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The diagnostic significance of glucose-6-phosphate isomerase (G6PI) antigen and anti-G6PI antibody in rheumatoid arthritis patients*

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ABSTRACT

Objective: To investigate whether glucose-6-phosphate isomerase (G6PI) antigen and anti-G6PI antibodies could be applied for the clinical diagnostic markers of rheumatoid arthritis (RA) and its associations with RA activity states. Methods: The levels of G6PI antigens and anti-G6PI Abs in sera from 176 RA patients in different states, 35 non-RA patients and 100 healthy donors and in synovia fluids from 33 patients and 11 non-RA patients were measured by ELISA. Results: The sensitivity and specificity of G6PI antigens in the RA patients were 75.0% and 93.3%, respectively. The levels of serum G6PI antigens in 176 RA patients were significantly higher than non-RA patients and the health controls. Especially, there was a significant difference between the active phase and the inactive phase in G6PI antigens levels. The levels of G6PI antigens in synovia fluid were also significantly higher in RA groups than in non-RA patients. With the values of the anti-G6PI Abs in sera, there were no marked differences among RA, non-RA patients and health controls. Also, there was no significant difference between the active phase and the inactive phase in RA patients. However, there were no significant differences of G6PI and anti-G6PI between RA patients and health controls in synovial fluid. Conclusions: G6PI is highly correlated with the activity states of RA, and could be applied for a clinical biomarker with high sensitivity and specificity for the diagnosis of RA.

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Keywords: Glucose-6-Phosphate Isomerase (G6PI); G6PI Antibody; Rheumatoid Arthritis (RA)

1. INTRODUCTION

RA is a common autoimmune inflammatory disease, characterized by chronic inflammation in the synovial membrane, cartilage and bone. Following the development of RA, hands, wrists, feet, and other small joints gradually become deformed, accompanied with skeletal muscle atrophy [1-4]. Based on the clinical observations, varieties of antigens and autoantibodies have been reported to be related to RA in patients. Reliable and specific diagnostic markers become more and more necessary to provide beneficial effects for clinical treatment with RA patients.

An increasing number of researchers focused on glucose-6-phosphate isomerase (G6PI) antigens and antibodies. In 1999, Matsumoto *et al*. firstly reported that K/BxNT cell receptor transgenic mice model produced autoantibodies against G6PI, which was demonstrated to be arthritogenic when it was injected into normal mice [5]. Later, Schaller *et al*. demonstrated that increasing of anti-G6PI Abs in most of RA patients, suggested a linkage between animal model and human RA [6]. However, they also found that anti-G6PI Abs were not unique to patients with RA but were present in many patients with inflammatory arthritis, proposing a notion that immune-based inflammatory arthritis induces increases of anti-G6PI Abs and G6PI/anti-G6PI immune complexes, which in turn influence proinflammatory cytokines release and involve in the development of inflammatory arthritis [7,8]. More recently, Fan *et al*. reported that human

serum G6PI alone or in combination with anti-cyclic citrullinated peptide Abs (anti-CCP Abs) may improve the clinical diagnosis of RA [9].

However, several other observations did not support this conception [10-13]. Kassahn *et al*. reported that few human autoimmune sera were detected with G6PI [10]. Herve *et al*. found that G6PI was not a specific autoantigen in RA [12]. Matsumoto *et al*. also reported the low prevalance of anti-G6PI Abs in patients with RA [13]. Taken together, existing data still argue the practical usage of G6PI antigen or anti-G6PI Abs as clinical diagnostic markers for RA patients.

In the present study, we analyzed serum and synovia fluid from patients with different autoimmune diseases to evaluate G6PI antigen and anti-G6PI Abs, and compared the expressions of G6PI antigen and anti-G6PI Abs in RA patients with different activity states. Our results show that G6PI antigen has high sensitivity and specificity, and is highly correlated with the activity states in RA patients, but not anti-G6PI Abs.

