JOJ
Urology & Nephrology
ISSN: 2476-0552

Juniper Publishers
key to the Researchers

Research article
Special Issue – January 2018
DOI: 10.19080/JOJUN.2018.04.555642

JOJ uro & nephron

Serum and Synovial Fluid AQP Levels in Rheumatoid Arthritis and the Effect of a Methotrexate on Dampness-Heat Block Syndrome

Dejan Spasovski*

University Clinic of Rheumatology, Republic of Macedonia, Skopje, Europe

Submission: September 27, 2017; **Published:** January 11, 2017

***Corresponding author:** Dejan Spasovski, University Clinic of Rheumatology, Republic of Macedonia, Skopje, Europe, Tel: +389023147-668; Email: drspasovski@yahoo.co.uk

Abstract

Aim: By measuring serum and synovial fluid aqua porin AQP1, AQP2 and AQP3 levels in rheumatoid arthritis (RA) patients, we aimed to study the effect of Methotrexate (MTX) on AQP expression in RA, and to explore the role of AQP sin the pathogenesis of RA and the mechanism of action of MTX. To determine if there is a change in the clinical indicators in the course of disease and if that change correlates with the dynamics of the quantity of excreted enzymes in urine, reactants of the acute phase and index of disease activity (DAS28).

Methods: Using the colorimetric method, as well as immunoturbidimetric assay for detection of AQP1, AQP2 and AQP3 levels, we examined samples of 90 participants (30 patients with knee joint effusion were assigned to the RA synovial fluid group). For the pre-treatment control groups, 30 healthy volunteers were recruited as the healthy control group and 30 osteoarthritis (OA) patients with knee joint effusion were included as the OA synovial fluid control group.

Results: After treatment for 2 weeks, serum AQP1, AQP2 and AQP3 levels were significantly increased in the combined treatment group ($P<0.05$), but there was no significant increase in the MTX group as compared to the pre-treatment control group ($P>0.05$).

Conclusion: AQPs have accuracy as a new therapeutic target for RA and to provide a new direction for RA research and treatment.

Keywords: Rheumathoid arthritis; C-reactive protein; Metotrexate

Abbreviations: AQPs: Aquaporins; ELISA: Enzyme-Linked Immunes or Bent Assay; ARA: Association for Rheumatism

Introduction

The recently identified Aquaporins (AQPs) are widely present on the cell membrane and belong to a family of specialized channel proteins for selective and efficient transport of water molecules, and there are 11 types of AQPs discovered so far. AQP scan significantly increase cell membrane water permeability and thus are involved in secretion, absorption and transcellular hemostasis of water, which are closely associated with the pathogenesis of certain diseases with water metabolism disorders [1].

Aquaporin1 (AQP1) is the primary type of water channel that is widely distributed in the circulatory system, digestive system and immune system [2]. Studies have shown that AQP1 and AQP3 express in the articular cartilage, synovial fluid and synovial cells both in R a patients and animal models [3,4], with articular cartel age being the main site of their expression [5]. Aquaporin2 (AQP-2) is a water channel identified by Fushimi in 1993 [6], which is located at the renal collecting duct chief cells at the luminal side and vesicles near the luminal side. AQP-2 is the key protein regulating water permeability at the renal collecting duct and thus is considered an essential protein for the maintenance of body water hemostasis [7]. However, currently there are no studies on AQP1 and AQP3 in rheumatoid arthritis TCM syndromes, or reports of AQP2 in rheumatoid arthritis. Therefore, research data are lacking in this area, and the role and regulatory mechanisms of AQPs in RA require in-depth studies to improve our understanding of the pathogenesis of RA and to discover additional therapeutic directions and drugs.

A long-term clinical study by our research group showed that a compound traditional

Chinese herbal medicine, had confirmed efficacy for the treatment of active RA [8]. Thus, based on our previous study, here we used an enzyme-linked immunes or bent assay (ELISA)

to detect serum and synovial fluid levels of water channel proteins AQP1, AQP2 and AQP3 in RA patients, and we observed the effect of MTX combined with SYD on AQP express ion levels.

