Expression of miRNA-223 and NLRP3 gene in IgA patients and intervention of traditional Chinese medicine

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ABSTRACT

Objective: The purpose of this study was to investigate the expression of miRNA-223 and NLRP3 in IgA patients and the intervention of traditional Chinese medicine (TCM), so as to realize the basic pathological changes of IgA patients, the expression of miRNA-223 and NLRP3 in IgA patients and the changes of patients’ body indexes before and after the treatment of TCM.

Methods: Firstly, according to the clinical data, patients with IgA nephropathy were divided into different groups according to their pathological changes. After that, the chemical sections and staining steps of the immune kidney were carried out. Immunohistochemical pv-9000 two-step method was used to stain it. By this method, miRNA-223 and NLRP3 genes in kidney were determined. After that, the image analysis method was used for semi quantitative experiment. Finally, the intervention of TCM was used to study the changes of indicators before and after treatment.

Results: miRNA-223 and NLRP3 genes could be found mainly in the cytoplasm of renal tubular epithelial cells and the interstitium of monocyte in renal tissue, and there were significant differences between miRNA-223 and NLRP3 genes in the expression levels of proteinuria alone, hematuria albuminuria alone and hematuria alone. There was a positive correlation between miRNA-223 and NLRP3 expression and 24-hour urinary protein in IgA nephropathy. In addition, it also had positive correlation with MCP-1 and IL-18.

Conclusion: This study could provide some direction and guidance for clinical diagnosis and treatment of IgA nephropathy.

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1. Introduction

IgA nephropathy is a kind of glomerulopathy which mainly occurs in mesangial area and mainly consists of IgA or IgA deposition (Chun et al., 2016; Wan et al., 2018). This disease is the most common type of primary glomerulonephritis. Among them, 30–40% of all nephrotic patients in China are renal biopsy patients, and 40–47.2% are primary glomerular diseases (Alfakeekh et al., 2019; Wan et al., 2018). In clinical diagnosis of IgA nephropathy, in addition to secondary IgA nephropathy, other diseases can also cause IgA deposition in mesangial area, such as Henoch Schonlein purpura nephritis, systemic lupus erythematosus, alcoholic liver disease, etc., which will cause IgA deposition in mesangial area (Hu et al., 2019). The clinical manifestations of this disease are not only complex and diverse, but also play a role in hematoma and proteinuria of different degrees. These clinical manifestations will even cause hypertension and obvious damage to renal function until the later stage of the disease, and eventually cause end-stage renal disease (Wang et al., 2020; Varghese et al., 2019). In the onset period, different stages of the patients will eventually lead to the emergence of proteinuria. However, microscopic hematuria is rarely seen (Cao et al., 2016). Some studies have found that if the prognosis of IgA nephropathy is not good, there will be a lot of proteinuria and renal function damage, which is the clinical indicator of diagnosis. The pathological indexes are glomerular sclerosis and interstitial fibrosis (Fei et al., 2016; Coucha et al., 2017).

At present, many experts and scholars carried out deep research on IgA nephropathy, and found that inflammatory complex plays a
significant role in kidney disease. However, NLRP3 inflammatory complex, as one of them, has a close relationship with the immune regulation and response of the body, which can have a certain sense of microorganisms in the cytoplasm and stress metabolism response (Li et al., 2018). Besides, it can also be activated by pathogen related molecular models, pathogens (PAMPs) and risk related molecular models (DAMPs), which play a great role in the development of many diseases, such as atherosclerosis, diabetes, amyloidosis, etc. (Wang et al., 2015). Some studies have shown that the expression level of NLRP3 mRNA will increase in renal biopsy tissues of IgA nephropathy patients, but the source of expression and the related expression mechanism are still unclear, lacking theoretical basis (Liu et al., 2017). Whether it has a close relationship with proteinuria, renal tubular atrophy, inflammatory infiltration of renal interstitium, and even the influence of traditional Chinese medicine (TCM) on the changes of some indexes of the body, etc., need further study.

