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Value of serum glucose-6-phosphate isomerase in patients with rheumatoid arthritis and correlation with disease activity: A case–control study

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Abstract:

BACKGROUND: Rheumatoid arthritis (RA) is a common multisystemic autoimmune disease with peripheral joint involvement. Many autoantibodies have been introduced in the course of RA; some of them have diagnostic and prognostic value. In this study, our aim is to determine the value of serum glucose-6-phosphate isomerase (G6PI) antigen (Ag) as a diagnostic and prognostic marker in RA.

MATERIALS AND METHODS: Eighty-seven known cases of RA who referred to an outpatient clinic of Shiraz University of Medical Sciences and 76 healthy controls were selected. Serum G6PI Ag was measured using sandwich enzyme-linked immunosorbent assay method, and the enzyme level was compared in the patient and control group, we also compared the enzyme level of patient group with disease activity, disease duration, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), and anti-cyclic citrullinated peptide (anti-CCP) antibody (Ab). The data were analyzed using SPSS V 16 software.

RESULTS: Positivity for G6PI was detected in 34.5% (30/87) of RA patients and 9.2% (7/76) of control group ($P < 0.001$). There was no significant correlation between enzyme level and disease activity, disease duration, ESR, CRP, RF, and anti-CCP Ab.

CONCLUSIONS: Overall, in our study, although there was a significant difference in serum G6PI Ag between patient and control group, no significant correlation was detected between serum G6PI level and disease activity score, ESR, CRP, and anti-CCP Ab, but relative correlation with ESR and disease duration could be challenging. G6PI Ag could be introduced as a diagnostic marker in RA, but its role as a prognostic marker is controversial.

Keywords:

Arthritis, glucose-6-phosphate isomerase antigen, glucose-6-phosphate isomerase, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is the most common chronic inflammatory arthritis, with annual incidence of around 40 per 100,000. Women are affected two to three times more often than men. The characteristic feature is symmetrical and erosive arthritis of small peripheral joints.[1,2]

Multiple features are known to be correlated with a greater likelihood of developing joint abnormalities including >20 inflamed joints, presence of rheumatoid nodule, significant titer of erythrocyte sedimentation rate (ESR), and high titers of serum rheumatoid factor (RF).[3]

Glucose-6-phosphate isomerase (G6PI) is a well-known glycolytic enzyme which catalyzes the interconversion of glucose-6-phosphate and fructose-6-phosphate.

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In addition to this enzymatic activity, it can act as an extracellular cytokine. Recently, it was shown that systemic immune response against G6PI antigen (Ag) induces joint-specific pathology in mices.[4]

By far, few studies have been done for assessing the diagnostic and prognostic significance of G6PI in human models and its role in human disease is a subject of controversy.[5,6]

In this study, our aim was to evaluate serum G6PI Ag level by enzyme-linked immunosorbent assay (ELISA) method in known patients of RA, to make a correlation between Ag level and disease activity, and to compare its level with a control group of normal population.

Materials and Methods

Patients

For determination of G6PI level in RA patients and comparison with control group, 98 known cases of RA (fulfilling the 1987 Revised American College of Rheumatology classification criteria) were selected from the patients referred to the Rheumatology Department of an outpatient clinic of Shiraz University of Medical Sciences, over a period of 1 year, during 2016–2017. Shiraz University of Medical Sciences approved code was 92-01-01-5646.

Patients with other inflammatory diseases, malignancies, and diseases of central nervous system, kidney, and liver besides RA were excluded from the study.

Eighty-three patients were female, 15 were male, and the mean age was 50 years (range, 25–73 years).

Sixteen patients were new cases of RA without any previous treatment.

Sera from age- and sex-matched apparently healthy control group including 80 members, consisting of 66 female and 14 male, with a mean age of 42.3 years (range, 27–67), normal laboratory data (complete blood count, ESR, C-reactive protein [CRP], and lipid profile), without any joint complaint, and family history of rheumatologic disease were also evaluated in this study.

All patients gave informed consent to participate in this study. The study was approved by the local ethics committee.

Sample collection and preparation

A volume of 5 cc venous clot blood sample was obtained from all participants and centrifuged at 2000 × *g* for 10 min, and sera were collected.

All serum samples were freezed at – 20°C until the time of evaluation.

Disease activity assessment

To determine disease activity, disease activity score in 28 joints (DAS 28) was used with the formula:[7]

$$0.56\sqrt{(28\text{TJC})} + 0.28\sqrt{(28\text{ SJC})} + 0.70\text{Ln (ESR/CRP)} + 0.014\text{ VAS}$$

where VAS represents visual analog scale – by asking the patients to make a vertical mark on a 100 mm VAS to their general health or global disease activity; TJC represents tender joint count; and SJC represents swollen joint count.

Then, the patients were classified according to the DAS 28 as below:

- No activity: ≤2.6
- Low disease activity: 2.6 < DAS ≤ 3.2
- Moderate disease activity: 3.2 < DAS ≤ 5.1
- High disease activity: DAS > 5.1.

