Mediterranean Journal of Hematology and Infectious Diseases

Scientific Letters

Association of Rheumatoid Arthritis with Glucose-6-Phosphate Dehydrogenase Deficiency: Results from a Case-Control Study

Keywords: Autoimmunity; Glucose-6-phosphate dehydrogenase deficiency, Oxidative stress; Inflammation; Joints; Connective tissue-diseases.

Published: July 01, 2024 **Received: May 03, 2024** **Accepted: June 14, 2024**

Citation: Dore M.P., Pes G.M., Mereu S., Piroddu J., Cavagna L., Erre G.L. Association of rheumatoid arthritis with glucose-6-phosphate dehydrogenase deficiency: results from a case-control study. Mediterr J Hematol Infect Dis 2024, 16(1): e2024056, DOI: http://dx.doi.org/10.4084/MJHID.2024.056

To the editor.

A decade ago, Gheita et al. reported a high frequency of glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) deficiency in patients with rheumatoid arthritis (R.A.) and concurrent metabolic syndrome.[1] More recently, in a retrospective cohort study using a large, nationwide database of individuals with known G6PD status, Israel et al. confirmed a significant association between the occurrence of R.A. and G6PD enzyme deficiency.[2] In Sardinia, Italy, hereditary G6PD deficiency reaches a frequency as high as 8% and 12% in the male and female population, respectively [3], which is among the highest in the world. It has already been proven that 95% of cases are due to the G6PD Med mutation ($Ser^{188}Phe$),[3] which entails a class II severe deficiency, according to the World Health Organization, because it is associated with a more than 90% reduction of catalytic activity.[4] Moreover, the prevalence of R.A. has been estimated at 552×10^5 in the general Sardinian population,[5] i.e., considerably higher than that recorded in the Italian peninsula (330×10^5).[6]

Based on this claim and the particularly favourable setting, the association between R.A. and G6PD deficiency was tested in the population of northern Sardinia.

Methods. This was a retrospective case-control study recruiting outpatients referred for upper endoscopy to the Gastroenterology section of a teaching hospital (University of Sassari, Italy). The section is the main referral centre for rheumatological patients with dyspeptic complaints. Data from January 2014 and January 2022 were retrieved from an electronic database. Collected data included sex, age, smoking habits, anthropometric parameters, and the presence of a defined diagnosis of R.A.

Rheumatoid Arthritis. R.A. was diagnosed by the rheumatologist according to national and international guidelines/expert consensuses progressively developed and used in clinical practice.[7] Rheumatoid factor (R.F.) levels and anti-citrullinated protein antibody (ACPA) titres were also available in a subset (n=291) of patients with R.A. Serum R.F. titres were measured in the hospital reference lab using a commercial ELISA kit (Abcam®, Cambridge, MA, U.S.A.),[8] and ACPA titres (Euro Diagnostica Immunoscan CCPlus®, Arnhem, The Netherlands) according to the manufacturer's instructions.

Glucose-6-Phosphate Dehydrogenase Deficiency. In all study participants, G6PD status had been assessed using a previously described laboratory test based on the measurement of the G6PD/6PGD ratio in red blood cells of peripheral venous blood samples [9]. Enzyme deficiency was classified as severe (<10% residual G6PD activity) or intermediate (between 10 and 80% residual activity). Molecular testing was not available.

Statistical Analysis. The body mass index (B.M.I.), calculated by using the formula weight (kg) / height $(m)^2$, allowed to stratify participants into normal, overweighted (B.M.I. between 25 and 29.9 kg/m^2), and obese (B.M.I.≥ 30 kg/m^2). According to smoking habits, patients and controls were divided into never smokers or former smokers/current smokers. Both severe and intermediate G6PD deficiency were merged into the same category to strengthen the analysis. Differences between means were evaluated by the Student's *t*-test for continuous variables and by the Pearson χ^2 test for categorical variables. The association between G6PD deficiency and R.A. was determined using univariable and multivariable logistic regression models by calculating unadjusted and adjusted odds ratios (O.R.s) and their 95% confidence intervals (CI). The analysis was conducted separately in males and females. All

Table 1. Features of the 5279 study participants stratified by G6PD status and sex.

