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The gelsolin level in patients with primary Sjogren's syndrome

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Abstract. – OBJECTIVE: Gelsolin (GSN) is a multifunctional protein that can regulate cell proliferation, apoptosis, inflammation and infection. GSN has been reported to be involved in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS) and many other diseases. The role of GSN in primary Sjogren's syndrome (pSS) remains still unclear. The aim of this study is to investigate the changes of GSN level in serum and whole blood cells of pSS patients and evaluate the relationship between GSN and fatigue or other clinical indicators.

PATIENTS AND METHODS: The cross-sectional study included 47 pSS patients (1 male and 46 females, average age: 52.83±12.63 years) and 51 healthy controls (all females, average age: 50.61±9.86 years). The patients were collected from the Second Affiliated Hospital of Harbin Medical University, China, without the age and sex differences. The levels of GSN in serum of pSS patients and the healthy controls were measured by Western blotting. The sequencing gene expression omnibus (GEO) data from National Center for Biotechnology Information (NCBI) about GSN levels in the whole blood cells of pSS patients and the healthy controls were analyzed by R language.

RESULTS: Compared with healthy controls, the level of GSN was significantly decreased in the serum of pSS patients (98.89 ± 28.94 *vs.* 131.6 ± 37.1 μg/ml, $p<0.001$). The expression of GSN in the whole blood cells of pSS patients was significantly lower than that in the healthy controls (6.4 ± 0.19 *vs.* 6.6 ± 0.17, $p<0.01$). Compared to non-fatigued pSS patients, the level of GSN was down-regulated in serum (85.69 ± 27.08 *vs.* 111.52 ± 24.71 μg/ml, $p<0.01$) and whole blood cells (6.43 ± 0.18 *vs.* 6.58 ± 0.21, $p<0.001$) in fatigue pSS patients. However, there was no significant correlation between the level of GSN and EULAR Sjogrens syndrome disease activity index (ESSDAI) in pSS patients ($p=0.73$).

CONCLUSIONS: GSN is decreased in serum and whole blood cells of pSS patients, and it is much lower in fatigue patients than that in non-fatigue patients. The correlation between the level of GSN and ESSDAI was not significant in pSS patients.

Key Words:
Gelsolin, Primary Sjogren's syndrome, Correlation, ESSDAI.

Introduction

Gelsolin (GSN), a member of the GSN family, was first identified in rabbit pulmonary macrophages by Yin and Stossel in 1979[1]. GSN has the ability to regulate actin assembly and disassembly[2], remove the actin released into the blood during tissue and cell damage, and reduce its damage to the body[3]. In addition, GSN was found to be involved in carcinogenesis, apoptosis, inflammation, aging and other biological process[4]. Recently, studies have shown that change of GSN level is associated with some autoimmune diseases. Compared with the healthy control, the level of GSN is decreased in serum of IgA nephropathy patients, and it is positively correlated with glomerular filtration rate[5]. In addition, GSN and IgA promote glomerular fibrosis through the transforming growth factor-β (TGF-β)1/Smads signaling pathway[6]. In multiple sclerosis (MS), the level of GSN in serum of patients is reduced. Moreover, in the experimental allergic encephalomyelitis (EAE) models, administrated exogenous GSN can decrease extracellular actin and myeloperoxidase activity in the brain, resulting in the delay of the onset of disease[7,8]. Besides, the level of GSN is lower in serum of patients with rheumatoid arthritis (RA) and systemic lupus erythematosu (SLE) than the healthy controls. In patients with SLE, the

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level of GSN in serum is negatively related with EULAR Systemic Lupus Erythematosus disease activity index (SLEDAI)[9]. However, the role of GSN in primary Sjogren's syndrome (pSS) patients remains unclear. Sjogren's syndrome (SS) is a chronic autoimmune disorder with common clinical manifestations such as dryness of the mouth and eyes, which results in the lymphocytic infiltration of the exocrine glands and polyclonal B-cell activation[10,11]. The primary SS patients (pSS) account for about 0.03–0.1% of the general population[12]. At present, it is believed that the genetic background and environmental factors trigger the production of auto-antibodies, thus leading to pathological damage[13,14]. A number of studies have also shown that chronic fatigue is a common symptom reported in about 68% of pSS patients[15-17]. In this study, we investigated the changes of the expression level of GSN in serum and whole blood cells of pSS patients and healthy controls. Latterly, we evaluated the relationship between GSN and fatigue in pSS patients, and the correlation between the level of GSN and ESSDAI.