2. PATIENTS AND METHODS

2.1. Patients Recruited for Evaluation of G6PI Antigen and Anti-G6PI Abs

From 2006 to 2009, inpatients and outpatients were recruited for the present study from Suzhou Chinese Traditional Medicine Hospital and Shanghai East Hospital. The research was in compliance with the Declaration of Helsinki and approved by the ethics committee of Soochow University. Informed consent was obtained from each patient. Diagnostic standard for RA patients was based on established clinical criteria [14]. RA patients include active stage (n = 96) and inactive stage (n = 80) (mean age 42.8 ± 15.2 years, range 18 - 94, male = 36, female = 140). RA active stage diagnostic criteria is according to three items among the following: 1) joint swelling ≥ 3; 2) joint tenderness ≥ 3; 3) morning stiffness ≥ 45 min. 4) average handgrip: male ≤192 mmHg, female ≤146 mmHg; 5) erythrocyte sedimentation rate: male ≥25 mm/h, female ≥30 mm/h. Otherwise patients were ascribed to inactive stage. Other autoimmune diseases patients include ankylosing spondylitis (AS) (n = 10), systemic lupus erythematosus (SLE) (n = 10), Sjogren syndrome (SS) (n = 7), mixed connective tissue disease (MCTD) (n = 5), which are in line with the New York Revised Criteria for AS in 1984, The Revised Criteria for Classification of SLE in 1997, The Diagnostic Criteria for SS in 1992 (the European Union), Sharp Criteria for MCTD in 1972, respectively. Health control samples were collected from physical examination center of Shanghai East Hospital (n = 100, mean age 44.3 ± 16.1 years , range 22 - 85, male = 55, female = 45).

2.2. ELISA for the Detection of G6PI Antigen

G6PI antigen assay kits were purchased from R&D System Co. Ltd. (Shanghai, China). Experiment procedure was according to the manufacture's instruction. In brief, add 50 µl samples, standard and blank into the 96-well plate, incubate for 2 hours at 37˚C, wash the plate for five times, add 50 µl Enzyme Conjugates to each well, incubate for 1 hours at 37˚C, wash the plate for five times, add 50 µl Substrate Solution, incubate for 20 minutes at 37˚C in dark place, add 50 µl Stop Solution, measure absorbance at 490 nm wave length, 630 nm as reference wave length in microplate reader (Bio-Rad Laboratories, Inc, Shanghai, China, Model 680). According to the standard curve, calculating the concentration for each sample, G6PI > 0.25 µg/ml is regarded as positive. G6PI standard is provided by Shanghai Beijia Biochemistry Reagent Co. Ltd. (Shanghai, China).

2.3. ELISA for the Detection of Anti-G6PI Abs

Recombinant human G6PI antigen (5 µg/ml, generous gift from Sigma-Aldrich Co. Ltd. Shanghai, China) is added into 96-well microplate, sealed and incubated for overnight at 4˚C, wash the plate for 5 times, add 3% BSA PBS buffer for 2 hours. Add 100 µl samples (1:300 diluted with 0.1% BSA PBS buffer) to the well. Incubate for 2 hours at 37˚C; wash the plates for 5 times. Add 100 µl HRP-linked rabbit anti-human IgG (1:8000 dilution), incubate 20 minutes at 37˚C, wash the plates 5 times, add 100 µl Substrates Solution, incubate at dark place for 20 minutes, add 100 µl Stop Solution, measure absorbance at 450 nm wavelength.

2.4. Statistical Analysis

Concentrations of G6PI antigen and anti-G6PI Abs expressed as mean ± SD. Data analyses were performed by using Sigma Stat (SPSS, Chicago, IL) software. Due to the unmoral distribution of sera and synovial fluids, correction of t-test was used between two groups. LSD and S-N-K ANOVA were performed among three or more groups. P value < 0.05 was considered statistical significant.

3. RESULTS

3.1. Expression of G6PI Antigen in Sera

The average concentration of G6PI in sera from RA is 4.34 ± 8.65 µg/ml, which is significantly higher than that from other autoimmune diseases (0.07 ± 0.07 µg/ml, P < 0.01), and also higher than that from health control group (0.34 ± 1.30 µg/ml, P < 0.01). In RA group, the mean concentration of G6PI of active state patients is 7.61 ± 10.69 µg/ml, which is markedly higher than that of inactive state patients (0.47 ± 0.47 µg/ml, P < 0.01)

(**Table 1**). Using the present ELISA method, we plot the receiver operating characteristic curve (ROC) of G6PI antigen (**Figure 1**). The ROC curve indicates that the best cut-off concentration for diagnosis of clinical RA is 0.25 μg/ml. The sensitivity and specificity of the present method are 75.0% and 93.3% respectively.