Glucose-6-phosphate isomerase concentration

To evaluate G6PI level, sera from patients and control group were assessed using a commercial ELISA kit (Abcam G-6-P-I human simple step kit), according to the recommended instructions.

Two 96-well ELISA kits were used in this study.

Other markers

ESR, CRP and RF, and anti-citrullinated C-peptide antibody (CCP Ab) were measured by Westergren method, semi-quantitative method, and ELISA, respectively. ESR values above age- and sex-matched reference range, CRP >6 mg/L, RF >8 IU/ml, and anti-CCP Ab >20 U/mL were defined as positivity according to the kit recommendations.

Assay procedure

1. Standards prepared from 1 to 7 according to the kit instructions
2. 50 µL serum sample (diluted at 1/50) and eight prepared standards were added to anti-G6PI precoated well
3. Then, 50 µL Ab cocktail was added to each well
4. After covering, plates were incubated for 1 h at room temperature on a plate shaker with 400 round/min (rpm)
5. The plates were washed three times with wash buffer
6. 100 µL tetramethylbenzidine substrate was added to each plate and again incubated in the dark for 10 min on shaker with 400 rpm
7. 100 µL stop solution was added to each well, and after 1 min shaking, the plates were read using ELISA reader, optical density was recorded, and concentrations (ng/ml) were calculated using the standard curve [Figure 1].

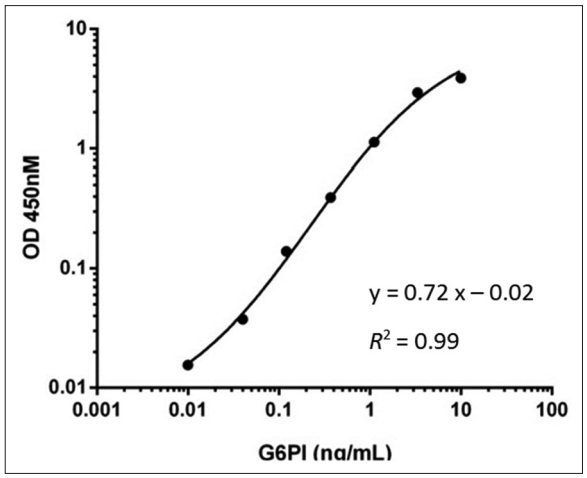

Figure 1: Standard curve of enzyme-linked immunosorbent assay kit showing glucose-6-phosphate isomerase concentration according to absorbance in 450 nm wavelength

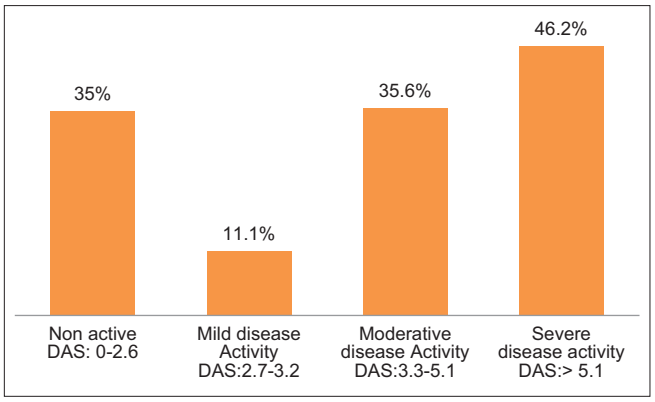

Figure 2: Percentage of serum glucose-6-phosphate isomerase positivity in patient group, classified according to disease activity score

Statistical analysis

The statistical analysis was performed using SPSS version 18 for windows. Data are presented as frequencies and percentages for categorical variables and mean ± standard deviation (SD) for continuous variables. Comparisons were done using Pearson's Chi-square test.

Results

Our study population was 98 patients diagnosed with RA and 80 healthy individuals as a control group.

Fifteen cases (11 RA patients and 4 controls) were removed from the study because of serum hemolysis and turbidity.

For determination of any difference in G6PI Ag level between RA patients and healthy controls, the ELISA results of both groups were compared.

Value of 1 SD above mean in the control group (5.4 ng/ml) was defined as positivity.[8]

Among 87 patients, 30 patients (34.5%) were positive for G6PI Ag, and among 76 controls, only 7 was positive (9.2%) with $P < 0.001$ [Table 1].

The patients were classified based on the DAS into nonactive, mild, moderate, and severe active groups. There was no significant correlation between disease activity and serum G6PI level in RA patients as shown in Figure 2.

Due to variable disease duration among patients, serum G6PI level was compared between different groups of patients (classified according to the disease duration as: new, 0–3 years duration, and >3 years duration). Patients with the longest disease duration had more serum G6PI concentration, although due to low volume sample it was not significant [Figure 3].

Values of ESR, CRP, RF, and anti-CCP Ab were recorded for each patient and compared with serum G6PI levels as revealed in Figure 4.