Patients and Methods

Patients and Samples

Blood samples were collected from 47 cases of pSS patients and 51 healthy controls in the Second Affiliated Hospital of Harbin Medical University (Harbin, China) from June 2019 to August 2019. pSS patients aged 18-65 years were diagnosed following the 2002 diagnostic criteria of American–European Consensus Group criteria (AECC). The exclusion criteria included overlapping SLE, systemic sclerosis, rheumatoid arthritis, and polymyositis. All of the patients provided informed consent. The characteristic of pSS patients and healthy controls was shown in Table I. This study obtained the approval of the Ethics Committee of Second Affiliated Hospital of Harbin Medical University in Heilongjiang Province, China (No. KY2019-200).

Clinical Parameters

EULAR Sjogrens syndrome disease activity index (ESSDAI) scale included 12 organ by organ domains. Each domain was divided into three or four levels according to the degree of activity, and scored as 0 (no activity), 1 (low activity), 2 (moderate activity), or 3 (high activity). These scores were then multiplied by an assigned weight factor, ranging from 1 to 6, with the total score ranging from 0 to 123 points. The ESSDAI score<5 was considered low disease activity, ESSDAI score = 5-13 was considered moderate activity, and ESSDAI score≥14 was considered high activity[18]. Fatigue severity was measured using the Profile of Fatigue (PROF) Questionnaire which has been validated for pSS[19].

GSN Concentration Measurement

The level of GSN was determined as our previous study[9]. Briefly, serum was isolated from blood of patients and diluted to 1:15 in 1×sample buffer (SB; 10% Glycerol, 2% SDS, 62.5mM Tris-HCl, 0.03% Bromophenol blue and 5% β-Mercaptoethanol, pH 6.8). Recombinant human gelsolin (rhp-GSN) (Cytoskeleton, Denver, USA) was diluted

Table I. pSS patients and healthy control's characteristic.

Characteristic	pSS patients (n = 47)	Healthy controls (n = 53)
Sex (Male/female)	1/46	0/51
Age, mean ± SD years	52.83 ± 12.63	50.61 ± 9.86
Dry mouth +/-	41/6 (87.2)	
Dry eye +/-	29/18 (61.7)	
Fatigue +/-	23/24 (48.9)	
ANA, no. +/-	42/5 (89.4)	
Ro (SSA), no. +/-	38/9 (80.8)	
La (SSB), no. +/-	16/31(34)	
ACA, no. +/-	10/37 (21.3)	
Schirmer's test +/-	39/8 (82.9)	
IgA (g/L) mean ± SD	3.51 ± 1.68	
IgM (g/L) mean ± SD	1.52 ± 1.08	

ANA: Antinuclear antibody; SSA: Sjögren's syndrome A antibodies; SSB: Sjögren's syndrome B antibodies; ACA: anticentromere antbody; Schirmer's test: negative test > 5 mm/5 min resulting in a negative test.

