

Molecular Hydrogen Capsule Therapy for Primary Biliary Cholangitis With Elevated IgG4: A Case Report on Immune Marker Normalization

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Abstract

Background/Aim: Primary biliary cholangitis (PBC) is a chronic autoimmune liver disease characterized by bile duct destruction, cholestasis, and inflammation, often leading to fibrosis and cirrhosis. While ursodeoxycholic acid (UDCA) is the standard treatment, some patients exhibit suboptimal responses, necessitating adjunctive therapies. Molecular hydrogen (H₂), known for its antioxidant and anti-inflammatory properties, has shown potential in mitigating oxidative stress and immune dysregulation in autoimmune liver diseases. This case report evaluates the therapeutic efficacy of H₂ capsules in managing PBC with elevated liver enzymes and immune dysregulation.

Case Report: A 44-year-old male with PBC, splenomegaly, and elevated IgG4 levels presented with acute cholestatic hepatitis. Laboratory tests revealed significantly elevated aspartate transaminase (AST) (279 U/l) and alanine

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aminotransferase (ALT) (183 U/l). Despite UDCA therapy, liver enzymes remained persistently high. On August 30, 2024, molecular hydrogen capsule therapy was introduced as adjunctive treatment. Over four months, AST and ALT levels declined to 95 U/l and 70 U/l, respectively, without adverse effects. Immune markers (KLRG-1, PD-1, and Tim3), previously reduced during PBC flares, normalized post-treatment. Imaging confirmed stable fibrosis, and IgG4 levels decreased, suggesting reduced autoimmune activity. The patient also reported improvements in fatigue and pruritus, enhancing overall quality of life.

Conclusion: Molecular hydrogen capsules therapy may serve as a safe and effective adjunctive treatment for PBC, contributing to improved liver enzyme levels, immune regulation, and patient well-being. Further studies are warranted to validate these findings and establish standardized treatment protocols in autoimmune liver diseases.

Keywords: Primary biliary cholangitis, molecular hydrogen, liver enzymes, IgG4, oxidative stress, immune modulation.

Introduction

Primary biliary cholangitis (PBC) is a chronic autoimmune liver disease characterized by the progressive destruction of small intrahepatic bile ducts, resulting in bile flow obstruction and cholestasis. This disruption triggers inflammation and fibrosis, which may progress to cirrhosis if untreated. While the exact etiology remains unclear, genetic predisposition, environmental triggers, and associations with other autoimmune conditions, such as Sjögren's syndrome and autoimmune thyroiditis, are believed to contribute to disease development (1, 2). The primary treatment for PBC is ursodeoxycholic acid (UDCA), a bile acid that enhances bile flow and slows disease progression. Most patients experience significant benefits from UDCA, particularly when initiated in the early stages of the disease (3, 4). Emerging therapies, including fibrates and molecular hydrogen (H₂), are being investigated for their potential to reduce cholestasis and modulate inflammation. The therapeutic effects of H₂ stem from its unique ability to function as a selective antioxidant and regulator of inflammation, oxidative stress, and cellular signaling (5). H₂ has been shown to directly neutralize reactive oxygen species, such as hydroxyl radicals and peroxynitrite, thereby mitigating oxidative stress, reducing inflammation, inhibiting apoptosis, and attenuating fibrosis (6).

Molecular hydrogen exerts anti-inflammatory effects by modulating key signaling pathways, including nuclear factor-

kappa B (NF-κB) (7, 8). By inhibiting NF-κB activation, hydrogen reduces the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 1 β (IL-1β), and interleukin 6 (IL-6), which play pivotal roles in autoimmune and inflammatory diseases (9, 10). Additionally, hydrogen activates pathways such as AMP-activated protein kinase (AMPK) and nuclear factor erythroid 2-related factor 2 (Nrf2), enhancing cellular repair mechanisms and maintaining redox homeostasis (11, 12). Nrf2 activation up-regulates antioxidant and detoxification enzymes, further amplifying hydrogen's protective effects (13). By mitigating oxidative stress, H₂ therapy alleviates cellular damage that contributes to inflammation and fibrosis in PBC. This reduction in oxidative injury slows bile duct damage and fibrosis progression, ultimately improving liver function (14). Although molecular hydrogen therapy remains in the early stages of application for liver diseases, its safety profile and ability to target key drivers of PBC, including oxidative stress and immune dysregulation, highlight its potential as an adjunct to standard treatments such as UDCA. Ongoing research continues to evaluate its efficacy and establish standardized therapeutic protocols. This report presents a case of PBC with significantly elevated liver enzymes successfully managed with adjunctive molecular hydrogen therapy.