The expression of miRNA-223 and NLRP3 in IgA patients and the intervention of TCM are studied. Firstly, according to the clinical data, patients with IgA nephropathy were divided into different groups according to their pathological changes. After that, the chemical sections and staining steps of the immune kidney were carried out. Immunohistochemical pv-9000 two-step method was used to stain it. By this method, miRNA-223 and NLRP3 genes in kidney were determined. After that, the image analysis method was used for semi quantitative experiment. Finally, the intervention of TCM was used to study the changes of indicators before and after treatment. miRNA-223 and NLRP3 genes could be found mainly in the cytoplasm of renal tubular epithelial cells and the interstitium of monocyte in renal tissue, and there were significant differences between miRNA-223 and NLRP3 genes in the expression levels of proteinuria alone, hematuria albuminuria alone and hematuria albuminuria. In addition, it also had positive correlation with MCP-1 and IL-18. This study could provide some direction and guidance for clinical diagnosis and treatment of IgA nephropathy.

2. Materials and methods

2.1. Research subject

Twenty male IgA patients and 30 female IgA patients were selected from Heilongjiang Hospital of traditional Chinese medicine. They aged 32.54 ± 9.65 years old, and the clinical data of all patients was collected. Before the study, informed consent was obtained from all patients and their families, and approved by the ethics committee of Heilongjiang Academy of Traditional Chinese Medicine.

Exclusion criteria: Patients with secondary IgA nephropathy such as Henoch Schonlein purpura nephritis, systemic lupus erythematosus, SJogren’s syndrome, ankylosing spondylitis, alcoholic cirrhosis and chronic hepatitis were excluded. In addition, before renal biopsy, none of the patients had been treated with immunosuppressant such as ACE inhibitor and adrenocortical hormone. All the remaining patients after exclusion criteria screening were included in this study.

Diagnostic scoring criteria: According to the pathological changes of the kidney under the light microscope, it needed to be scored, including the scope of inflammatory cell infiltration in the renal interstitium, the area of renal tubular atrophy and the area of renal interstitial fibrosis. Among them, the scoring criteria of renal tubules and interstitial lesions were as follows. If there was no corresponding pathological change, it was 0 point. The corresponding pathological changes were less than 25%, 1 point. The corresponding pathological changes were 25–50%, 2 points. The corresponding pathological changes were more than 51%, 3 points.

Grouping: According to the group of hematuria and albuminuria, it was divided into four groups: simple Hematuria group, simple albuminuria group, hematuria and albuminuria group and normal control group. These four groups were represented by A, B, C and D respectively. According to the pathological changes under the light microscope, it could be divided into two groups: the focal segmental sclerosis group and the mild lesions group. FSGS (focal segmental glomerulosclerosis) and MGA (minor glomerular abnormalities) were used in the group of focal segmental sclerosis.

Th1, Th2, CD4+, and CD25Treg levels were measured before and after the intervention of TCM to reflect the health status of patients.

2.2. Immunohistochemical section

10% neutral formaldehyde solution was fixed, and the kidney tissue embedded in paraffin (Leica company, Germany) was cut into 4 μm thick slices by rotary microtome. In 45 °C warm water, the film exhibition machine (Leica company, Germany) was used for film exhibition. Then, the prepared adhesive slide was used to remove the slide. Baking machine (Tianjin hanglida industry and Trade Co., Ltd.) modulation temperature was 70 °C. The baking time was 1.5 h. It was dried for standby.