to form a protein gradient (50 ng, 100 ng, 150 ng and 200 ng). Samples (15 μL) were run with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose (NC) membranes. For the determination of GSN, the membranes were incubated with the mouse monoclonal anti-gelsolin antibody for 2 h at 37°C (1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA). Then, the secondary antibody goat anti-mouse IgG-horseradish peroxidase (HRP) was treated for 1 h at 37°C (1:2000 dilution; Wuhan Boster Biological Technology Co., Ltd., Wuhan, China). Chemiluminescence detection was performed with Super-Signal West Pico Chemiluminescent Substrate (Pierce, Rockford, CA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) version 19.0 (IBM, Armonk, NY, USA) was used for data analysis. Data were expressed as mean ± standard deviation (SD). Kolmogorov-smirnov was used to detect whether the data conformed to normal distribution. Independent sample *t*-test was performed for data conforming to normal distribution, while Mann-Whitney U test was performed for data not conforming to normal distribution. Regression analysis was performed by SPSS19.0. In addition, in order to eliminate batch effect between GEO website data of different platforms, homogenization was performed using Sva package of R language, and then *t*-test was performed using limma package of R language. $p<0.05$ is considered statistically significant.

Results

The Expression of GSN is Decreased in Whole Blood Cells of pSS Patients

To evaluate the change of GSN expression in pSS patients, we searched pSS related data (All these pSS patients were considered to be clearly diagnosed, but information about clinical symptoms of patients could not be found on the website) on GEO website (GSE66795 and GSE84844) and collected the data from 30 healthy controls and 190 pSS patients[20] to analyze the expression of GSN. The results showed that the expression of GSN in the whole blood cells was significantly decreased in pSS patients compared to healthy controls (Figure 1).

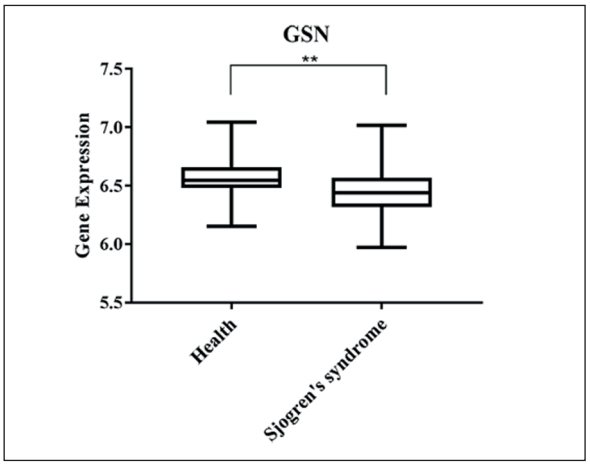

Figure 1. The expression of GSN in whole blood cells in pSS patients and health control. The value is expressed as mean±SD. $^{**}p<0.01$, Sjogren's syndrome *vs.* health.

The Level of GSN in Serum of pSS Patients is Lower Than the Healthy Controls

In order to verify the result of GEO database, the level of GSN in serum of pSS patients was detected by Western blotting. The result showed that the level of GSN in serum of the pSS patients is lower than that in the healthy controls (Figure 2).

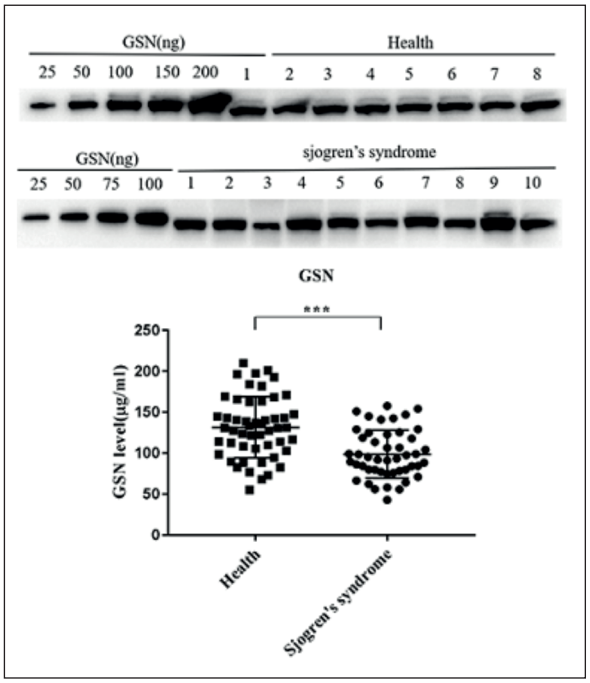

Figure 2. The level of GSN in serum from the pSS patients and the healthy controls. The value is expressed as mean±SD. $^{***}p<0.001$, Sjogren's syndrome *vs.* health.