In this case, the patient received hydrogen capsules (PURE HYDROGEN) supplied by HoHo Biotech Co., Ltd. (Taipei, Taiwan, ROC). Each capsule contained 170 mg of

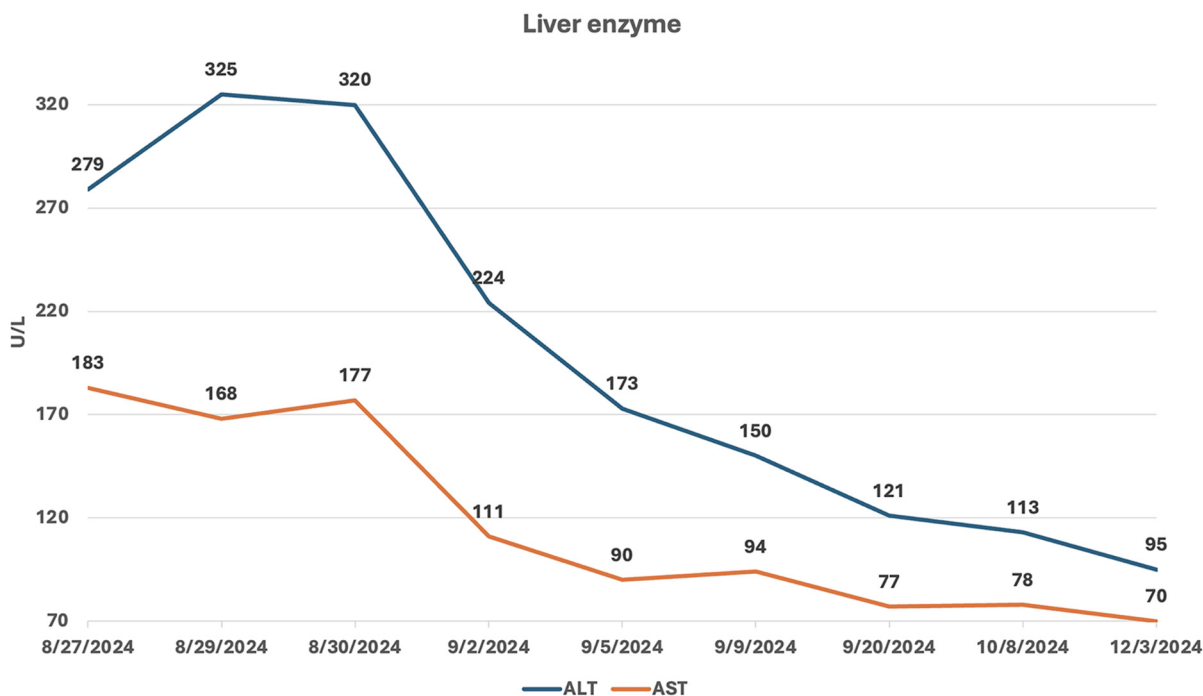


Figure 1. Changes in serum levels of aspartate transaminase (AST) and alanine aminotransferase (ALT) before (August 27, 2024) and after the initiation of molecular hydrogen therapy. Both AST and ALT levels exhibited decreasing trends following the commencement of molecular hydrogen therapy on August 30, 2024.

hydrogen-enriched calcium, delivering approximately $1.7 \times 1,021$ molecules of hydrogen-equivalent to the hydrogen concentration found in 24 cups (200 ml each) of water containing 1,200 ppb (0.6 mM) of dissolved hydrogen. This study was approved by the Institutional Review Board (IRB) of Tri-Service General Hospital, National Defense Medical Center, Taiwan (IRB approval number: B202105106; approval date: July 18, 2023) and conducted in accordance with all relevant ethical guidelines. Written informed consent was obtained from the patient for publication of this case report. The study adhered to the ethical standards of the institution, the 1964 Declaration of Helsinki, and its subsequent amendments or comparable ethical standards.

Case Report

A 44-year-old male with a history of primary biliary cholangitis (PBC), splenomegaly (long-axis diameter: 16.1 cm), and elevated IgG4 levels was referred for the

management of acute cholestatic hepatitis. Initial laboratory tests revealed markedly elevated liver enzyme levels [aspartate transaminase (AST): 279 U/l, alanine aminotransferase (ALT): 183 U/l; Figure 1]. Serological analysis demonstrated positive anti-mitochondrial antibodies (45 U/ml; reference range <20 U/ml), a high antinuclear antibody (ANA) titer (1:1,280), and elevated IgG4 levels (93.00 mg/dl; reference range=8-140 mg/dl). Despite adherence to conventional therapy with ursodeoxycholic acid (UDCA) and dietary modifications, liver function showed only minimal improvement.