Material and Methods

The diagnosis of the patients included in the study is based on the revised diagnostic criteria for classification of Rheumatoid arthritis proposed in 1987 by the American Association for Rheumatism (ARA) [9]. In order to include the patient in the group with RA, he should fulfill at least 4 of the 7 criteria. Criteria 1-4 should persist at least 6 months.

Using the colorimetric method, as well as immunoturbidimetric assay for detection of AQP1, AQP2 and AQP3 levels, we examined samples of 90 participants (30 patients with knee joint effusion were assigned to the RA synovial fluid group). For the pre-treatment control groups, 30 healthy volunteers were recruited as the healthy control group and 30 osteoarthritis (OA) patients with knee joint effusion were included as the OA synovial fluid control group. Other patients negated use of other drugs such as golden salts, antibiotics or diuretics. Specimens are collected in the period of 2 years.

Inclusion criteria

In the study are included patients with RA, aged 18-65 years, not previously treated with NSAIDs or DMARDs.

Exclusion criteria

From the study are excluded patients with diseases or conditions that could influence results directly or indirectly:

- Patient younger than 18 years.
- Patients with previous history of disease of the spleen, thyroid gland, liver, kidneys, hematological, cardiovascular, neurological, autoimmune and lung diseases.
- Patients with diabetes mellitus, febrile conditions, acute infections, neoplasms.
- Patients with uric arthritis, SLE, mixed connective tissue disese, vasculitis.
- Patients with history of blood transfusion and patients with body overweight.
- Patients with history of use of drugs from the base line.
- Patients that in 0 point had increase level of glucose, serum ind urine urea and creatinine, blood hypertension, smokers and blood and enzyme disorders
- Patients previously treated with salycilates, antibiotics, golden salts or diuretics
- All the patients took part in this study voluntarily, so the ethic criteria for this study are fulfuled.

Clinical estimation of disease activity

Clinical estimation is made by subspecialist in the fild. Disease activity is estimated using DAS 28 index (Disease Activity Score - DAS 28). The index uses mathematical formula to obtain unique composite quantitative score, which consists of: palpabile painful joints (maximal niumber 28), swallen joints (maximal niumber 28), Erythrocyte sedimentation rate (ESR) and patient's estimation for disease activity (0-100 mm). Visual Analogue Scale - VAS) and morning stiffness (minutes). DAS 28 index ranges from 0 to 10 and score <3.2 qualifies the disease as low active.

Laboratory estimation

For clinical estimation of the disease it is necessary to examine following laboratory variables: complete blood count and differential, reactants of the acute phase, ACPA-antibodies, C-reactive protein (CRP), Rheumatoid factor (RF) and Erythrocyte sedimentation rate (ESR), alcaline phosphatase (AP), aspartat aminotransferase (AST), alanin aminotransferase (ALT), creatin kinase (CK), laktat dehydrogenase (LDH), serum urea, serum creatinin. Urine samples are taken not only for rutine analyses, but also for determination of NAG and microalbuminuria.

Statistical analysis

To test the significance of the differences between two aritmetical means i.e.,proportions is used the Student t-test. To compare the mean values of certain numerical parameters between two groups was used Wilcoxon-matched test for independent species. Sensitivity and predictivity for positive and negative test of the examined markers is determined with the sensitivity and specificity test. P-value between 0.05 and 0.1 is considered statistically significant. Data analysis is performed with statistical package Statistica 7.0.

Results

Levels of AQP1expression

Table 1: Comparison of AQP1, AQP2 patient in each group of patients with RA.

	Group AQP1 Expression		Group AQP2 Expression	
RA serum group	1.13±0.5413	12.91±10.075	0.93±0.4813	16.35±7.415
Healthy control group	1.17±0.4818	14.1±1.075	1.27±0.4718	18.80±0.337
RA joint fluid group	1.19±0.6719	11.91±11.234	1.58±1.4023	16.50±9.694
OA joint fluid groupc	1.20±1.0420	12.08±10.682	1.80±0.3326	1.80±0.3326

How to cite this article: Dejan S. Serum and Synovial Fluid AQP Levels in Rheumatoid Arthritis and the Effect of a Methotrexate on Dampness-Heat Block Syndrome. JOJ uro & nephron. 2018; 4(4): 555642. DOI: 10.19080/JOJUN.2018.04.555642.

