in patients with Meniere's disease

Table 1 Primer sequence

Gene name	Upstream primer 5'-3'	Downstream primer 5'-3'
AQP8	GTGGCCTGCGTGGTCTACTAG	GCCCGCGCAGGTCACCACGTG
GAPDH	CGAGCTGGCACTTGTACACA	GTGGCGACTGACACGAGTGAC

Table 2 Comparison of serum AQP8 mRNA expression between control group and Meniere's disease group(±)

Groups	Number of cases(n)	AQP8 mRNA/GAPDH
Control group	102	1.01±0.21
Meniere's disease group	105	2.36±0.45
t	-	27.525
P	-	0.000

Table 3 Comparison of serum AQP8 mRNA expression in patients with Meniere's disease with different degrees of labyrinthine hydrops(±)

Groups	Number of cases(n)	AQP8 mRNA/GAPDH
No endolymphatic hydrops group	29	2.07±0.39
Mild hydrops group	48	2.34±0.49[a]
Severe hydrops group	28	2.69±0.50[ab]
F	-	12.598
P	-	0.000

Note: compared with the no endolymphatic hydrops group,[a]P ¡0.05; Compared with mild hydrops group,[b]P ¡0.05

Table 4 Comparison of general data and serum AQP8 mRNA expression between good prognosis group and poor prognosis group[(±)/n(%)]

index	Good prognosis group(n=84)	Poor prognosis group(n=21)	$t/$	P
Male[n(%)]	33(39.29)	7(33.33)	0.252	0.615
Age(year)	60.45±7.34	60.13±8.62	0.172	0.863
Body mass index(kg/m^2)	22.13±2.27	22.38±2.37	0.448	0.655
Course of disease(year)	9.75±2.92	11.24±2.67	2.126	0.036

index	Good prognosis group(n=84)	Poor prognosis group(n=21)	*t/*	*P*
Smoking history[n(%)]	26(30.95)	6(28.57)	0.045	0.832
Drinking history[n(%)]	21(25.00)	5(23.81)	0.013	0.910
Hypertension[n(%)]	46(54.76)	12(57.14)	0.039	0.844
Hyperlipidemia[n(%)]	25(29.76)	6(28.57)	0.011	0.915
Diabetes[n(%)]	13(15.48)	3(14.29)	0.018	0.892
Degree of labyrinthine hydrops[n(%)]			21.617	0.000
No endolymphatic hydrops	27(32.14)	2(9.52)		
Mild hydrops	43(51.19)	5(23.81)		
Severe hydrops	14(16.67)	14(66.67)		
Diseased part[n(%)]			0.343	0.558
Left ear	42(50.00)	12(57.14)		
Right ear	42(50.00)	9(42.86)		
AQP8 mRNA/GAPDH	2.26±0.37	2.76±0.45	5.298	0.000

Table 5 Multivariate logistic regression analysis of poor prognosis in patients with Meniere's disease

Influence factor	*B*	*SE*	*Wald*	*OR*	*95%CI*	*P*
AQP8 mRNA	1.006	0.349	8.317	2.736	1.381˜5.422	0.004
Course of disease	0.070	0.218	0.104	1.073	0.700˜1.645	0.747
Degree of labyrinthine hydrops	0.100	0.120	0.692	1.105	0.873˜1.398	0.405

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`Tables.docx` available at https://authorea.com/users/508269/articles/586223-expression-of-aqp8-in-serum-of-patients-with-meniere-s-disease-and-its-value-in-evaluating-the-degree-of-hydrolabyrinth-and-predicting-prognosis