On August 30, 2024, molecular hydrogen capsule therapy was initiated as an adjunctive treatment. The patient was prescribed one of H₂ capsules daily. Over four months, significant biochemical improvements were observed, with AST levels decreasing to 95 U/l and ALT to 70 U/l by December 2024 (Figure 1). No adverse effects were reported, and the patient noted an overall improvement in well-being. Imaging studies, including liver ultrasound and

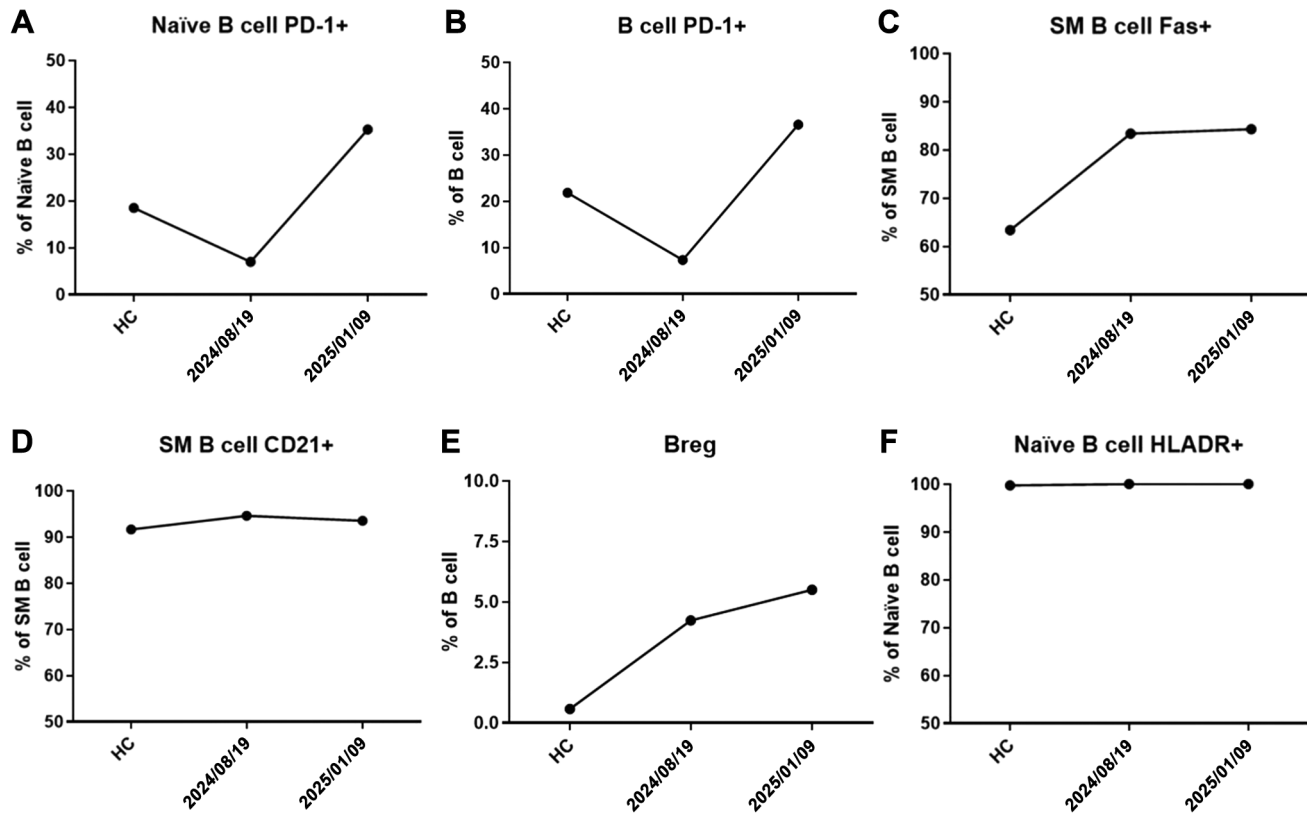


Figure 2. Immunophenotypic changes of B cells following molecular hydrogen therapy. Whole blood analysis was conducted before and after molecular hydrogen therapy with the Health Control (HC) group shown for comparison (far left). Panels (A, B, and E) show that naïve B cell PD-1+, total B cell PD-1+, and regulatory B cell (Breg) populations significantly increased after molecular hydrogen therapy, surpassing the levels observed in the HC group. Panels (C, D, and F) demonstrate stable conditions for switched memory (SM) B cell Fas+, SM B cell CD21+, and naïve B cell HLADR+ populations following therapy.