Compared with the healthy control group, peripheral blood AQP1 levels were significantly decreased in dampness-heat block age type RA patients (t=2.29, P=0.04). AQP1 was detected in the synovial fluid of both the RA dampness-heat block age patients and the OA patients, but the rewash no significant difference between the two groups (t=0.05, P=0.963). The peripheral blood and synovial fluid AQP1 levels were not significantly different in dampness-heat block age type RA patients (t=1.07, P=0.305) (Table 1).

Levels of AQP2 expression

Compared with the healthy control group, peripheral blood AQP2 levels were decreased in the dampness-heat block age type RA patients and the difference was statistically significant (t=2.17, P=0.04). Compared with the OA synovial fluid group, the synvovial fluid AQP2 level of the RA dampness-heat block age patients was not significantly different (t=0.6, P=0.558). In addition, there was no significant difference between peripheral blood and synovial fluid AQP2 levels in the dampness-heat block age type RA patients (t=0.56, P=0.585).

Levels of AQP3 expression

Compared with the healthy control group, peripheral blood AQP3 decreased in the dampness heat block age type RA patients and the difference was statistically significant(t=2.58, P=0.03).

Compared with the OA synovial fluid group, the synovial fluid AQP3 level in the RA dampness heat block age patients was not significantly different (t=0.24, P=0.816). Furthermore, there was no significant difference between peripheral blood and synovial fluid AQP3 levels in the dampness-heat block age type RA patients (t=0.55, P=0.59).

Discussion

Rheumatoid arthritis (RA) is a common multi-system rheumatological disease. In the warring States period, traditional Chinese medicine (TCM) categorized RA as one of the paralytic diseases, and pointed out its close relationship with «dampness». The earliest extant medical literature «Non include» first proposed the definition of paralytic disease, and provided detailed discussion of its pathogenesis, syndrome classification, outcome and prognosis as follows: «the so-called paralytic diseases are caused by invasion of severe wind, cold and dampness at various stages"; «mixed wind, cold and dampness invades the body and causes paralytic diseases"; and «hefty dampness takes over and causes paralytic disease" [10]. The "Analytical Dictionary of Characters" also states that "paralytic disease is a condition of dampness". Chapter 11 of the "Analytical Dictionary of Characters" defined dampness as «originated from water and so it can cover; when water covers the earth, dampness forms" [11]. In another word, «dampness» is «water".

The «No include-Essentials on disease and therapy «states that: «all spasms and neck rigidity area scribed to dampness" [12]. Normal water metabolism is coordinated by the lung, spleen and kidney. Specifically, water is distributed to the whole body through dissipation and precipitation through the lung, conversion and transportation through the spleen and filtration and transpiration through the kidney. The «Medical Law» states that: «water-related diseases are mainly related to the lung, spleen and kidney" [1]. Dysfunction of the lung, spleen and kidney often leads to moisture accumulation and internal blockage. Overall, dampness initiates the pathogenesis of RA, which is frequently the result of wind-chill or wind-heat and affects multijoints of the body, causing symptoms such as joint swelling and pain, stiffness and limited flexion and extension. Water metabolism disorders and water accumulation and block age are common pathogenic factors of RA. Chronic accumulation of dampness could transform to heat so dampness-heat blockage syndrome is commonly seen in RA patients in the clinic.

