ISSN: 2692-5206
AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

UO'K:: 616.36-002-07-08-052:378.047-021.65

IMMUNOBLOT EXAMINATION OF THE IMMUNE MARKER IN AUTOIMMUNE HEPATITIS

Tairova G.B.

babakulovnaguzal@gmail.com

Sayfutdinova Z.A.

zuxra.sayfutdinova.91@bk.ru

Toshkent tibbiyot akademiyasi, 100109 Toshkent, O‘zbekiston

Farobiy ko’chasi 2, Tel: +998781507825 E-mail: info@tma.uz

Abstract: Autoantibodies that are not related to the organ, such as antinuclear antibodies (ANA) and smooth muscle antibodies (SMA), are highly prevalent in patients with autoimmune hepatitis. The liver and kidney microsomal antibody type 1 (LKM1) is a serological marker of type 2 autoimmune hepatitis (AIH-2). Antibodies against liver cell cytosol, namely type 1 antibodies (LC1), are present alongside or separately from anti-LKM1, both indicating the clinical condition of AIH-2. Antibodies against the soluble liver antigen (anti-SLA) are found in type 3 AIH when anti-LC1, ANA, SMA, and anti-LKM1 are negative. However, subsequent studies have shown that this marker can also appear together with other specific antibodies for different types of AIH.

Keywords: autoimmune hepatitis, autoimmune hepatitis liver cirrhosis, LKM-1/LC1, sp100, gp210, *AMA-M2.*

Autoimmune hepatitis occurs when the immune system begins attacking the liver tissue. In this condition, immune responses against external factors such as viruses are triggered, but these reactions are more likely to occur in individuals with a genetic predisposition. Type 2 autoimmune hepatitis represents a specific condition, as molecular mimicry (i.e., molecules from viruses or bacteria resembling molecules in the body) is observed. As a result of this mimicry, the body produces antibodies against its liver microsomes, specifically against liver/kidney type 1 microsomes. These antibodies target the liver enzyme cytochrome P450 2D6 (CYP2D6). Thus, type 2 autoimmune hepatitis is triggered through a molecular mimicry mechanism, stimulating immune responses against liver enzymes[9].

Brazilian scientists have studied the occurrence of autoimmune hepatitis (AIH) in patients with chronic hepatitis B infection. In the study, liver biopsies from 1,759 patients were taken, and 92 were confirmed to have AIH. Additionally, in patients with chronic hepatitis C infection, the following results were observed: 66% of patients had SMA (smooth muscle antibodies), 41% had LKM (liver kidney microsomal) antibodies, and ANA (antinuclear antibody) was positive in a percentage of cases. These results suggest that hepatitis viruses can coexist with autoimmune hepatitis. This research points to the simultaneous presence of hepatitis viruses and autoimmune hepatitis, meaning that viruses might stimulate

ISSN: 2692-5206
AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

autoimmune reactions and lead to hepatitis. The main therapeutic agents used in treating AIH are corticosteroids and azathioprine, which help normalize liver enzymes and immunoglobulin G (IgG) levels, leading to remission (the period when the disease enters its inactive phase) [2].

Furthermore, 40% of patients with AIH have a family history of autoimmune diseases, which supports the genetic inheritance of autoimmune conditions. This suggests a genetic basis for the disease and indicates the potential for familial transmission. Although AIH and other autoimmune diseases are related to genetic inheritance, they do not follow Mendelian inheritance. In Mendelian inheritance, diseases are passed through genes located on specific chromosomes, whereas autoimmune diseases are often influenced by multiple genetic factors and environmental factors[10].

Thus, while individuals with a family history of autoimmune diseases may carry genetic risk factors, environmental factors (such as infections, stress, diet, and other factors) also play a significant role. These factors can cause the immune system to react improperly, leading to the development of autoimmune diseases. Autoimmune hepatitis in children is primarily associated with genetic predisposition. The connection between human leukocyte antigens (HLA) and type 1 AIH was first identified 30 years ago [1].

In 1990, Donaldson and colleagues studied the impact of HLA in 96 patients with AIH and 14 patients who had undergone liver transplantation. In AIH patients, HLA-DR3 and HLA-DR4 antigens were found. AIH patients with the HLA DR3 antigen had a poor prognosis, with low treatment efficacy and a high need for liver transplantation [5].

ANA, SMA, anti-LKM1, anti-LC1, and antimitochondrial antibodies were first identified in tissue samples (kidney, liver, and stomach) using human IgG conjugates as detection reagents [7].

Methods. The aim of the study was to analyze the samples from patients who gave consent for participation. We studied 52 patients with AIH type 1, collecting samples at the time of diagnosis. All 52 patients (78% women, average age 33, range 28-51) were diagnosed with AIH according to the International Autoimmune Hepatitis Group criteria.