MRI, showed no evidence of new biliary obstruction or significant fibrosis progression of fibrosis during this period. Follow-up FibroScan results indicated stable liver stiffness measurements, corroborating the biochemical improvements. Additionally, IgG4 levels decreased from their initial elevated levels, suggesting reduced autoimmune activity. The patient's quality of life, assessed using a structured questionnaire, showed significant improvement, with reductions in fatigue and pruritus, common symptoms of PBC. During PBC flares, key immune markers such as killer cell lectin like receptor G1 (KLRG-1), programmed death-1 (PD-1), and T-cell immunoglobulin and mucin domain 3 (Tim3) were notably reduced (Figure 2 and Figure 3), correlating with disease activity. After supplementation

with molecular hydrogen, these markers returned to normal levels, indicating an immunomodulatory effect of H₂.

Discussion

This case highlights the potential of H₂ therapy as an adjunctive treatment for PBC, particularly in patients with a limited response to conventional therapies such as UDCA. The patient's significant biochemical improvement, coupled with enhanced quality of life and reduced autoimmune activity, underscores the multifaceted therapeutic effects of H₂ in managing oxidative stress, inflammation, and immune dysregulation (7, 15). The selective antioxidant properties of H₂ likely contributed to the observed reduction in liver

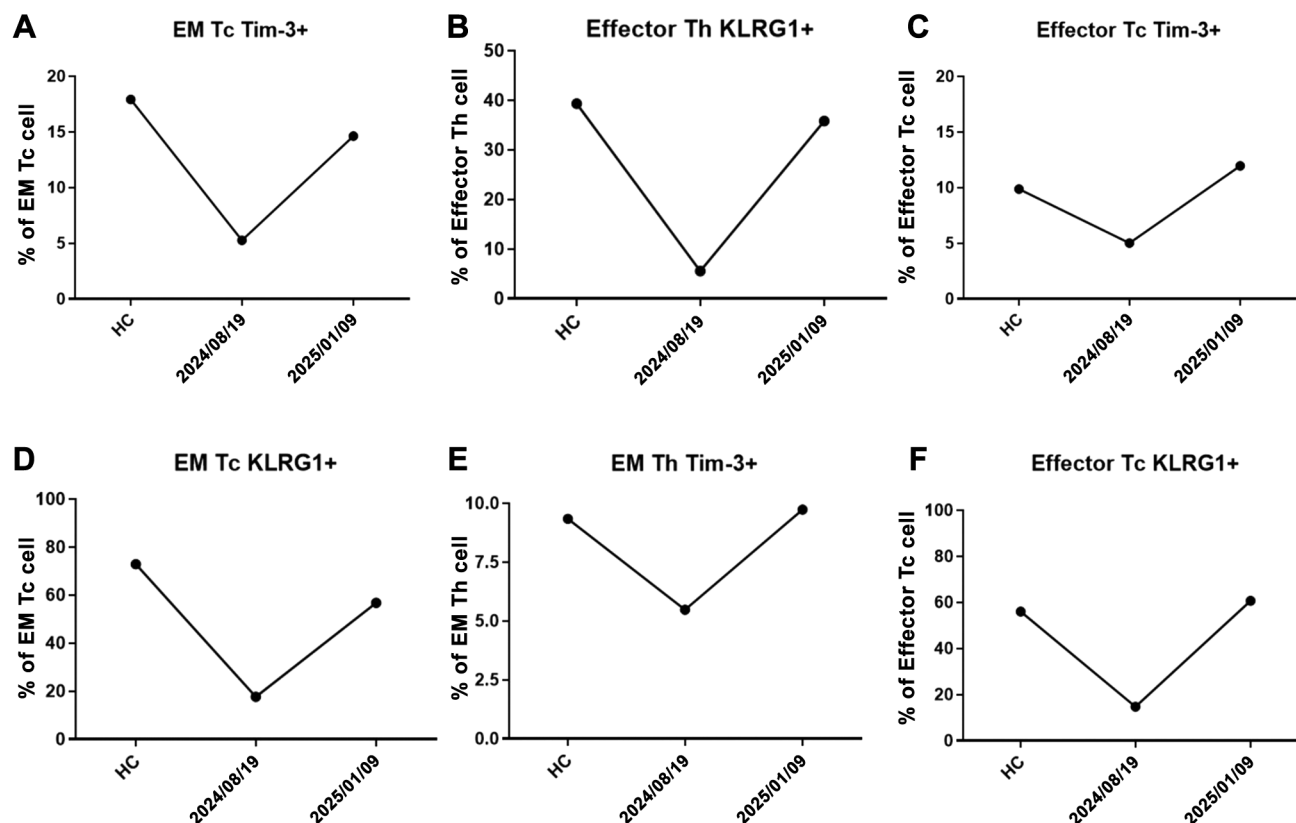


Figure 3. Immunophenotypic changes of T cells following molecular hydrogen therapy. Whole blood analysis was conducted before and after molecular hydrogen therapy, with the Health Control (HC) group shown for comparison (far left). Panels (A, B, C, D, E, and F) show that the percentage of effector memory (EM) cytotoxic T cells (Tc) Tim-3+, effector helper T cells (Th) KLRG1+, effector Tc Tim-3+, EM Tc KLRG1+, EM Th Tim-3+, and effector Tc KLRG1+ increased following molecular hydrogen therapy, eventually returning to levels comparable to those in the HC group.

enzyme levels. By neutralizing hydroxyl radicals and peroxynitrite, H₂ mitigates oxidative stress, a primary driver of liver injury in PBC (16, 17). Additionally, its ability to modulate inflammatory pathways, such as NF- κ B and AMPK/Nrf2, not only reduces pro-inflammatory cytokines but also enhances the production of cytoprotective enzymes, thereby promoting cellular repair and maintaining redox balance (18, 19).

