

INNATE IMMUNE RESPONSE IN NON-ALCOHOLIC FATTY LIVER DISEASE: AN OVERVIEW OF ALTERATIONS IN TLR 9, MACROPHAGES AND TNF α

Novianti Supriatna^{1*}, Nurjati Chairani Siregar²

¹Master Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

²Departement of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta Indonesia

pISSN: 2828-4070

<https://doi.org/10.69951/proceedingsbookofficeonimeri.v6i-.131>

Proceedings ICE on IMERI. 2022.

Received: October 5th, 2021

Accepted: November 15th, 2021

Published online: February 14th, 2022

Corresponding Author

Name : Novianti Supriatna
Email : novianti.supriatna@ui.ac.id
Mailing address : Jakarta, Indonesia
Telephone : 081287553468
Fax : -
ORCID ID : 0000-0003-4985-6267

Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) and Alcoholic Liver Disease (ALD) are two causes of chronic liver disease. In Indonesia, NAFLD is the most common case of hepatic disease for Indonesian people. Risk factors such as obesity, diabetes mellitus, and metabolic syndrome increase the risk of developing NAFLD. About 1.5 billion people worldwide suffer from fatty liver, with a prevalence of 25-30%. Hence, it will continue to increase sharply if risk factors or diseases are not mitigated with pharmacological treatment and diet. The innate immune response induces abnormalities in fatty liver, mediated by Kupffer cells and TLR9 (Toll-Like Receptor 9), leading to inflammation via proinflammatory cytokines, like TNF α . Hepatocyte injury releases signals in the form of mitochondrial DNA enclosed in microparticles, which travels into the plasma, and are then captured by TLR9. Activation of TLR9 on Kupffer cells is the starting point of the inflammatory process in the pathogenesis of NAFLD, and it triggers other immune responses that encourage the development of steatohepatitis until a more severe liver injury occurs. The latest study found that analysis is needed to determine the immunological mechanisms and therapeutic targets associated with inflammation in fatty liver disease as evidenced by various immunological approaches.

Keywords: innate immune response, TLR 9, macrophages, TNF α , Nonalcoholic Fatty Liver Disease

Introduction

Nonalcoholic Fatty liver Disease (NAFLD) is becoming a significant health problem for approximately 25% of the world's adult population. The abundance of cases results

from unanticipated complications and the absence of early detection.^{1,2} The most significant risk factor for NAFLD is metabolic syndrome. The prevalence of metabolic syndrome in Indonesia reaches 23%. Since 2013 to 2018, metabolic syndrome is associated with obesity which increased from 14.8% to 21.8%. It is also associated with hypertension, the prevalence of which increased from 25.8% to 34.1%. Diabetes mellitus is another contributing factor, the prevalence of which increased from 6.9% to 8.5% in Indonesia.³

NAFLD is a chronic liver disease afflicting children and adults; it is caused by genetics and epigenetics factors, unhealthy dietary habits, and low habitual physical activity, leading to chronic liver disease. Environmental factors can lead to insulin resistance, oxidative stress, and lipotoxicity, which induce the formation of the fatty liver spectrum.³ NAFLD is indicated with lesser consumption of alcohol: less than 20 grams/per day in women, and less than 30 grams/per day in men.^{1,4}

Inflammation promotes the progression of simple fatty liver to steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma, and cardiovascular disease. The mechanism of inflammation begins with receptor activation, which then activates signaling pathways via transcriptional phosphorylation mechanisms, releasing inflammatory mediators that form the causal mechanism in fatty liver disease. The classic inflammatory and metabolic receptor of TLR9 through NfκB activation will release various proinflammatory mediators.^{5,6}