3.2. Expression of G6PI Antigen in Synovial Fluid

In the synovial fluid, the concentration of G6PI from RA patients is 1.39 ± 1.58 μg/ml, which is significantly higher than that from other autoimmune diseases patients (0.16 ± 0.17 μg/ml, $P < 0.01$) (**Figure 2(a)**).

3.3. Expression of Anti-G6PI Abs in Sera

The mean optical density (OD) value of anti-G6PI abs in sera from RA group is 0.79 ± 0.56, in active RA is 0.87 ± 0.63, in inactive RA is 0.69 ± 0.44, in other autoimmune diseases is 0.58 ± 0.25 and in health control group is 0.70 ± 0.46, respectively. There is no significant difference among RA, other autoimmune diseases and health control groups, also there is no significant difference between active and inactive groups in RA (**Table 2**).

Table 1. Concentration of G6PI antigen in RA, other autoimmune diseases and health groups.

Group	Patients	G6PI (μg/ml)	G6PI positive rate	
			Patients (n)	Percentage (%)
RA	176	4.34 ± 8.65*	132	75.0
Active RA	96	7.61 ± 10.69#	84	87.5
Inactive RA	80	0.47 ± 0.47	48	60.0
Other autoimmune diseases	35	0.07 ± 0.07	0	0.00
Health	100	0.34 ± 1.30	9	91.0

G6PI concentrations are expressed as mean ± SD. *$P < 0.01$ as compared with other autoimmune diseases and health; #$P < 0.01$ as compared with inactive RA.

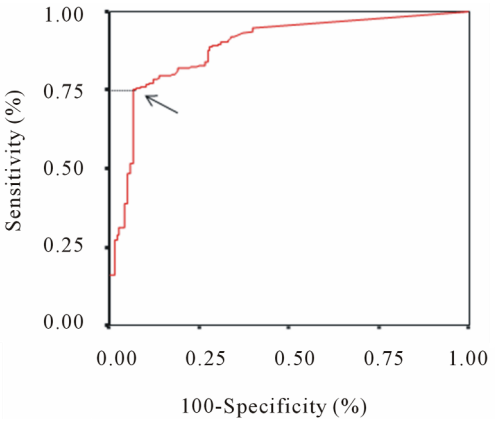

Figure 1. Receiver operating characteristic curve of G6PI ELISA. 100 health control and 176 rheumatoid arthritis sera were used to plot the curve. Arrow indicates the cut-off point.

3.4. Expression of Anti-G6PI Abs in Synovial Fluid

The mean OD value of anti-G6PI abs in synovial fluid from RA group is 0.73 ± 0.52, in other autoimmune diseases is 0.66 ± 0.41, there is no statistical difference between these two groups (**Figure 2(b)**).

4. DISCUSSION

In the present study, we clearly demonstrate that G6PI antigen level is higher in RA patients than other autoimmune diseases patients and health control, it is also highly related with the RA states, and active phase shows higher levels of antigen than that of inactive phase. However, we could not found any difference of anti-G6PI Abs among all groups, suggesting by using the present method, G6PI could be applied as another biochemistry marker for RA besides rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP Abs).

RA is a kind of unknown etiology, attacking small joints of limbs as autoimmune disease, it is a worldwide, high disabling disease, so clarifying the cause and investigating the mechanism of RA will provide beneficial effects for the early diagnosis and guide treatment for RA patients. Recently, researchers argued whether G6PI and anti-G6PI Abs could be applied as clinical diagnostic markers of RA.

In 1968, it was firstly realized that G6PI was a key enzyme for glycolysis and gluconeogenesis in the study of non-spherocytic hemolytic anemia. As a kind of multifunctional proteins in cells, it could induce the bone marrow stem cells to become different from mononuclear cells and B cell to become different from plasmacytes [15]. Yu *et al*. reported G6PI regulated matrix metalloproteinase-3 (MMP-3), and increased the expression of MMP-3 in the synovial cells and endothelial cells in RA patients, and promoted bone destruction. It was speculated

Table 2. OD value of anti-G6PI Abs in RA, other autoimmune diseases and health groups.

Group	Patients (n)	Anti-G6PI Abs
RA	77	0.79 ± 0.56
Active RA	42	0.87 ± 0.63
Inactive RA	35	0.69 ± 0.44
Other autoimmune diseases	23	0.58 ± 0.25
Health	100	0.70 ± 0.46

OD indicates optical density; data are expressed as mean ± SD.