The observed reduction in IgG4 levels is particularly noteworthy, as elevated IgG4 levels are linked to autoimmune activity in certain hepatitis patients (20, 21). The immunomodulatory effects of H₂, evidenced by the normalization of key immune markers such as KLRG-1, PD-1, and Tim3, suggest a role in regulating the immune responses that contribute to hepatocyte injury. These findings are consistent

with preclinical studies demonstrating H₂'s ability to attenuate immune-mediated damage. The absence of adverse effects in this patient further supports the safety profile of molecular hydrogen therapy. Its non-invasive nature and ease of administration in the form of oral capsules make it an attractive option for long-term management of chronic liver diseases. However, this case also underscores the need for larger, controlled studies to validate these findings and establish standardized dosing protocols.

Conclusion

Molecular hydrogen therapy appears to be a safe and effective adjunctive treatment for PBC, especially in patients with an inadequate response to conventional therapies. By

targeting oxidative stress, inflammation, and immune dysregulation, H₂ offers a comprehensive approach to slowing disease progression and enhancing patient outcomes. While this case report contributes to the growing body of evidence supporting its therapeutic potential, further research is necessary to confirm its efficacy, optimize its use, and explore its role in other autoimmune liver diseases.

Conflicts of Interest

The Authors declare that they have no conflicts of interest or competing interests related to this study.

Authors' Contributions

YTL: Conceptualization, methodology, writing – original draft, writing review, and editing. JWJL: Conceptualization, methodology, writing original draft, writing review, and editing. YJH: Conceptualization, methodology, project administration, writing original draft, writing review, and editing. SWL: Conceptualization, methodology, writing original draft, writing review, and editing. TYH: Conceptualization, methodology, writing original draft, writing review, and editing. FCL: Conceptualization, investigation, supervision, writing, review, and editing.

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References

- 1 Houri I, Hirschfield GM: Primary biliary cholangitis: pathophysiology. *Clin Liver Dis* 28(1): 79-92, 2024. DOI: 10.1016/j.cld.2023.06.006
- 2 Trivella J, John BV, Levy C: Primary biliary cholangitis: Epidemiology, prognosis, and treatment. *Hepatol Commun* 7(6): e0179, 2023. DOI: 10.1097/HC9.000000000000179