Innate Immune Response in NAFLD

The pathogenesis of NAFLD involves a plethora of mediators, including cytokines, chemokines, neurotransmitters, adiponectin, TNFα, micro-RNA (miRNA), Extracellular vesicles (EVs), and metabolites. These mediators activate immune cells, which release proinflammatory chemokines, and cytokines, thus causing hepatocyte apoptosis.⁵ The gut microbiota also can trigger an immune response in NAFLD. Intestinal barrier dysfunction worsens NAFLD and increases bacterial translocation. Destruction of the barrier by the gut microbiota causes bacterial products, such as endotoxins, to enter the blood circulation through the gut-liver axis, which then causes inflammation of the liver.⁵

The liver and adipose tissue can affect insulin resistance and systemic metabolism. Adipose tissue secretes adiponectin, leptin, TNFα, and IL6, as well as several lipid groups (ceramide, palmitic acid). These cytokines inhibit the function of the mitochondria and endoplasmic reticulum, causing stress and apoptosis of hepatocytes. Kupffer cells will produce TNF-related apoptosis-inducing ligand (TRAIL), Tumor necrosis factor (TNF), and Fas ligand (FasL) through phagocytosis to encourage hepatocyte apoptosis, steatohepatitis, and fibrosis.⁵

Alterations in TLR9, Macrophages, and TNFα

The metabolic system is derived pathways of the TLR receptor and NLR. PRR, such as TLR and NLR, is responsible for introducing immunogenic signals, representing significant changes in the liver.⁶

TLR9/Toll-Like Receptor 9

Toll-like receptor 9 (TLR9) is a receptor with primordial functions in inflammatory and metabolic processes. TLR9 regulates homeostasis under acute stress pressure, working on unmethylated CPG DNA.⁷ TLR9 activation induces progression of NAFL to NASH. A study revealed that when mitochondrial DNA enclosed by microparticles is released into the plasma in steatotic hepatocytes, it will activate TLR9 in endosomes to undergo hyperactivation and produce inflammatory cytokines, such as IL6, TNF α , and IL1 β , encouraging the development of steatohepatitis. It also stimulates exacerbation by strengthening inflammation and liver injury.⁸

By default, most TLR9 in resting conditions is localized in the endoplasmic reticulum of adipose tissue, intestines, and liver with adiponectin, mtDNA, and Peroxisome Proliferator-Activated Receptor (PPAR) levels under normal conditions, and NF κ B is not activated.⁹ When hepatosteatosis begins, the secretion of mtDNA will activate TLR9. Adipose infiltration relies on the activation of TLR9 in Kupffer cells, which could occur by intestinal epithelium damage that causes endotoxins to enter the portal circulation or translocation of bacterial cells. Furthermore, when mtDNA is increased in the systemic circulation, adipocytes synthesis will be more depressed, accompanied by decreased adiponectin levels and decreased Adenosine Monophosphate Activated Protein Kinase (AMPK) activation. Increased activation of TLR9 will reduce PPAR activity and activate NF κ B, producing proinflammatory cytokines. If the hepatic, gut, and visceral adipose tissue compartments are depressed, the proinflammatory positive feedback loops that rely on TLR9 will become stronger.^{9,10} When the amount of mtDNA in circulation is higher than that of adiponectin, TLR9 will suppress PPAR activation. Besides that, adiponectin levels that are too low cannot dampen the TLR9 signaling. Moreover, the availability of free cholesterol in cells will strengthen TLR9 signaling. Activation of Hepatic Stellate Cells (HSCs) indicates that liver fibrosis has begun. However, if there is antagonism of TLR9, it will prevent inflammatory paracrine loops in the tissue compartment, resulting in attenuation of fibrosis from the activation of HSCs. TLR9 antagonism reduces fat, inflammation, and fibrosis. It is mediated by adiponectin, which systematically increases energy expenditure and reduces fat, inflammation, and fibrosis. Furthermore, the PPAR signal will regain homeostatic function by attenuating TLR9 activation (Figure 1).⁹

