ANNOTATION

of the dissertation work Amirkulova Ainura Askarbekovna on the topic: "Clinical and Genetic Characteristics of Non-Alcoholic Steatohepatitis in the Republic of Kazakhstan" submitted for the degree of Doctor of Philosophy (PhD) in the specialty 8D10102 – "Medicine."

Relevance of the Research Topic:

Non-alcoholic fatty liver disease (NAFLD) is one of the most common forms of chronic liver disease worldwide, affecting up to 25% of the adult population [Sepanlou et.al., 2020]. In recent decades, NAFLD has become an integral part of metabolic syndrome and is closely associated with conditions such as obesity, type 2 diabetes, and cardiovascular diseases [Watt M.J. et al., 2019]. According to the WHO, the incidence of metabolic syndrome and obesity worldwide has significantly increased, leading to a rise in patients with NAFLD and its complications, including non-alcoholic steatohepatitis (NASH), liver fibrosis, and hepatocellular carcinoma [WHO, Geneva, 2021].

The problem of NAFLD becomes especially relevant in the context of Kazakhstan. According to local studies, the prevalence of NAFLD among the adult population of Kazakhstan continues to rise, requiring the development of local strategies for diagnosis and treatment [Nersesov V.A. et.al., 2017]. Numerous studies highlight that the pathogenesis of NAFLD is related to several genetic factors, such as the polymorphisms of PNPLA3, TM6SF2, and MBOAT7, which affect lipid metabolism in the liver and the risk of fibrosis [Sookoian S. et.al., 2023].

The gut microbiome also has a significant impact on the development and progression of NAFLD. Dysbiosis can contribute to insulin resistance, inflammation, and the development of liver fibrosis [Rau M. et al., 2018]. Recent studies emphasize that the microbiome can modify genetic predisposition and influence the clinical course of the disease [Pirola C.J. et al., 2022].

The relevance of our research is increased by the need to develop personalized approaches to the diagnosis and treatment of NAFLD based on genetic and microbiota data from patients with the disease.

Research Aim:

To investigate the genetic and microbiotic characteristics of non-alcoholic steatohepatitis (NASH) in the population of the Republic of Kazakhstan and their impact on clinical-laboratory manifestations and disease progression, with the aim of developing an algorithm for early diagnosis and personalized treatment.

Research Objectives:

1. To investigate the impact of genetic markers PNPLA3 and TM6SF2 on the laboratory and clinical manifestations of non-alcoholic steatohepatitis (NASH) in the population of Kazakhstan.
2. To analyze the influence of PNPLA3 and TM6SF2 gene polymorphisms on the progression of non-alcoholic steatohepatitis.
3. To study the gut microbiome status and its relationship with laboratory indicators in patients with non-alcoholic steatohepatitis.

4. To develop, based on the obtained data, an algorithm for early diagnosis of steatohepatitis in clinical practice, grounded in patients' individual genetic and clinical characteristics.

Study Design:

An observational analytical case-control study.

At the initial stage, the study included 197 patients with liver disease. Following a general clinical assessment (including symptom survey, medical and life history collection, and physical examination) and diagnostic procedures, patients with other causes of hepatitis (such as viral hepatitis, drug-induced hepatitis, autoimmune hepatitis, etc.) were excluded from the study.

At the next stage, 61 patients were enrolled for an in-depth collection of clinical and physical examination data, laboratory and instrumental investigations, genetic profiling, and analysis of the gut microbiome to assess the influence of various mutations on the clinical course of steatohepatitis.

Research Methods:

1. Clinical: Collection of clinical and anamnestic data and physical examination.
2. Instrumental: Abdominal ultrasound (US), liver FibroScan, esophagogastroduodenoscopy (EGD).
3. Laboratory: Complete blood count (CBC), blood biochemical analysis, enzyme-linked immunosorbent assay (ELISA) for hepatitis viruses, autoimmune markers, alpha-fetoprotein (AFP).
4. Genetic: Blood sampling for genetic testing to identify PNPLA3 and TM6SF2 gene polymorphisms.
5. Microbiome Analysis: Stool sequencing to study the colonic microbiota.
6. Statistical: Statistical processing and analysis of the collected data.