Variables	Patients with G6PD deficiency (N=661)		Patients without G6PD deficiency (N=4618)		P-value*
	Males (n=160)	Females (n=501)	Males (n=1610)	Females (n=3008)	
Mean age, years	52.7 ± 16.6	51.4 ± 16.0	53.1 ± 16.2	51.6 ± 17.2	0.536
Smoking habits, n (%)					
No	87 (54.4)	309 (61.7)	786 (48.8)	1819 (60.4)	0.269
Current or former smoker	73 (45.6)	192 (38.3)	824 (51.2)	1189 (39.6)	
Body mass index, kg/m²					
< 25	81 (50.4)	292 (58.4)	797 (49.6)	1772 (58.9)	
25–29.5	55 (34.4)	136 (27.1)	615 (38.2)	854 (28.4)	0.385
≥ 30	54 (15.2)	73 (14.5)	198 (12.3)	382 (12.7)	
Rheumatoid arthritis, n (%)					
None	137 (85.6)	418 (83.4)	1474 (91.5)	2656 (88.3)	**<0.0001**
Yes	23 (14.4)	83 (16.6)	136 (8.5)	352 (11.7)	

*P values refer to the comparison between G6PD deficient and G6PD normal without sex difference; in bold are statistically significant; # Glucose-6-phosphate dehydrogenase.

Table 2. Unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (CI) for rheumatoid arthritis in 5,279 study participants.

Covariates	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
Sex		
Males	Ref.	Ref.
Females	1.32 (1.09–1.59) **	1.36 (1.01–2.08) *
Age		
< 60	Ref.	Ref.
≥ 60	2.96 (2.48–3.52) **	3.28 (2.40–4.47) **
Body mass index		
< 25 kg/m²	Ref.	Ref.
25 – 29 kg/m²	0.94 (0.68–1.30)	0.77 (0.55–1.08)
≥ 30 kg/m²	0.81 (0.48–1.37)	0.67 (0.40–1.13)
Smoke		
No	Ref.	Ref.
Yes	1.59 (1.21–2.17) **	1.81 (1.34–2.44) **
G6PD# deficiency		
No	Ref.	Ref.
Yes	1.61 (1.29–2.03) **	2.21 (1.54–3.18) **

* P<0.05; **P<0.001; # Glucose-6-phosphate dehydrogenase

statistical analyses were performed using SPSS statistical software (version 22.0, Chicago, IL, U.S.A.). P-values lower than 0.05 were considered statistically significant.

Ethical Considerations. Ethical review and approval were waived for this study due to observational retrospective design by the Italian law (GU No. 76 31/Mar/2008). The procedures followed in the study were in accordance with the ethical standards of the Declaration of Helsinki.

Results. A total of 5,279 study participants (mean age 53.0 ± 17.1 years; 66.5% female) were included in the analysis. In 594 participants, a diagnosis of R.A. (11.3%) was posed by the rheumatologist. **Table 1** shows the features of the study participants separately in males and females. There were 661 patients (12%) with G6PD deficiency, reflecting the regional frequency,[3] with an F: M ratio of 1.86, and 4618 patients without. No significant differences were observed in B.M.I. and smoking habits. The prevalence of R.A. was 14.4% in males and 16.6% in females, respectively, among carriers of G6PD deficiency, whereas it was 8.5% and 11.7% among males and females with normal enzyme activity ($p < 0.0001$).

The results of univariable and multivariable logistic regression analysis, using the presence or absence of R.A. as the outcome, according to the exposure to G6PD deficiency, are reported in **Table 2**. The odds for an R.A. diagnosis in subjects with G6PD deficiency were statistically significant (OR 2.21, 95%CI 1.54–3.18) after adjusting for the covariates included in the study (**Table 2**) and higher in males [OR 1.82, 95%CI 1.13–2.93] compared with females [OR 1.49, 95%CI 1.15–1.94].

The subset of 291 patients with R.A. and availability of immunological markers was stratified according to R.F. and ACPA positivity (**Table 3**). Interestingly, the frequency of G6PD deficiency was significantly increased in R.A. patients positive for both markers compared with controls (17.6% vs 12.1%, $p = 0.029$). In

Table 3. Selected variables in 291 rheumatoid arthritis patients stratified according to immunological markers including rheumatoid factor (RA) and anti-citrullinated protein antibodies (ACPA).