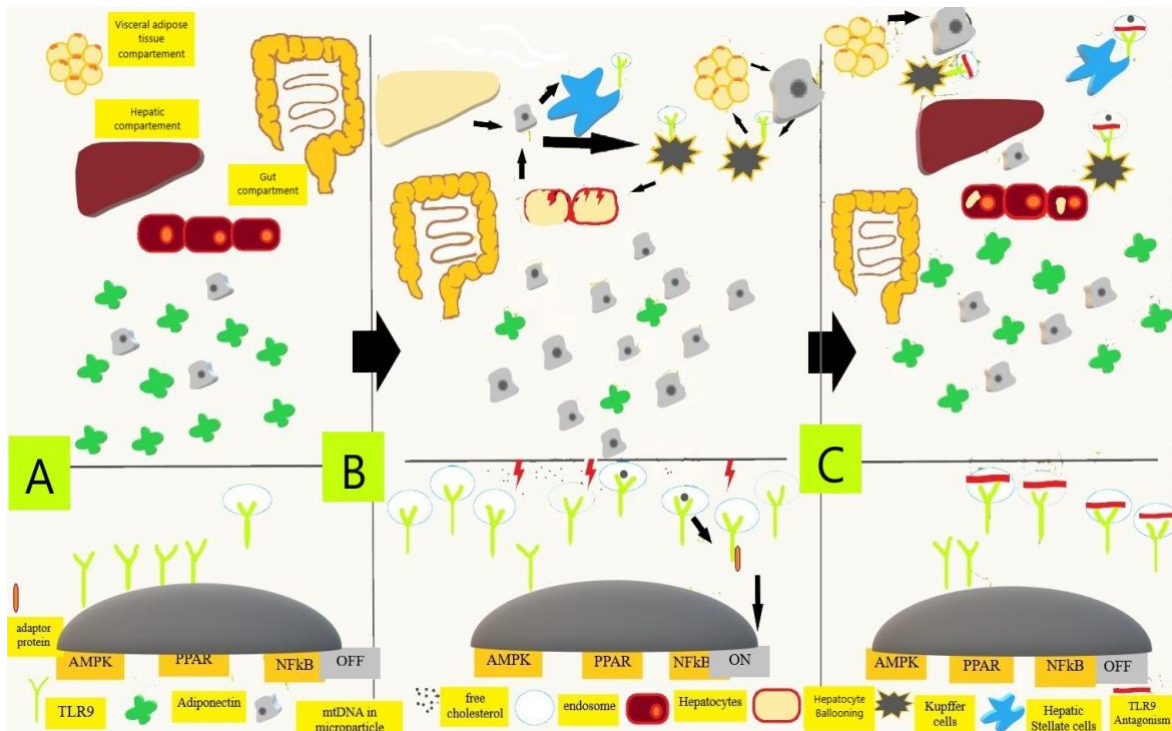


Figure 1. Role of Toll-like receptor 9 (TLR9) which is promote hepatic inflammatory process, and role of TLR9 antagonism in steatohepatitis which is reduce hepatic inflammation

Role of Kupffer Cells in The Inflammation Process

Macrophages that reside in the liver are known as Kupffer cells. They represent approximately 90% of all tissue macrophages and 35% of non-parenchymal cells in the liver. There is a large number of intravascular macrophages in the hepatic tissue, where they fill the sinusoidal lumen and recognize antigens in the blood that are present along the cell surface. Kupffer cells have the extraordinary capacity to admit millions of red blood cells, platelets, and immune cells within the circulation. It also selects pathogens or products of the pathogens, which penetrate through the epithelial cells and enter the bloodstream toward the gut-liver axis, causing hepatocytes damage.¹¹

Kupffer cells can be activated by certain signal expressions, either in the form of Pathogen Associated Molecular Pattern (PAMP) or Damaged Associated Molecular Pattern (DAMP). These signals are captured by receptors on the surface of the macrophage membrane called TLR or receptors in the cytoplasm called NLR.¹² The role of Kupffer cells, among others, includes inducing inflammation by secreting inflammatory mediators, recruiting chemokines such as CCL2 and CXCL10, and recruiting fibrogenic monocytes into injured liver tissue.⁷ The release of DAMP, including mitochondrial DNA by apoptosis, causes hepatocytes to directly activate TLR9 on the surface of Kupffer cells and trigger an inflammatory cascade. (Figure2)⁷