2.3. Immunohistochemical staining

The steps of immunohistochemical staining were as follows:

(1) Firstly, dewaxing was required. However, before this, the tissue slices needed to be placed at normal ambient temperature for 60 min. Or, it was put into a 60 °C incubator (Shanghai Experimental Instrument Company) and baked for 20 min. Then, in xylene, 80% ethanol was put into it for soaking for 6 min, a total of five times. The soaking time was the same. Then, the tissue slices were put into 3% H2O2 solution, 95% ethanol, 80% ethanol and 75% ethanol for further immersion. The soaking time was 6 s except for 10 min when the solution was put into 3% H2O2. After immersion, it needed to be rinsed, and the rinses used were tap water and distilled water.

(2) After the above operations, the next step was to carry out tissue antigen repair. Firstly, PH6.0 citrate antigen repair buffer was added to the pressure cooker (Zhejiang SUPOR Co., Ltd.). It should be noted that it should be sufficient to submerge the whole slice. After that, it was necessary to put the viscose glass in the plastic dyeing basket, and then put it into the pressure cooker for heating and pressure. In this process, the speed should not be too fast, it should be slow. It was necessary to put the slide into the buffer and soak it for five minutes. After that, the lid was locked and the small valve was raised for a total of 10 min. After that, it was necessary to put it in cold water. After the small valve sank, the cover could be opened. After that, it was taken out and cooled at normal ambient temperature for 20 min. Note that it should be placed in a plastic repair box and then rinsed with distilled water and PBS solution. Among them, the number of times of washing with distilled water was two times, three minutes each time. The number of times PBS solution was used for washing was three times, five minutes each time.

(3) After the above operations were completed, an anti-virus working fluid was dripped into it. After that, it was put into a refrigerator with a temperature of 4 °C for a whole night. The concentration of the working solution used was determined according to the pre experiment. After that, Tris
solution was added to mix at a concentration of 1 µg/ml. After that, PBS was used again to wash the tissue adhesive slides (Shitai Experimental Equipment Co., Ltd.) four times for four minutes each time. After that, the polymer peroxidise-anti-Mouse IgG was added to it for incubation. Normal room temperature was at 25 °C for 20 min. Then, the PBS needed to be used again for washing for 3 times. After that, DAB substrate working solution was added to each slice. In the process of drooping, it needed to be evenly distributed.

(4) Then, the microscope (Japanese Olympus cx42) was used to observe the results. When there was a specific staining result on the positive image but the background was not stained, the film needs to be collected. In this experiment and the pre experiment, the time for the positive film to develop color and receive film was two minutes, and the time for the negative film to receive film without coloring the background was five minutes. After the complete color rendering, it was washed with tap water to make the color rendering stop. In the process of re-dyeing, hematoxylin dye solution was used for three minutes. Then, it needed to be rinsed with tap water. After that, the differentiation operation was needed, which used 1% hydrochloric acid alcohol for one minute. Then, it needed to be rinsed again, with tap water and distilled water. Among them, the washing time with distilled water is three minutes. Then, the tissue adhesive slides were immersed in different concentrations of ethanol for three minutes. Then, it was soaked in xylene for five minutes. Finally, it needed to be dried and sealed with neutral gum.

2.4. The judgment of immunohistochemical staining results

Single blind method was used. The results of immunohistochemistry were evaluated by a senior doctor of pathology. The scoring standard referred to the method introduced by olaussen Ka, i.e. 5 high power (X400) visual fields were randomly observed and 100 tumor cells were counted for each section. The half quantitative H-score score was obtained by the product of the cell color intensity score and the percentage of positive tumor cells as the relative intensity of protein expression. The specific scoring method was as follows. According to the degree of color development, it could be divided into four levels: 0 for negative (non staining), 1 for weak positive (light yellow), 2 for positive (brown), and 3 for strong positive (brown). Then, according to the percentage of positive tumor cells, the score was 0, 1–9%, 0.1, 10–50%, 0.5, > 50% and 1.0.

2.5. Analysis of antigen expression

The expression of inflammatory corpuscles was measured semi quantitatively by using the system of color pathological image and text analysis. Each immunohistochemical section was photographed in multiple visual fields randomly selected under the microscope and the microscope and stored in a computer. Then, the image analysis system was used to separate the positive target from the background color, and the average optical density was used to represent the expression of the corresponding measurement index, and the area and integrated optical density were measured respectively.