Discussion

RA is a multisystemic autoimmune disease with a wide variety of autoantibodies; some of them have diagnostic and prognostic value. Detection of abnormal auto-Ab in RA before presentation of clinical signs and symptoms is called "preclinical RA."[9]

Among these diverse antibodies, RF and anti-CCP Ab are more investigated and both of them have been involved as criteria for diagnosis of RA.[10] G6PI is a glycolytic enzyme in human that can act as a cytokine too. G6PI deficiency can cause an autosomal recessive nonspherocytic hemolytic anemia.[11] Furthermore, G6PI is used as a prognostic marker in colorectal, breast, lung, kidney, gastrointestinal, and some other cancers.[12] Recently, some studies have examined G6PI level in the form of serum and synovial fluid Ag, Ab, and molecular study in patients with RA.[13,14]

We measured the serum concentration of G6PI Ag in RA patients and a healthy control group. In our study, of 87 RA patients, 30 (34.5%) showed positivity for G6PI Ag compared with control group, 7 positive in 76 cases (9.2%) ($P < 0.001$). We also compared the level of G6PI Ag in patients with DAS, disease duration, ESR, CRP, RF, and anti-CCP Ab. No significant correlation was achieved between these parameters.

Schaller *et al.* in 2006 confirmed high levels of G6PI Ag and enzymatic activity in immune-mediated rheumatologic diseases, particularly RA patients.[15] They also had a same experience in 2001.[5] van Gaalen *et al.* in 2004 found that autoantibodies against G6PI are associated with

Table 1: Mean of serum glucose-6-phosphate isomerase concentration and percentage of positivity in patient and control groups

Group	*n*	Sex		Mean of age (year)	G6PI level (ng/mL)				Number of G6PI positivity (%)
		Female (%)	Male (%)		Minimum	Maximum	Mean±SD	Median	
Patient	87	86.2	13.8	50.0	0.48	10.4	5.0±1.7	4.3	30 (34.5)
Control	76	82.9	17.1	42.3	0.29	9.3	3.7±1.8	3.8	7 (9.2)

G6PI=Glucose-6-phosphate isomerase, SD=Standard deviation

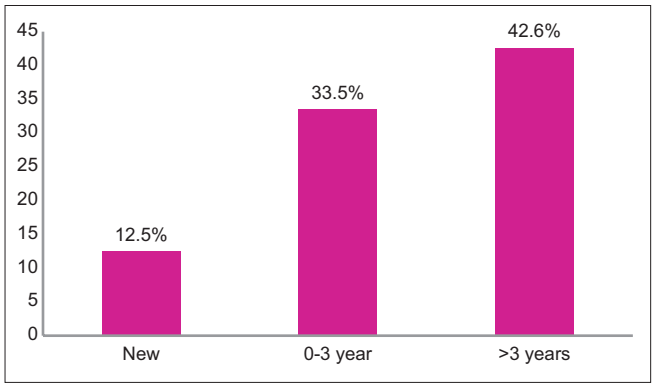

Figure 3: Percentage of serum glucose-6-phosphate isomerase positivity in patient group, classified according to disease duration, $P = 0.091$

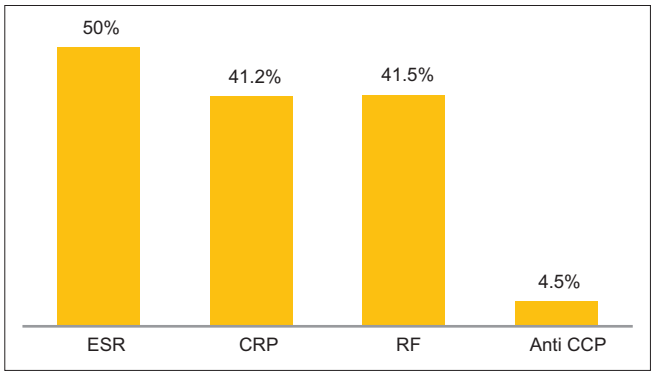

Figure 4: Percentage of serum glucose-6-phosphate isomerase positivity in patients with increased erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, and anti- citrullinated C-peptide antibody

occurrence of extra-articular manifestations.[6] Although other later studies in 2010 showed the same results about G6PI Ag level in RA patients and control group, they showed some controversies about the correlation between Ag level and disease activity.[3,16]

The last published investigation before our study was done by Yang *et al*. in 2013 and evaluated serum G6PI Ag and Ab in 176 RA patients, 35 nonRA patients, and 100 healthy controls. They found positive G6PI Ag in 75% (137/176) of RA patients, 9.1% (9/100) of healthy controls, and 0 in non-RA rheumatologic patients and also significant correlation between serum G6PI Ag level and disease activity. About serum G6PI Ab, the study showed no remarkable difference between RA patients, non-RA rheumatologic patients, and healthy controls.[8]

Overall, in our study, there was significant difference in serum G6PI concentration, between patient and control group, so it seems to be a reliable marker for diagnosis of RA till now; however, due to controversies about correlation with disease activity, more studies with larger sample size are needed including level of G6PI Ag and Ab in serum and synovial fluid and also tissue biopsy.

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Conflicts of interest

There are no conflicts of interest.

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