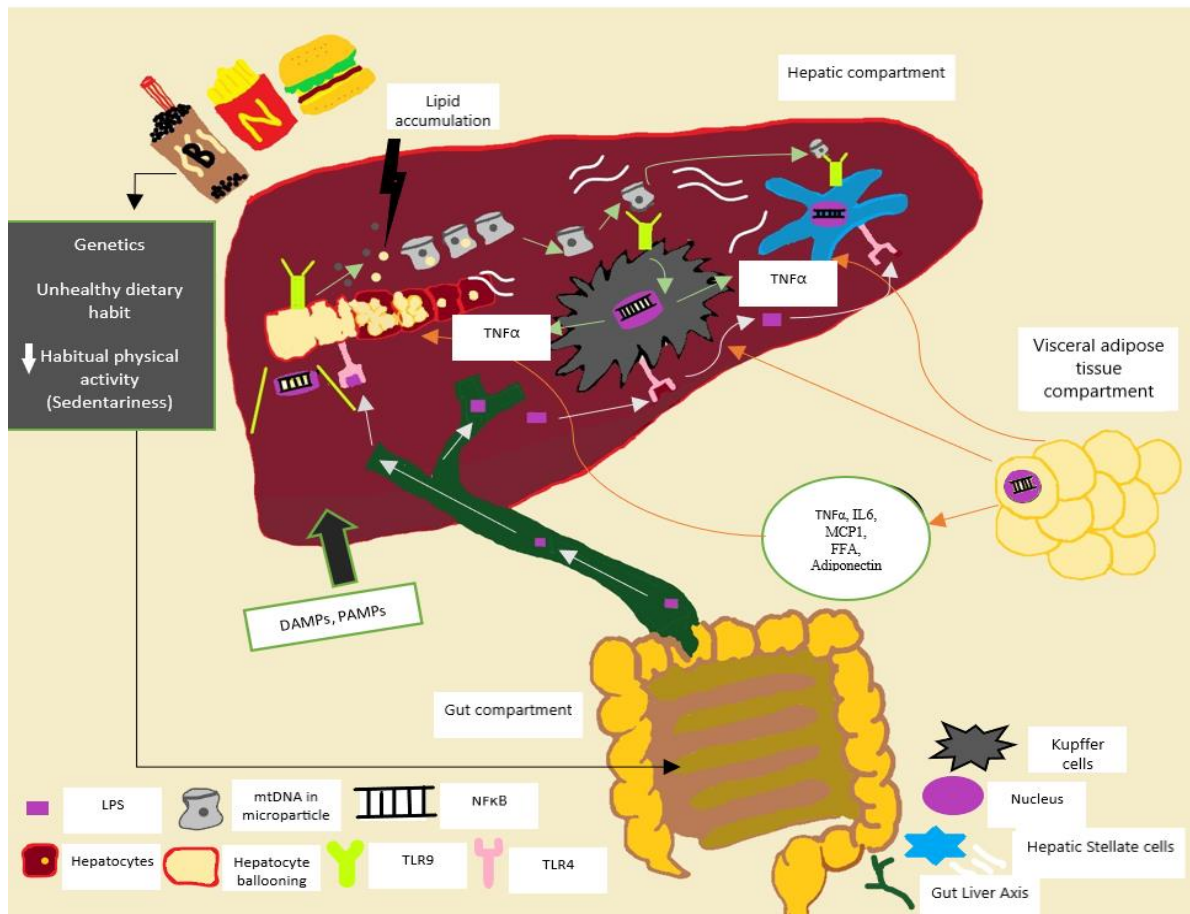


Figure 2. Interaction of Kupffer cells with hepatocytes and Hepatic Stellate Cells in Non-Alcoholic Fatty Liver Disease. In addition, there is a contribution of bacterial products from the gut to the liver that enter through the gut-liver axis and also an involvement of visceral adipose tissue compartments in the hepatic inflammatory process