2.6. Statistical methods

All the measured data were input into SPSS20.0 for statistical analysis. The counting data was expressed in the rate (%). The comparison between groups was performed by x² test. The difference was statistically significant with P < 0.05, and the test level was α = 0.05.

3. Results

3.1. Expression of miRNA-223 and NLRP3 in different clinical types of IgA nephropathy

According to the final immune results, miRNA-223 and NLRP3 genes could be found mainly in the cytoplasm of renal tubular epithelial cells and the interstitium of monocyte in renal tissue. Compared with IgA group and control group, the expression level of miRNA-223 and NLRP3 gene in the cytoplasm of renal tubular epithelial cells and the interstitium of monocyte in IgA group increased significantly. However, the gene expression of miRNA-223 and NLRP3 in the control group was not significant at all, and the difference between them was statistically significant.

According to Fig. 1, miRNA-223 and NLRP3 were significantly different in the pure albuminuria group, the expression level of hematuria albuminuria group and the pure hematuria group. The expression level of miRNA-223 and NLRP3 in pure hematuria group was the lowest, which was statistically significant.

3.2. Expression of miRNA-223 and NLRP3 in different pathological types of IgA nephropathy

According to Fig. 2, the expression of NLRP3 gene in different renal tissue types was also different. Compared with the mild lesion group, the expression of NLRP3 gene was generally high in the mild lesion group, but relatively low in the mild lesion group. There was significant statistical difference between the two groups.

According to Fig. 3, Fig. 4 and Fig. 5, it can be seen that NLRP3 had a positive correlation with its expression in FSGS group, whether it was interstitial inflammation, tubular atrophy or interstitial fibrosis. The expression level fluctuated from 0.3 to 0.9. It could be seen that the expression of NLRP3 gene could effectively show the location and type of renal damage, which had a great guiding significance for renal damage.

3.3. The relationship between miRNA-223 and NLRP3, 24-hour urinary protein, MCP-1 and IL-18 in IgA nephropathy

According to the correlation chart of NLRP3 and 24-hour urinary protein, MCP-1 and IL-18 in IgA nephropathy in Fig. 6, it could be seen that the expression of miRNA-223 and NLRP3 was positively correlated with 24-hour urinary protein, MCP-1 and IL-18 in IgA nephropathy.
and NLRP3 in renal tissue of IgA nephropathy patients had a certain relationship with 24-hour urinary protein, and there was a positive correlation between them. The clinical results showed that the expression of miRNA-223 and NLRP3 had no definite correlation with serum creatinine, urea nitrogen and other clinical indicators, and there was no statistical difference between them.

According to Fig. 7, Fig. 8 and Fig. 9, it could be seen that the expression of NLRP3 in renal tissue of IgA nephropathy patients has a certain relationship with MCP-1 in hematuria albuminuria group, hematuria group and albuminuria group, and there was a positive correlation between them. Therefore, it could be seen that NLRP3 could be used as the relevant standard for IgA nephropathy diagnosis. By detecting MCP-1, the expression of NLRP3 could be realized.
According to Fig. 10, Fig. 11 and Fig. 12, the expression of NLRP3 in renal tissue of IgA nephropathy patients had a certain relationship with the IL-18 in hematuria albuminuria group, hematuria group and albuminuria group, and there was a positive correlation between them. Therefore, it could be known that NLRP3 could be used as the relevant standard for IgA nephropathy diagnosis. By detecting IL-18, the expression of NLRP3 can be roughly realized.

3.4. The effect of TCM before and after intervention on Th1, Th2, CD4+, CD25Treg in patients

According to Table 1, after the application of TCM, Th1, Th2, CD4+, CD25Treg of patients had changed to some extent. Among them, Th1 of patients before and after treatment had a very significant statistical difference. After treatment, the value of Th1 was significantly decreased, but for Th2 and CD4+, CD25Treg of patients was not very significant.