The Expression of GSN is Down-Regulated in pSS Patients with Fatigue

To investigate whether GSN is associated with fatigue in pSS patients, we searched the relevant data from GEO website (GSE66795 and GSE84844). The results showed that compared to healthy controls and non-fatigued patients, the expression of GSN was significantly prohibited in fatigued patients (Figure 3). Furthermore, in order to verify the results of GEO database, all pSS patients were divided into two groups (fatigued and non-fatigued pSS patients' groups) to investigate the relationship between GSN and fatigue. It was found that the level of GSN in fatigue pSS patients is significantly down-regulated compared to the healthy controls or non-fatigued pSS patients (Figure 4).

Relationship Between the Level of GSN and Clinical Indicators in pSS Patients

ESSDAI score was performed in pSS patients, and the correlation between GSN and ESSDAI was analyzed. The results showed that there is no correlation between the level of GSN and ESSDAI in pSS patients (**Supplementary Figure 1**). Meanwhile, the presence or absence of pulmonary interstitial lesions (**Supplementary Figure 2A**), abnormal immunoglobulin and complement (**Supplementary Figure 2B**), blood system involvement (**Supplementary Figure 2C**) and renal involvement (**Supplementary Figure 2D**) has no effect on GSN level in pSS patients.

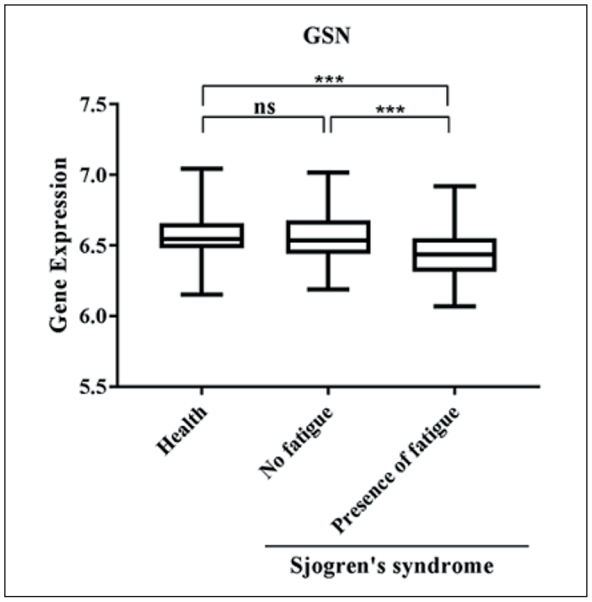

Figure 3. The expression of GSN in health control and pSS patients with fatigue or non-fatigue. The value is expressed as mean±SEM. NS:no significantly difference; ***p<0.01, pSS patients with fatigue *vs.* pSS patients with non-fatigue or health control.

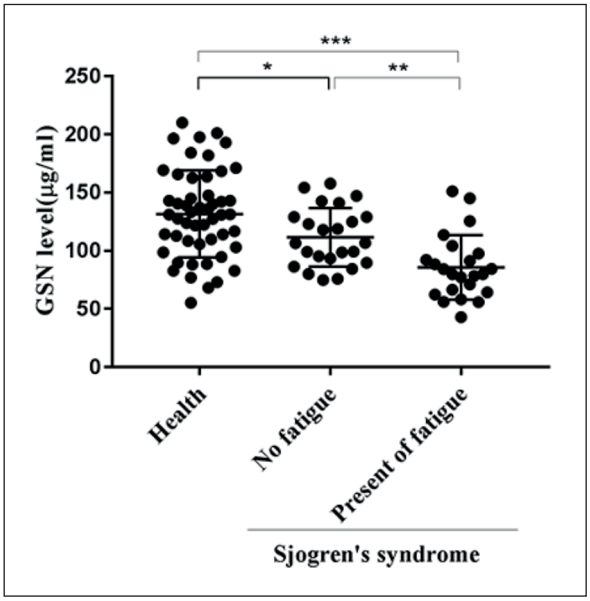

Figure 4. The level of GSN in health control and pSS patients with fatigue or non-fatigue. The value is expressed as mean±SD. *p<0.05, **p<0.01, ***p<0.001, pSS patients with fatigue *vs.* pSS patients with non-fatigue or health control.