When hepatocytes are damaged, it releases a signal in the form of mitochondrial DNA that TLR9 will capture. The signal serves as a danger sign and activates Kupffer cells. Activated Kupffer cells will release various inflammatory mediators, causes apoptosis, and begins the process of fibrogenesis (Figure2).⁸ The mitochondrial DNA of hepatocytes serves as ligands, resulting in active TLR9 in Kupffer cells.⁷ In the pathophysiology of NAFLD, risk factors such as obesity and metabolic syndrome increase adipose tissue damage, thereby releasing Free Fatty Acids (FFAs) to the portal circulation, leading to triglycerides (TGs) accumulation in hepatocytes. Moreover, FFAs from adipocytes release elevated levels of cytokines and chemokines, such as MCP1, TNFα, IL6, as well as lower levels of functional adipokines such as adiponectin.¹³ Increased accumulation of triglycerides in hepatocytes leads to inflammation and apoptosis in liver cells, after which HSCs promote progressivity to fibrosis by accelerating the development of steatohepatitis to fibrosis. (Figure2).⁸

In addition to adipose tissue disorders, the gut contributes to the release of bacterial products as PAMP, such as endotoxins or lipopolysaccharides, as well as molecular patterns associated with damage to other tissues that express DAMP in the liver, thereby activating Kupffer cell receptors to produce cytokines such as IL1β, TNFα, and IL6, which

increase liver injury.⁸ When DAMP and PAMP bind to TLR, it activates MYD88 or TRIF signaling pathway, which activates NF κ B and Janus kinase (JNK). Activated NF κ B secretes proinflammatory cytokines, leading to lipid accumulation, cell injury, and apoptosis. Meanwhile, when DAMP and PAMP bind to NLR in the cytoplasm, it activates NOD Like Receptor Family Pyrin Domain Containing 3 (NLRP3), the adaptor protein ASC, and Caspase1 axis to regulate IL1 β production through the assembly of inflammasome complexes. This process leads to the production of proinflammatory cytokines, such as IL1 β and IL18, causing liver inflammation and apoptosis.^{7,9}

Correlation of Increased TNF α with NAFLD Incidence

The cytokine TNF α , thought initially to be a classic inflammatory cytokine, is currently associated with steatosis, insulin resistance, and inflammatory diseases. Activation of proinflammatory cytokines such as TNF α in the liver and adipose tissue plays a vital role in the pathogenesis and progression of NAFLD. Insulin resistance correlates with higher serum TNF α levels in NASH patients. The stress on the endoplasmic reticulum (ER) disrupts the insulin receptor substrate-1 (IRS1), resulting in insulin resistance. Insulin resistance will impair metabolic regulation, prompting the unfolded protein response (UPR) to activate the gene that encodes transcription factor NF κ B to release inflammatory mediators, such as TNF α .^{14,15} Tumor Necrosis Factor Receptor 1 (TNFR1) expression, as a receptor for TNF α , also correlates with disease activity and fibrosis. TNF α signal transduction is mediated by TNFR1 and Tumor Necrosis Factor Receptor 2 (TNFR2). TNFR1 is expressed in multiple sites, whereas TNFR2 expression is more restricted to endothelial cells, neurons, and immune cells. When TNF α binds to TNFR1, it leads to the formation of protein complexes by transmitting signals through cytoplasmic adaptor proteins. Moreover, TNF α -TNFR1 complexes can interact with the adaptor protein Fas Associated Protein with Death Domain (FADD), leading to activation of initiator caspase 8. Activated caspase 8 promotes the activation of downstream effector caspases, cleaves cellular substrates, and induces apoptosis.¹⁵