3.5. The effect of TCM before and after intervention on the number of urine red blood cells and urine protein

According to Table 2, after the application of TCM, the number of urine red blood cells and urine protein of patients had changed to some extent. Among them, there was a very significant statistical difference in the number of urine red blood cells before and after treatment. After treatment, the value of urine red blood cells decreased significantly, which also had a great impact on the urine protein of patients. There was a certain statistical significance in

Table 1

| Index type | Th1     | Th2     | Treg    |
|------------|---------|---------|---------|
| Before treatment | 17.8 ± 10.42 | 2.67 ± 1.45 | 5.3 ± 1.54 |
| After treatment  | 13.1 ± 6.3  | 5.2 ± 1.43  | 3.68 ± 1.2  |
| T        | 2.412    | 1.051    | 0.952    |
| p        | 0.02     | 0.31     | 0.346     |
Table 2
The effect of TCM before and after intervention on the number of urine red blood cells and urine protein.

| Index | Number of RBC in urine (pieces/UL) | 24-hour urine protein quantification (g) |
|-------|-------------------------------------|----------------------------------------|
| Before treatment | 34.53 ± 39.37 | 1.29 ± 0.49 |
| After treatment | 22.79 ± 26.3 | 0.75 ± 0.35 |
| T | 2.136 | 2.245 |
| P | 0.02 | 0.026 |

the value before and after treatment, and the amount of urine protein decreased after treatment.

4. Discussion

IgA nephropathy was the most common type of primary glomerulonephritis. If the prognosis of IgA nephropathy was not good, it would produce a lot of proteinuria and renal function damage, which was the clinical indicator of diagnosis. The pathological indexes were glomerular sclerosis and interstitial fibrosis to some extent (Chi et al., 2017). It had been found that inflammatory complex played an important role in renal diseases. However, NLRP3 inflammatory complex, as one of them, was closely related to the immune regulation and response of the body. It could make a certain sense of the microorganisms in the cell cytoplasm and stress metabolism response (Bian et al., 2017; Barbier et al., 2015). However, the source and mechanism of its expression were still unclear and lack of theoretical basis. Therefore, it was very important and necessary to study the relationship between NLRP3 inflammatory complex and miRNA-223 and proteinuria, renal tubular atrophy, inflammatory infiltration of renal interstitium, and the influence of TCM on the changes of some indexes of the body, which could help people better understand the pathogenesis of IgA nephropathy and explore it from the micro perspective.

The expression of miRNA-223 and NLRP3 in IgA patients and the intervention of TCM were studied. Through this study, it could be known that miRNA-223 and NLRP3 genes could be found mainly in the cytoplasm of renal tubular epithelial cells and in the interstitium of monocyte. Moreover, there were significant differences between miRNA-223 and NLRP3 in proteinuria group, hematuria albuminuria group and hematuria group. There was a positive correlation between miRNA-223 and NLRP3 expression and 24-hour urinary protein in IgA nephropathy. This is basically consistent with the results of the causal relationship between NLRP3 inflammatory corpuscles and IgA pathogenesis studied by Tsai et al. (2017). In addition, it had positive correlation with MCP-1 and IL-18. After the treatment with TCM, the number of urine red blood cells and the amount of urine protein decreased significantly. In addition, the T1h value of the patients began to decrease after treatment, indicating that TCM had a good effect on improving kidney disease. miRNA-223 and NLRP3 genes could reflect the type of disease and the location of kidney damage to a certain extent, and help prevent kidney disease. This study could provide some direction and guidance for clinical diagnosis and treatment of IgA nephropathy. Although some achievements have been made in this study, there are still some deficiencies. There are few research samples included, so the research in the future will continue to expand the research scope to consolidate the results of this study.

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