In addition, 21 control groups were also studied: 12 patients with AIH-2, 10 patients with primary biliary cholangitis, and 10 healthy individuals as controls. All autoimmune hepatitis patients underwent clinical, laboratory, and immunological evaluation (severity of hepatitis and/or cirrhosis). Autoantibodies were tested according to the manufacturer's instructions using commercially available organ-specific immunoblot kits. According to the guidelines, purified F-actin was preloaded onto special lanes to detect the antigen. Positive and negative control serum samples were also used in the tests.

Anti-F-actin antibodies were detected by multiplying the sample's absorption value by 25, compared to the negative control. Results were considered positive if the optical density was greater than 30, suspicious if it was between 25-30, and negative if it was less than 25. For the purpose of adjusting the sensitivity and specificity of the immunoblot method, we tested 50 serum samples and set the cutoff value as the average optical density at 450 nm. We re-examined 36 out of the 52 AIH-1 patients (48.9%). Autoantibody tests were conducted once

ISSN: 2692-5206
AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

during the follow-up period (3-6 months after starting immunosuppressive treatment) to evaluate changes in autoantibodies, which were then compared to disease activity and remission or relapse phases.

Results: Using the immunoblot method, 43 out of 52 AIH patients (82%) tested positive for SMA, 7 (13.5%) for LKM-1/LC1, and 2 (3.8%) for sp100 and gp210 autoantibodies. The specificity of the method was high across all patients, with IgG background. The optical density in 10 blood donors averaged 0.21. During the study, gamma-globulin levels in AIH patients ranged up to 49 g/l, and IgG levels were found to exceed 40 mg/dl.

In AIH-1 patients, clinical (age, gender, remission, relapse periods), laboratory (ALT, AST, alkaline phosphatase, bilirubin, gamma-globulin), and histological parameters were associated. The average follow-up time for AIH patients after diagnosis was 6 months. Among the 52 AIH patients, 11 maintained positive autoantibody levels, and 3 remained seronegative during the follow-up period.

Conclusion: The immunoblot method is a standard test for detecting autoantibodies and forms the core of autoimmune hepatitis immunoserology. Antibodies against liver cell cytosol type 1 and the soluble liver antigen are considered diagnostic markers for AIH. In recent years, kits for detecting anti-LC1 and anti-SLA by enzyme-linked immunosorbent assay (ELISA) have become available. In patients with autoimmune hepatitis, 10% to 15% of those with positive or negative anti-LKM1 showed the presence of these autoantibodies. Similarly, anti-SLA antibodies were found in 28% of those with positive anti-LKM1 and 12% in those with negative anti-LKM1. These findings suggest that these autoantibodies are specific to liver autoimmune diseases. Anti-LC1 and anti-SLA antibodies may be considered serological markers of autoimmune hepatitis and should be studied in autoimmune processes, particularly in AIH.

References

1. Bastian Engel, Alena Laschtowitz, Maciej K. Janik et al. Genetic aspects of adult and pediatric autoimmune hepatitis: A concise review. European Journal of Medical Genetics 2021; 64:e104214
2. Christen, U., Hintermann, E., 2018. Autoantibodies in autoimmune hepatitis: can epitopes tell us about the etiology of the disease? Front. Immunol. 9 (FEB), 1–10. https://doi.org/10.3389/fimmu.2018.00163
3. Moy L., Levine J. Autoimmune hepatitis: a classic autoimmune liver disease, Curr. Probl. Pediatr. Adolesc. Health Care 44 (2014) 341–346
4. Smyk, D.S., 2013. Risk factors for autoimmune hepatitis: from genesand pregnancy to vaccinations and pollutants. Immuno Gastroenterol. https://doi.org/10.7178/ig.32.
5. Stephens, C., Castiella, A., Gomez-Moreno, E.M., et al., 2016a. Autoantibody presentation in drug-induced liver injury and idiopathic autoimmune hepatitis: the influence of human leucocyte antigen alleles. Pharmacogenetics Genom. 26 (9), 414–422. https://doi.org/10.1097/FPC.0000000000000230
6. Tairova G.B. Autoimmun gepatitning davolash prinsiplarini takomillashtirish. Amaliy va tibbiyot ilmiy jurnali. 2023;11(02): 443-445

ISSN: 2692-5206
AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

7. Tairova, G. B., Kurbonova Z. Ch. "Improvement Of Laboratory Diagnosis Of Autoimmune Hepatitis." *Galaxy International Interdisciplinary Research Journal* 10.12 (2022): 1667–1671.
8. Tairova G.B. Improving The Principles of Treatment Of Autoimmune Hepatitis. Eurasian Medical Research Periodical. 2023;26:p110-112
9. Taubert R., Hardtke-Wolenski M., Noyan F., Wilms A. et al. Intrahepatic regulatory T cells in autoimmune hepatitis are associated with treatment response and depleted with current therapies, J. Hepatol. 61 (2014) 1106–1114
10. Ulff-Møller, C.J., Svendsen, A.J., Viemose, L.N., Jacobsen, S., 2018. Concordance of autoimmune disease in a nationwide Danish systemic lupus erythematosus twin cohort. Semin. Arthritis Rheum. https://doi.org/10.1016/j.semarthrit.2017.06.007.