In a study conducted by Neeta et al. in 2020, the curve of TNF α and adiponectin was built to predict the severity of fatty liver. The cut-off value of TNF α from the ROC curve was 11.2 pg/ml for NAFLD, with a sensitivity of 90.05%, specificity of 100%, and accuracy of 91.80%. The cut-off value of adiponectin from the ROC curve was < 4.2 μ g/ml for NAFLD with a sensitivity of 80.49%, specificity of 85%, and accuracy of 80.32%. Studies show that there has been a significant increase in TNF α values, accompanied by a decrease in adiponectin in serum of people with fatty liver disease, which correlates with the degree of liver steatosis based on diagnostic tests using ultrasonography. In another study, obese children who had NAFLD significantly showed higher serum concentrations of TNF α compared to controls, which shows that TNF α is associated with metabolic syndrome.^{16,17} However, definitive diagnostics of fatty liver disease in adults require a histopathological picture for diagnostic accuracy.¹⁷

A study in children and adolescents by Pacifico et al. in 2014 showed an increase in interventricular disturbances after examination of vascular imaging with noninvasive ultrasonography in 41 subjects from 54 subjects with obesity and NAFLD. There is increased septal thickness with left ventricular (LV) systolic and diastolic dysfunction. The NAFLD group also showed an increase in epicardial fat thickness. Fat thickness correlates with NAFLD and atherosclerosis in obese children and adolescents. Increased free fatty acids released by adipocytes in visceral organs, such as the heart and the liver, promote low-grade chronic inflammation, releasing of proinflammatory cytokines, such as TNF α .¹⁸

Pharmacological Interventions to Prevent The Progression of NAFL to NASH

The therapy of fatty liver disease is determined at the level of the disease. Most fatty liver diseases have fallen into fibrosis or even hepatocellular carcinoma (HCC), thus requiring chemotherapy. Therefore, early prevention is needed to prevent the progression of NAFL to NASH. In addition to managing metabolic diseases, several pharmacological agents have been trialed in animals and humans, showing promising results. These agents include probiotics, pentoxifylin, prednisolone, and vitamin E.¹⁹

The therapeutic agents target the signaling pathway of innate immune responses, from receptors to proinflammatory cytokines released by Kupffer cells. According to Wang et al., in 2021, only vitamin E effectively prevents NAFLD, judging by improvements in the histopathological condition of the liver cells that experience inflammation. Therapeutic targets are already known; however, further analysis is needed to develop pharmacological interventions in NAFLD.¹⁹

Conclusion

NAFLD is caused by many factors, such as genetics and epigenetics. TLR9 activation promotes the transcription factor Nf κ B on Kupffer cells to release inflammatory mediators, like TNF α . The innate immune response in NAFLD is followed by lipid accumulation, cell injury, and apoptosis of hepatocytes. The chronic inflammatory process will promote more severe liver injury; therefore, analysis is needed to determine the immunological mechanism associated with inflammation in fatty liver disease and to develop pharmacological intervention.²⁰

Competing Interests

There is no competing interest in this study.

Acknowledgments

This article was presented in the 6th International Conference and Exhibition on Indonesian Medical Education and Research Institute (6th ICE on IMERI), Faculty of

Medicine, Universitas Indonesia. We thank the 6th ICE on IMERI committee, who had supported the peer-review and manuscript preparation.