	Patients with rheumatoid arthritis				**Patients without rheumatoid arthritis (N=4685)**
Variables	**RF+, ACPA+** (N=170)	**RF+, ACPA-** (N=18)	**RF-, ACPA+** (N=49)	**RF-, ACPA-** (N=54)	
Sex, n (%)					
Male	37 (21.8)	5 (27.8)	15 (30.6)	14 (25.9)	1623 (34.6)
Female	133 (78.2)	13 (72.2)	34 (69.4)	40 (74.1)	3062 (65.4)
Mean age, years	59.3 ± 14.93	67.0 ± 5.6	65.5 ± 9.7	50.7 ± 14.5	51.8 ± 16.7
CRP[§], n(%)					
Normal	154 (90.6)	18 (100)	46 (93.9)	51 (94.4)	N/A[‡]
Increased	16 (9.4)	0 (0.0)	3 (6.1)	3 (5.6)	
ESR[†]					
Normal	31 (18.2)	2 (11.1)	8 (16.3)	16 (29.6)	N/A
Increased	139 (81.8)	16 (88.9)	41 (83.4)	38 (70.4)	
G6PD[#] deficiency, n (%)					
None	140 (82.4)	15 (83.3)	39 (79.6)	46 (85.2)	4120 (87.9)
Yes	30 (17.6)*	3 (16.7)	10 (20.4)	8 (14.8)*	565 (12.1)

[§] C-reactive protein: normal values [<1.0 mg/dL]; [†]Erythrocyte sedimentation rate, normal values [<25 mm/h]; [#] Glucose-6-phosphate dehydrogenase. [‡] Inflammatory markers were unavailable in patients without rheumatoid arthritis. *Differences between RA patients with G6PD deficiency and RF+, ACPA+ and RF-, ACPA- were statistically significant.

comparison, the frequency of G6PD deficiency was non-significantly increased (14.8% vs 12.1%, p = 0.537) in patients negative for RA-specific markers. However, the small number of patients with G6PD deficiency in this subset did not allow us to draw definitive conclusions.

Discussion. The present study involved a large cohort of patients. Our findings suggest that similar to Israel's results,[2] in the Northern Sardinian population, G6PD deficiency significantly increased the odds of R.A. This association was found in both sexes, especially in male patients. It is reasonable considering that among females, a higher frequency of heterozygosity was reported, resulting in greater residual enzyme activity. Interestingly, in a subanalysis, the increased frequency of G6PD deficiency was detected in R.A. patients with at least one positive immunologic marker.

Antioxidant mechanisms protecting the body from the harmful action of reactive oxygen species (R.O.S.) are hinged on several enzymes found in most cells, one of the most important being G6PD. More specifically, in the first reaction of the pentose phosphate pathway, G6PD supplies reducing equivalents (NADPH) necessary to maintain high intracellular levels of reduced glutathione (G.S.H.), a thiol-containing tripeptide active against R.O.S., especially the superoxide anion.[10] Subjects with G6PD deficiency are generally asymptomatic, but they may experience more or less serious haemolytic crises following the intake of specific drugs, especially NSAIDs, or the consumption of certain foods, such as fava beans.[4] Beyond haemolytic crises and neonatal jaundice, more recent literature has reported how G6PD deficiency, affecting any cell of the organism, can be implicated in other disorders, including the cardiovascular system,[11] as well as various autoimmune diseases[12] and, more specifically, R.A. Pathogenic pathways underlying R.A. onset and progression are largely unknown, with different mechanisms, including genetic predisposition, environmental risk factors, microbial exposure, and increased oxidative stress, being proposed until now.[13,14] The R.A. is characterized by impaired antioxidant defense, although the precise mechanisms involved are relatively poorly understood. Experimental[15] and human studies[16] seem to corroborate the notion that a defect in antioxidant mechanisms, whatever the cause, concurs with inflammation in determining joint tissue injury as well as systemic damage.

Several studies and meta-analyses have shown that impaired antioxidant defense is one of the pathogenic hallmarks of R.A. disease.[17] Suggested hypotheses to explain how failure to counteract oxidative stress can contribute to maintaining the autoimmune mechanism in R.A. are structured along two lines of reasoning: (i) a decrease in the intracellular glutathione, whose sulfhydryl (–SH) moiety is responsible for R.O.S. neutralization, and (ii) the establishment of a chronic inflammatory dysregulation, globally affecting cell signalling including that of the immune system. Evidence from different studies highlighted the large amount of R.O.S. produced by monocytes from R.A. patients. In particular, oxygen and nitrogen-reactive species can directly degrade some components of the synovial tissues—more specifically, hyaluronic acid and other proteoglycans—contributing to joint damage. Furthermore, activated T-cells themselves are highly sensitive to R.O.S. damage, and this might contribute to

the establishment of an altered immune response in R.A. via a self-sustained pathogenic loop. In such circumstances, it is reasonable to assume that any alteration of the antioxidant system due to inherited defects can exacerbate the intracellular redox status, thereby interfering with the mechanisms involved in immune tolerance and increasing the chance of developing R.A. Consequently, tentative speculation to explain our findings could be that G6PD deficiency, via intracellular NADPH depletion, may hamper the conversion of GSSG to G.S.H. necessary for R.O.S. disposal. Some intracellular components, critical for immune tolerance, may be permanently modified and trigger the sustained immune activation conducive to R.A.