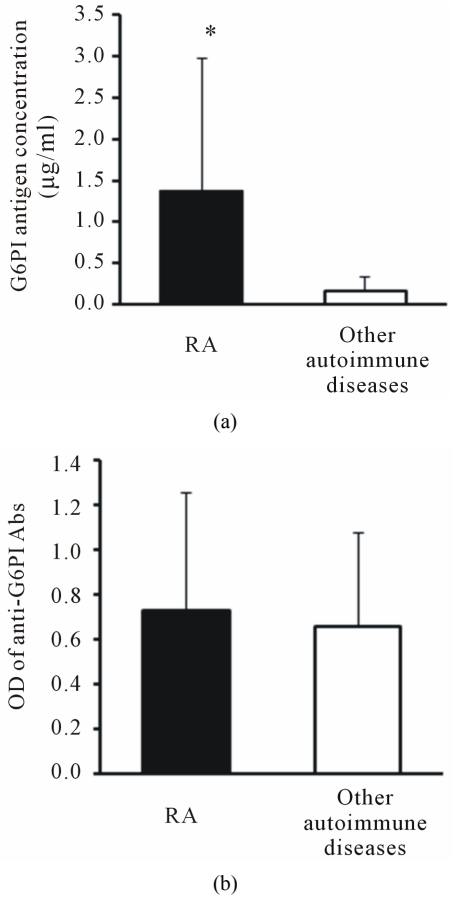

Figure 2. Concentration of G6PI antigen and anti-G6PI Abs in synovial fluids. (a) Concentration of G6PI antigen in synovial fluids from RA (n = 33) and other autoimmune diseases (n = 11). $^{*}P < 0.01$ vs other autoimmune diseases group; (b) OD value of anti-G6PI Abs in synovial fluids from RA (n = 33) and other autoimmune diseases at 450 nm wavelength.

that G6PI might play a role in vascular proliferation, synovial proliferation and bone destruction in RA patients [16]. However, it was reported that few human autoimmune sera could be detected with G6PI [10]. Herve *et al*. reported that glucose-6-phosphate isomerase is not a specific autoantigen in rheumatoid arthritis [12]. Other study also showed that G6PI concentration in sera and synovial fluids was increased in inflammatory arthritic diseases [8]. Recently, it was reported that G6PI was a useful marker for diagnosis of RA patients; it is positively correlated with anti-G6PI Abs, G6PI-containing immune complexes, G6PI mRNA, anti-CCP Abs, RF, C-reactive protein and ESR, respectively [9]. The EILSA data showed high sensitivity and specificity for RA patients, revealing the present method is reliable. Our data also showed that expression of G6PI in sera and synovial fluids from RA patients is higher than that of other autoimmune diseases and health control, suggesting the diagnostic application of G6PI in clinical. Discrepancy among the different laboratory for expression of G6PI in human sera and synovial fluids may be due to the different patients included, detection methods and sample pretreatment. Further study still needs to compare the difference between each method and the specificity of G6PI including all kinds of arthritis patients.

The most interesting finding of the present study is expression of G6PI is highly correlated with RA activity state. All of active RA patients showed higher levels of G6PI both in sera and synovial fluids compared with inactive RA patients. These data suggested that G6PI at least played a role in the development of RA, through which mechanisms still need further investigation. The present data will provide guide message for treatment with clinical patients under different stages.

Most of the researchers focused on the expression of anti-G6PI Abs. Matsumoto *et al*. demonstrated that anti-G6PI Abs produced in K/BxNT cell receptor transgenic mice was arthritogenic when it was injected into normal mice [5]. Later, although Schaller *et al*. demonstrated an increase of anti-G6PI Abs in most of RA patients, they also found that anti-G6PI Abs were not unique to patients with RA but were present in many patients with inflammatory arthritis, suggesting the unspecificity of anti-G6PI Abs for RA patients [7,8]. Matsumoto *et al*. also reported the low prevalance of anti-G6PI Abs in patients with RA [13]. Our present data also demonstrated that anti-G6PI Abs were not different among all groups, suggesting anti-G6PI Abs were not good markers for diagnosis of RA.

Taken together, our present study demonstrates that G6PI could be applied for a clinical biomarker with high sensitivity and specificity for the diagnosis of RA. The increased expression of G6PI is highly correlated with the activity states of RA. On the contrary, anti-G6PI has little clinical value for the diagnosis of RA.

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