References

1. Idalsoaga F, Kulkarni A V., Mousa OY, Arrese M, Arab JP. Nonalcoholic Fatty Liver Disease and Alcohol-Related Liver Disease: Two Intertwined Entities. *Front Med.* 2020;7(August):1–17.
2. De Vries M, Westerink J, Kaasjager KHAH, De Valk HW. Prevalence of Nonalcoholic Fatty Liver Disease (NAFLD) in patients with type 1 diabetes mellitus: A systematic review and meta-analysis. *J Clin Endocrinol Metab.* 2020;105(12):3842–53.
3. Riskesdas K. Hasil Utama Riset Kesehatan Dasar (RISKESDAS). *J Phys A Math Theor* 2018;44(8):1–200.
4. McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe.* 2012;12(3):324–33.
5. Arab JP, Roblero JP, Altamirano J, Bessone F, Chaves Araujo R, Higuera-De la Tijera F, et al. Alcohol-related liver disease: Clinical practice guidelines by the Latin American Association for the Study of the Liver (ALEH). *Ann Hepatol.* 2019;18(3):518–35.
6. Poznyak V, Fleischmann A, Rekke D, Rylett M, Rehm J, Gmel G. The World Health Organization's global monitoring system on alcohol and health. *Alcohol Res Curr Rev.* 2013;35(2):244–9.
7. Feng D. The alteration of immune cells in the pathogenesis of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Liver Res.* 2020;4(1):23–7.
8. Handa P, Vemulakonda A, Kowdley K V., Uribe M, Méndez-Sánchez N. Mitochondrial DNA from hepatocytes as a ligand for TLR9: Drivers of nonalcoholic steatohepatitis? *World J Gastroenterol.* 2016;22(31):6965–71.
9. Shepard CR. TLR9 in MAFLD and NASH: At the Intersection of Inflammation and Metabolism. *Front Endocrinol (Lausanne).* 2021;11(January):1–21
10. Gieben U, Group N, City SL, Dusseldorf U, Einrichtungen M. Liver and Heps Everything. Vol. 185, *Advances in Anatomy Embryology and Cell Biology.* 2007. 1–73 p.
11. Lee WY, Sanz MJ, Wong CHY, Hardy PO, Salman-Dilgimen A, Moriarty TJ, et al. Invariant natural killer T cells act as an extravascular cytotoxic barrier for joint-invading Lyme Borrelia. *Proc Natl Acad Sci U S A.* 2014;111(38):13936–41.
12. Mehal WZ, Azzaroli F, Crispe IN. Immunology of the healthy liver: Old questions and new insights. *Gastroenterology.* 2001;120(1):250–60.
13. Cai J, Zhang XJ, Li H. The Role of Innate Immune Cells in Nonalcoholic Steatohepatitis. *Hepatology.* 2019;70(3):1026–37.
14. Masarone M, Rosato V, Dallio M, Gravina AG, Aglitti A, et al. Role of Oxidative Stress in Pathophysiology of Nonalcoholic Fatty Liver Disease. 2018(2018):1-14.

15. Wandrer F, Leibig S, Marhenke S, Vogel A, John K, Manns PM et al. TNF-Receptor-1 inhibition reduces liver steatosis, hepatocellular injury and fibrosis in NAFLD mice. *Cell Death and Disease*. 2020;11:212.
16. Neeta C, Agr AW, Amit K B. Journal I. Evaluation of proinflammatory markers-tumor necrosis factor alpha (TNF α) and adiponectin in nonalcoholic fatty liver disease. Department of Biochemistry, Index Medical College, Indore. *Depart*. 2020;3(9):148–54.
17. Wang YL, Liu LJ, Zhao WH, Li JX. Intervening TNF- via PPAR γ with Gegenqinlian Decoction in Experimental Nonalcoholic Fatty Liver Disease. *Evidence-based Complement Altern Med*. 2015;2015.
18. Pacifico L, Chiesa C, Anania C, De Merulis A, Osborn JF, Romaggioli S, et al. Nonalcoholic fatty liver disease and the heart in children and adolescents. *World J Gastroenterol*. 2014;(27):9055–71.
19. Wang H, Mehal W, Nagy LE, Rotman Y. Immunological mechanisms and therapeutic targets of fatty liver diseases. *Cell Mol Immunol* 2021;18(1):73–91.
20. Singh S, Osna NA, Kharbanda KK. Treatment options for alcoholic and nonalcoholic fatty liver disease: A review. *World J Gastroenterol*. 2017;23(36):6549–70.