Novelty of the Study:

1. For the first time, a comprehensive analysis of the clinical and genetic characteristics of steatohepatitis has been conducted in the Kazakhstani population, taking into account the genetic polymorphisms of the PNPLA3 and TM6SF2 genes.
2. For the first time, the impact of the colonic microbiota on the clinical presentation and progression of the disease was assessed, along with the investigation of the relationship between genetic traits and microbiota composition in Kazakhstani patients with steatohepatitis using next-generation sequencing (NGS).
3. For the first time, a trend toward an increased risk of liver fibrosis was identified in patients with the TM6SF2 gene polymorphism in the context of non-alcoholic steatohepatitis, which opens up new opportunities for predicting disease progression and developing personalized approaches for treatment and early identification of high-risk groups.

Key Findings Presented for Defense:

1. The study demonstrated that the presence of the TM6SF2 gene polymorphism may be one of the factors determining the severity of cytolysis in patients with non-alcoholic steatohepatitis (NASH). A significant difference in laboratory indicators such as alanine aminotransferase (ALT) was observed between the compared groups. At the same time, the TM6SF2 polymorphism had no effect on other laboratory parameters

such as gamma-glutamyl transpeptidase (GGT), bilirubin and its fractions, or lipid metabolism markers in patients with NASH.
2. Patients with the TM6SF2 polymorphism showed greater diversity in gut microbiome enterotypes compared to patients without the polymorphism. Specifically, Enterotype III was identified in patients with the polymorphism, whereas it was absent in the non-polymorphic group. This suggests a potential influence of genetic factors on the intestinal microbiota composition and highlights the need for further research in this area.
3. The PNPLA3 gene polymorphism was found to affect LDL cholesterol levels, indicating a predisposition in this group of patients to disturbances in hepatic lipid metabolism, which in turn increases the risk of developing steatohepatitis and other metabolically associated disorders.

Practical Significance:

1. A scientifically grounded and clinically applicable algorithm for risk stratification, early diagnosis, and individualized management of patients with NASH has been developed for use within the healthcare system of the Republic of Kazakhstan.
2. Based on a comprehensive assessment of genetic markers (TM6SF2, PNPLA3), microbiome enterotypes, as well as clinical and biochemical characteristics of the disease, the feasibility of implementing a personalized model of medical monitoring for high-risk NASH patients has been substantiated.
3. The obtained data demonstrate the potential of using genetic and microbiota-related markers as predictors of liver fibrosis progression, paving the way for the individualization of therapeutic and diagnostic algorithms. Criteria have been identified for intensified monitoring and the application of comprehensive pathogenetic therapy aimed at correcting metabolic, inflammatory, and microbiome-related disturbances.

Author's Contribution to the Research:

Throughout the entire course of the study, the doctoral candidate actively participated in formulating the research topic, aims, and objectives, developed the research methodology, and was responsible for recruiting patients with hepatitis. She independently conducted a literature review related to the dissertation topic, wrote the dissertation chapters, and performed data collection and synthesis of the research findings. The author also carried out the interpretation of clinical-laboratory, morphological, and instrumental data of the patients.
The research findings were prepared and published by the author in journals recommended by the Committee for Quality Assurance in the Sphere of Education and Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan, as well as presented at international scientific-practical conferences and published in foreign journals. The results of the dissertation work have been implemented into the clinical practice of the RSE "Hospital of the Medical Center of the Administration of the President of the Republic of Kazakhstan" and may be used in educational programs, clinical protocols, and quality management systems for medical care of patients with NASH.