- 3 DeMart A: Radiology drugs: R&D continues, approval lags. *Hospitals* 62(22): 79, 1988.
- 4 Cazzagon N, Floreani A: Primary biliary cholangitis: treatment. *Curr Opin Gastroenterol* 37(2): 99-104, 2021. DOI: 10.1097/MOG.0000000000000708
- 5 Johnsen HM, Hiorth M, Klaveness J: Molecular hydrogen therapy-a review on clinical studies and outcomes. *Molecules* 28(23): 7785, 2023. DOI: 10.3390/molecules28237785
- 6 Yuan T, Zhao JN, Bao NR: Hydrogen applications: advances in the field of medical therapy. *Med Gas Res* 13(3): 99-107, 2023. DOI: 10.4103/2045-9912.344978
- 7 Zheng CM, Hou YC, Liao MT, Tsai KW, Hu WC, Yeh CC, Lu KC: Potential role of molecular hydrogen therapy on oxidative stress and redox signaling in chronic kidney disease. *Biomed Pharmacother* 176: 116802, 2024. DOI: 10.1016/j.biopha.2024.116802
- 8 Gao X, Niu S, Li L, Zhang X, Cao X, Zhang X, Pan W, Sun M, Zhao G, Zheng X, Song G, Zhang Y: Hydrogen therapy promotes macrophage polarization to the M2 subtype in radiation lung injury by inhibiting the NF-κB signalling pathway. *Heliyon* 10(10): e30902, 2024. DOI: 10.1016/j.heliyon.2024.e30902
- 9 Hirano T: IL-6 in inflammation, autoimmunity and cancer. *Int Immunol* 33(3): 127-148, 2021. DOI: 10.1093/intimm/dxaa078
- 10 Jang DI, Lee AH, Shin HY, Song HR, Park JH, Kang TB, Lee SR, Yang SH: The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics. *Int J Mol Sci* 22(5): 2719, 2021. DOI: 10.3390/ijms22052719
- 11 Li Q, Shi M, Ang Y, Yu P, Wan B, Lin B, Chen W, Yue Z, Shi Y, Liu F, Wang H, Duan M, Long Y, Bao H: Hydrogen ameliorates endotoxin-induced acute lung injury through AMPK-mediated bidirectional regulation of Caspase3. *Mol Immunol* 168: 64-74, 2024. DOI: 10.1016/j.molimm.2024.02.001
- 12 Tabibzadeh S: Signaling pathways and effectors of aging. *Front Biosci (Landmark Ed)* 26(1): 50-96, 2021. DOI: 10.2741/4889
- 13 Fang W, Tang L, Wang G, Lin J, Liao W, Pan W, Xu J: Molecular hydrogen protects human melanocytes from oxidative stress by activating Nrf2 signaling. *J Invest Dermatol* 140(11): 2230-2241.e9, 2020. DOI: 10.1016/j.jid.2019.03.1165
- 14 Shi J, Duncan B, Kuang X: Hydrogen treatment: a novel option in liver diseases. *Clin Med (Lond)* 21(2): e223-e227, 2021. DOI: 10.7861/clinmed.2020-0370
- 15 Slezak J, Kura B, LeBaron TW, Singal PK, Buday J, Barancik M: Oxidative stress and pathways of molecular hydrogen effects in medicine. *Curr Pharm Des* 27(5): 610-625, 2021. DOI: 10.2174/1381612826666200821114016
- 16 Wasik U, Milkiewicz M, Kempinska-Podhorodecka A, Milkiewicz P: Protection against oxidative stress mediated by the Nrf2/Keap1 axis is impaired in Primary Biliary Cholangitis. *Sci Rep* 7: 44769, 2017. DOI: 10.1038/srep44769
- 17 Dallio M, Romeo M, Cipullo M, Ventriglia L, Scognamiglio F, Vaia P, Iadanza G, Coppola A, Federico A: Systemic oxidative

- balance reflects the liver disease progression status for primary biliary cholangitis (Pbc): the narcissus fountain. *Antioxidants (Basel)* 13(4): 387, 2024. DOI: 10.3390/antiox13040387
- 18 Sies H, Berndt C, Jones DP: Oxidative stress. *Annu Rev Biochem* 86(1): 715-748, 2017. DOI: 10.1146/annurev-biochem-061516-045037
- 19 Ishibashi T: Molecular hydrogen: new antioxidant and anti-inflammatory therapy for rheumatoid arthritis and related diseases. *Curr Pharm Des* 19(35): 6375-6381, 2013. DOI: 10.2174/13816128113199990507
- 20 Tanaka A, Notohara K: Immunoglobulin G4 (IgG4)-related autoimmune hepatitis and IgG4-hepatopathy: A histopathological and clinical perspective. *Hepatol Res* 51(8): 850-859, 2021. DOI: 10.1111/hepr.13683
- 21 Capurso G, Pedica F, Palumbo D, Della-Torre E: IgG4-related autoimmune liver disease. *Minerva Gastroenterol (Torino)* 69(1): 23-49, 2023. DOI: 10.23736/S2724-5895.20.02794-4