G6PD deficiency might also enhance R.A. risk via additional mechanisms, such as dysregulation of the inflammatory response. Several in vitro studies have demonstrated that G6PD-deficient cells release several regulatory cytokines in excess, such as the transforming growth factor beta (TGF-β), which plays a major role in inflammation and oxidative stress, as confirmed by the lowering R.O.S. effect of TGF-β inhibitors.[18] Clinical evidence suggests that TGF-β regulates the function of fibroblasts and might have a pathogenic role in R.A.[19] Bira et al. reported an increased TGF-β in the synovial tissue and fluids of R.A. patients.[20] Through its action on pivotal mechanisms of innate (natural killer cells) and adaptive (Tregs cells) immunity, TGF-β upregulation may contribute to the autoimmune response mounted in R.A.[20]

The present study has some limitations due to its retrospective design and the lack of molecular typing of G6PD deficiency. However, it is reasonable to assume that the majority of patients carried the Mediterranean variant.[3] The frequency of G6PD deficiency in patients without R.A. was comparable to that reported for the Sardinian population in the same area,[12] making a bias unlike. Although the database used included patients with clinical complaints requiring endoscopy, we are confident there were no reasons to think that the comorbidity distribution was dissimilar between subjects with and without G6PD deficiency.

Conclusions. Our study confirmed previous results of an association between G6PD deficiency and R.A., especially in patients with R.A. positive for R.F. and/or ACPA. At present, it is not justified to recommend systematic screening for G6PD in patients with R.A., and further evidence from a larger case series is needed. Nonetheless, the identification of a new risk factor, such as G6PD deficiency, opens a new avenue in research to understand R.A. pathogenesis better.

Author contributions statement: G.M.P. and M.P.D. were responsible for conceptualization, study design, literature search, analysis, interpretation, writing the original draft, reviewing, and editing. G.M.P. contributed with formal analysis. S.M., J.P., and L.C. collected data and performed data curation and writing. G.L.E. contributed to the analysis, data interpretation, writing, review, and editing. All authors had full access to all the data in the study and were the final ones responsible for deciding to submit for publication.

Maria Pina Dore[1,2], Giovanni Mario Pes[1,3], Sandro Mereu[1], Jessica Piroddu[1], Lorenzo Cavagna[4] and Gian Luca Erre[1].

[1] Dipartimento di Medicina, Chirurgia e Farmacia, University of Sassari, Sassari 07100, Italy.
[2] Baylor College of Medicine, 77030 Houston, Texas, USA.
[3] Sardinia Blue Zone Longevity Observatory, Ogliastra, Italy.
[4] Division of Rheumatology, IRCCS Fondazione Policlinico San Matteo, Pavia, Italy.
M.P. Dore and G.M. Pes contributed equally.

Competing interests: The authors declare no conflict of Interest.

Correspondence to: Prof. Maria Pina Dore. Clinica Medica, Viale San Pietro - 8, PO Box 07100 Sassari, Italy. Tel.: +39 347 4539532. E-mail: mpdore@uniss.it

References:

1. Gheita TA, Kenawy SA, El Sisi RW, Gheita HA, Khalil H. Subclinical reduced G6PD activity in rheumatoid arthritis and Sjogren's Syndrome patients: relation to clinical characteristics, disease activity and metabolic syndrome. Mod Rheumatol. Jul 2014;24(4):612-7. https://doi.org/10.3109/14397595.2013.851639 PMid:24252052
2. Israel A, Schäffer AA, Berkovitch M, et al. Glucose-6-phosphate dehydrogenase deficiency and long-term risk of immune-related disorders. Original Research. Frontiers in Immunology. 2023-September-11 2023;14 https://doi.org/10.3389/fimmu.2023.1232560 PMid:37753082 PMCid:PMC10518697
3. Fiorelli G, Meloni T, Palomba V, Manoussakis C, Villa S, Cappellini MD. Gene frequency of glucose-6-phosphate dehydrogenase (G6PD) polymorphic variants in Sardinia. Gene Geogr. Dec 1990;4(3):139-42.
4. Luzzatto L, Ally M, Notaro R. Glucose-6-phosphate dehydrogenase deficiency. Blood. Sep 10 2020;136(11):1225-1240. https://doi.org/10.1182/blood.2019000944 PMid:32702756
5. Sardu C, Cocco E, Mereu A, et al. Population based study of 12 autoimmune diseases in Sardinia, Italy: prevalence and comorbidity. PLoS One. 2012;7(3):e32487. https://doi.org/10.1371/journal.pone.0032487 PMid:22396771 PMCid:PMC3292563
6. Rossini M, Rossi E, Bernardi D, et al. Prevalence and incidence of rheumatoid arthritis in Italy. Rheumatol Int. May 2014;34(5):659-64. https://doi.org/10.1007/s00296-014-2974-6 PMid:24610538

7. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. Sep 2010;62(9):2569-81.
8. Bampton JL, Cawston TE, Kyle MV, Hazleman BL. Measurement of rheumatoid factors by an enzyme-linked immunosorbent assay (ELISA) and comparison with other methods. Ann Rheum Dis. Jan 1985;44(1):13-9.
https://doi.org/10.1136/ard.44.1.13
PMid:3970590 PMCid:PMC1001560
9. Mosca A, Paleari R, Rosti E, et al. Simultaneous automated determination of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in whole blood. Eur J Clin Chem Clin Biochem. May 1996;34(5):431-8.
https://doi.org/10.1515/cclm.1996.34.5.431
PMid:8790979
10. Weiss SJ. The role of superoxide in the destruction of erythrocyte targets by human neutrophils. J Biol Chem. Oct 25 1980;255(20):9912-7.
https://doi.org/10.1016/S0021-9258(18)43479-5
PMid:6253458
11. Dore MP, Portoghese M, Pes GM. The Elderly with Glucose-6-Phosphate Dehydrogenase Deficiency are More Susceptible to Cardiovascular Disease. J Atheroscler Thromb. Jun 1 2021;28(6):604-610.
https://doi.org/10.5551/jat.56531
PMid:32908034 PMCid:PMC8219535
12. Dore MP, Fanciulli G, Pes GM. Is Glucose-6-Phosphate Dehydrogenase Deficiency a Risk Factor for Autoimmune Thyroid Disease? A Retrospective Case-Control Study. Int J Environ Res Public Health. Feb 3 2023;20(3)
https://doi.org/10.3390/ijerph20032709
PMid:36768075 PMCid:PMC9916078
13. Erre GL, Mameli G, Cossu D, et al. Increased Epstein-Barr Virus D.N.A. Load and Antibodies Against EBNA1 and E.A. in Sardinian Patients with Rheumatoid Arthritis. Viral Immunol. Sep 2015;28(7):385-90.
https://doi.org/10.1089/vim.2015.0035
PMid:26083265
14. Bo M, Erre GL, Bach H, et al. PtpA and PknG Proteins Secreted by Mycobacterium avium subsp. paratuberculosis are Recognized by Sera from Patients with Rheumatoid Arthritis: A Case-Control Study. J Inflamm Res. 2019;12:301-308.
https://doi.org/10.2147/JIR.S220960
PMid:31819587 PMCid:PMC6899068
15. Wruck CJ, Fragoulis A, Gurzynski A, et al. Role of oxidative stress in rheumatoid arthritis: insights from the Nrf2-knockout mice. Ann Rheum Dis. May 2011;70(5):844-50.
https://doi.org/10.1136/ard.2010.132720
PMid:21173018
16. Hassan MQ, Hadi RA, Al-Rawi ZS, Padron VA, Stohs SJ. The glutathione defense system in the pathogenesis of rheumatoid arthritis. J Appl Toxicol. Jan-Feb 2001;21(1):69-73.
https://doi.org/10.1002/jat.736
PMid:11180282
17. Quinonez-Flores CM, Gonzalez-Chavez SA, Del Rio Najera D, Pacheco-Tena C. Oxidative Stress Relevance in the Pathogenesis of the Rheumatoid Arthritis: A Systematic Review. Biomed Res Int. 2016;2016:6097417.
https://doi.org/10.1155/2016/6097417
PMid:27340664 PMCid:PMC4906181
18. Sanna F, Bonatesta RR, Frongia B, et al. Production of inflammatory molecules in peripheral blood mononuclear cells from severely glucose-6-phosphate dehydrogenase-deficient subjects. J Vasc Res. 2007;44(4):253-63.
https://doi.org/10.1159/000100903
PMid:17361089
19. Parsanathan R, Jain SK. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is linked with cardiovascular disease. Hypertens Res. Jun 2020;43(6):582-584. doi:10.1038/s41440-020-0402-8
https://doi.org/10.1038/s41440-020-0402-8
PMid:31974484
20. Bira Y, Tani K, Nishioka Y, et al. Transforming growth factor beta stimulates rheumatoid synovial fibroblasts via the type II receptor. Mod Rheumatol. 2005;15(2):108-13.
https://doi.org/10.3109/s10165-004-0378-2
PMid:17029045