Discussion

Gelsolin (GSN), a known actin modulator, has therapeutic effects in many diseases including sepsis, burn hurts, multiple sclerosis and diabetes[21]. In sepsis mice, the exogenous GSN can reduce disease activity score and improve the survival rate[22,23]. In the rat model of MS, administrated exogenous GSN reduces the disease through inhibiting the activity of extracellular actin and MPO induced by S1P[8]. Exogenous GSN (8 mg/mL) can prevent acetic acid-induced pain and relieve carrageenan-induced foot swelling in mice, which has equivalent effects of 10 mg/kg of diclofenac sodium. GSN also reduces the expression level of TNF-α and IL-6[24]. The changes of gelsolin levels are related to diseases and can be used to predict the prognosis of diseases. For instance, the plasma gelsolin concentrations are significantly lower among burn patients with sepsis than those without sepsis, and

it is negatively associated with the occurrence of sepsis[25]. GSN levels are significantly decreased at 6 h post-operation in acute kidney injury (AKI) patients, and GSN in post-cardiopulmonary bypass (CPB) patients may have beneficial effects on diminishing inflammatory responses[26]. In this study, it is demonstrated that the expression level of GSN in both serum and whole blood cells is decreased in pSS patients compared with healthy controls. Fatigue is a prominent feature in majority pSS patients [27,28]. Previous reports have shown that fatigue accounts for about 68% patients[17,18] and accounts for 48.9% in this study. The change of related genes expression level is thought to be involved in fatigue development[29,30]. Norheim et al[29] have demonstrated that single nucleotide polymorphism (SNP) variations are associated with fatigue in pSS patients and revealed that the SLC25A40 is stronger in pSS high fatigue patients than controls. In addition, fatigue may be related with inflammation. Although Hartkamp et al[31] did not show any association between fatigue and the serum inflammatory factors. Howard et al[27] found that interferon (IFN)-γ-induced protein-10 (IP-10), IFN-α, IFN-γ and lymphotoxin-α (LT-α) are significantly higher in patients with pSS than non-fatigued controls. Davies et al[32] revealed that the pain, depression, and pro-inflammatory cytokines are considered to be the most powerful predictors of fatigue in pSS. In this study, we discovered that pSS patients with low serum GSN are more prone to fatigue. In patients with RA and SLE, the serum GSN is also lower than that in healthy controls[9], and patients also often experienced fatigue. Therefore, we speculated that GSN might be related with fatigue in pSS patients. Nowadays, the ESSDAI used as a gold standard to elevate disease activity in clinical studies[33]. In our study, GSN is neither correlated with disease activity (ESSDAI), nor with other clinical indicators, including Lung damage, blood system damage and others.

There are still limitations in this study. First, fatigue is one of the common complaints of pSS patients, and decreased expression of GSN must have some effects on it. We found that the decrease of serum GSN in pSS patients is related to fatigue, but the specific mechanism is still unclear. Second, whether GSN is also associated with fatigue in these patients of other autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus remains unknown. Therefore, more investigations need to explore the importance of GSN for pSS fatigue, how GSN plays a role in it, and whether supplemented GSN can relieve symptoms of fatigue in pSS patients.

Conclusions

Our results showed that the level of GSN is significantly lower in pSS patients than healthy controls. The pSS patients with low serum GSN level are more prone to fatigue. It is preliminarily believed that GSN can be an indicator used for the diagnosis of pSS.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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