Publications Related to the Dissertation Topic:

A total of 10 scientific papers have been published on the topic of the dissertation

research, including 1 publication in a journal indexed in the Scopus database (Q2), and 3 publications in journals recommended by the Committee for Quality Assurance in the Sphere of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan. Additionally, 4 abstracts were published in collections of international scientific conferences. A certificate of state registration of copyright in the Republic of Kazakhstan was obtained, and 2 acts of implementation of new technologies (innovations) into the practice of healthcare organizations were issued.

The research results and key findings of the dissertation were presented and discussed at the following international scientific-practical conferences:

– The republican scientific-practical conference with international participation dedicated to the 60th anniversary of NJSC "Astana Medical University": "Topical Issues of Primary Health Care: Current Trends, Problems, and Solutions";

– The 5th International Congress "Gastroenterology – 2024";

– The International Scientific and Practical Conference "High-Tech Methods of Diagnosis and Treatment of Malignant Neoplasms";

– The 4th International Conference "Gastroenterology – 2023";

– The 1st Scientific-Practical Conference of Students and Young Scientists "Chronic Inflammatory Skin Conditions: Interdisciplinary Challenges".

Conclusions Drawn from the Study:

1. Among patients with non-alcoholic steatohepatitis (NASH) carrying the PNPLA3 gene polymorphism (rs738409 G allele), a statistically significant increase in LDL levels was observed ($p = 0.016$), indicating the gene's influence on triglyceride and LDL metabolism. This may serve as a marker of a more aggressive disease course and a higher risk of progression, as well as elevated cardiometabolic risk. These patients also showed an increased risk of developing liver cirrhosis, observed in 21% of cases compared to 10% in patients without the mutation.
2. Analysis of alanine aminotransferase (ALT) levels based on TM6SF2 gene polymorphism (rs58542926 allele) revealed statistically significant differences between comparison groups ($p = 0.024$), supporting an association between this genetic variant and the severity of cytolytic syndrome in NASH patients. These findings confirm the role of TM6SF2 in the pathogenic mechanisms of liver parenchymal damage. Patients with the TM6SF2 polymorphism demonstrated a stronger tendency toward disease progression and liver fibrosis. Fibrosis was present in 33.3% of patients with the TM6SF2 mutation—8.3% higher than in those without the mutation.
3. No statistically significant clinical or laboratory differences ($p = 0.625$) were found between NASH patients with and without the TM6SF2 polymorphism (genotypes C/C and C/T). Laboratory indicators such as GGT, total and direct bilirubin, alkaline phosphatase (ALP), glucose levels, and lipid profile showed no significant variations.
4. Gut microbiota analysis revealed significant differences in microbiota composition between patients with different genetic mutations. Patients with the TM6SF2 polymorphism exhibited the presence of a third enterotype, which was absent in patients without the mutation—indicating a link between genetic factors and microbiota composition. In the group without the TM6SF2 polymorphism, the first

enterotype predominated (59%), while in the mutation group, enterotypes were more evenly distributed (Type I — 68.2%, Type II — 27.3%, Type III — 4.5%).

5. Based on these findings, an algorithm for the use of genetic testing in the early diagnosis of steatohepatitis among the Kazakhstani population has been developed. It is recommended to include genetic testing for PNPLA3 and TM6SF2 polymorphisms in diagnostic protocols for patients at high risk of developing advanced NAFLD stages. For patients with identified mutations, regular monitoring of gut microbiota is advised to detect potential complications and prevent fibrosis progression.

Practical Recommendations:

1. It is recommended to classify NASH patients with TM6SF2 (rs58542926) and PNPLA3 (rs738409) polymorphisms into a separate clinical high-risk group for liver fibrosis progression. These patients should undergo targeted and more frequent metabolic monitoring, including weight, glycemia, lipid profile, and ALT/AST levels.
2. For patients with the TM6SF2 polymorphism, microbiome monitoring is advised, including enterotype analysis, as the presence of a specific Type III enterotype may be associated with a more severe course of NASH.
3. Genetic testing for PNPLA3 and TM6SF2 is recommended not as a population-wide screening measure but rather based on clinical and epidemiological indications, with the aim of risk stratification and planning of personalized treatment strategies.