Trujillo et al. found that AQP1 expressed at both synovial cells and chondrocytes in deep layers of articular cartilage and their expressions were upregulated in RA as compared to normal tissues, implying that AQP splay an import antrolein maintaining homeostasis of the synovial fluid. A study by Song Weietal [8] showed that, the RA synovium had expressed AQP1, and AQP1 mRNA expression was reduced with symptom relief and resolved inflammatory markers through effective treatment. A study by Mobasheri et al. [5], confirmed these findings and proposed that an important role of AQP1 in the pathogenesis of RA may be that its abnormal expression is one of the leading reasons for synovitis, joint effusion and pannus formation. A study by Yanlin Yueetal [6] also found that AQP1 and AQP3 mRNA as were expressed in RA synovial cells, and the expression of AQP1 mRNA was significantly higher than the expression of AQP3 mRNA. Mengetal showed that, AQP3 presented on the articular cartilage and synovial membrane, which was correlated with joint effusion formation, articular cartilage and synovium degeneration, and swelling of surrounding joint tissues. A study by Song Weietal [8] also found that factors, such as dampness-heat, caused increased AQP3 expression, and thus they suggested that abnormal AQP3 expression may be one of the pathogenic factors in dampness-heat syndrome.

Conclusion

Pre-and post-treatment AQP changes in control synovial cells need to be examined to further explore the signaling pathways and regulatory factors for AQPs in the pathogenesis of RA.

References

1. Zhang BY (1985) Zhong Yi, NeiKe Xu (1st edn) Shanghai: shanghai science & technology press, China pp: 232.

2. Verkman AS (2007) Role of aquaporins in lung liquid physiology. Respir Physiol Neurobiol 159(3): 324-330.

3. Trujillo E, Gonzalez T, Marin R, Martín-Vasallo P, Marples D, et al. (2004) Human articular chondrocytes synoviocytes and synovial microvessels express aquapodn water channels; upregulation of AQP1 in rheumatoid arthritis Histol Histopathol 19(2): 435-444.

How to cite this article: Dejan S. Serum and Synovial Fluid AQP Levels in Rheumatoid Arthritis and the Effect of a Methotrexate on Dampness-Heat Block Syndrome. JOJ uro & nephron. 2018; 4(4): 555642. DOI: 10.19080/JOJUN.2018.04.555642.

4. Wei S, Liu J, Qiu Le (2008) Effect of Tou-Bi-Zhuan-Qi on the expression of aquaporin-1 in the synovial tissue of joint in patients with rheumatoid arthritis. Journal of Clinical Rehabilitative Tissue Engineering Research 12(28): 5444-5447.

5. Mobasheri A, Wray S, Marples D (2005) Distribution of AQP2 and AQP3 water channels in human tissue microarrays. J MolHistol 36(1-2): 1-14.

6. Yue YL, Liu JP, Liu QS, Jiang H, Xie C (2013) Effect of acetazolamide on AQP1 and AQP3 expressions in fibroblast-like synoviocytes of rheumatoid arthritis. Chin J Cel and Mol Immunol 29(11): 1177-1180.

7. Li Xuejun, Yu Heming (1996) Molecular biological study of the water channels [J]. Progress in Physiological Sciences 27(1): 19-24.

8. Wei S, Lao SX, Huang ZX, et al. (2008) Effect of Dampness-heat Factor on the Expression of Aquaporin 3, 4 Gene in Gastric Mucosa of Rats [J]. Chinese Journal of Information on Traditional Chinese Medicine 15(6): 34-36.

9. van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH (1996) Development and validation of the European league against rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American college of rheumatology and the world health organization/international league against rheumatism criteria. Arthritis Rheum 39(1): 34-40.

10. Wang QQ (2003) Nei Jing, Xuan Du (1st edn) Beijing: Traditional Chinese Medicine Publishing House of China pp. 151.

11. Xu S, Shuo W, Jie Zi (1963) Beijing: Zhonghua Edition pp. 155.

12. Wang QQ (203) Nei Jing, Xuan Du (1st edn) Beijing: Traditional Chinese Medicine Publishing House of China pp.126.

DOI: 10.19080/JOJUN.2018.04.555642

How to cite this article: Dejan S. Serum and Synovial Fluid AQP Levels in Rheumatoid Arthritis and the Effect of a Methotrexate on Dampness-Heat Block Syndrome. JOJ uro & nephron. 2018; 4(4): 555642. DOI: 10.19080/JOJUN.2018.04.555642.