



Alcohol-Induced Hepatic Fibrosis and the Relation between Hepcidin and Liver Fibrosis

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Abstract

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Abstract: Alcoholic liver disease (ALD) is a worldwide health problem that may lead to development of fatty liver steatosis, hepatitis and cirrhosis. Alcohol is known to exert a harmful effect on a variety of human tissues. In particular, the liver is the major site of alcohol-induced damage because it is the direct recipient of the blood that contains elevated levels of alcohol, and it is the major organ responsible for alcohol metabolism. The damage caused by ethanol is mainly attributed to its metabolic process that results in production of acetaldehyde and reactive oxygen species (ROS) such as hydrogen peroxide, superoxide and free hydroxyl radical. These metabolites cause depletion of reduced glutathione (GSH), peroxidation of cellular membranes, oxidation of macro-molecules such as proteins and nucleic acids, and eventually lead to progressive injury of hepatocytes. Additionally, ethanol and its metabolic products enhance the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6). The enhanced production of those inflammation factors; stimulated partially by oxidative stress; results in cytokine imbalance and immune disorders, leading to further hepatic damage. Thus, agents with anti-inflammatory and anti-oxidative properties might be potential candidates for protection against alcohol-induced liver disease. The metabolic functions of the alcoholic liver are seriously affected. Disorders in iron metabolism are characteristic of ALD. Abnormal levels of iron, ferritin, and transferrin were reported in ALD. Hepcidin, the principle hepatic regulator of the metabolism of iron, is decreased in ALD.

Keywords: Alcoholic liver disease; fibrosis; alcohol; cirrhosis; hepcidin.

1. Introduction

Alcoholic liver disease (ALD) is characterized by liver injury, inflammation, fibrosis, cirrhosis, and cancer caused by long-term or large volumes of alcohol intake. ALD is classified into several stages

according to the pathological features of the liver. The first and most widespread stage, alcoholic fatty liver, is a result of increased lipid accumulation in the liver. The second stage, alcoholic hepatitis, manifests as changes to hepatocytes that give them a balloon like appearance, a large number of

Mallory Denk bodies, and liver infiltration by monocytes, neutrophils, and other inflammatory cells. In the third stage, alcoholic liver fibrosis, collagen fibrosis extends into the lobules of the liver, and then, the final two stages of ALD, cirrhosis, and hepatocellular carcinoma (HCC), may develop (Singal et al., 2011).

Hepcidin is a principle hepatic regulator of the metabolism of iron. The expression of hepcidin in the liver is downregulated by both acute and chronic exposure to alcohol (Sangkhae and Nemeth, 2018). Ethanol consumption increases absorption of iron, presumably by downregulation of hepcidin expression, leading to increased ferritin levels. The increased levels of iron and ferritin may play a role in the progression of ALD to cirrhosis and eventually HCC (Guo et al., 2013).

2. Mechanisms of ethanol-induced liver diseases

The mechanisms of ethanol induced liver injury are complex and involve multiple signaling pathways. Therefore, Alcohol-induced liver injury mainly involves structural damage to liver cells, lipid accumulation, and inflammation. Studies have demonstrated that transcription factors, kinases, and microRNAs (miRNAs) play critical regulatory roles in ALD. Ethanol, a two-carbon amphiphilic small-molecule compound, is oxidized and metabolized into an intermediate, acetaldehyde, by ethanol dehydrogenases, microsomal oxidases, and catalases in the liver. In addition to being metabolized into acetaldehyde, ethanol metabolism leads to an increase in reactive oxygen species (ROS) levels and decreases in the nicotinamide adenosine dinucleotide (NAD)⁺/NADH ratio. Under physiological conditions, most ethanol (80%–90%) is metabolized in the cytoplasm by alcohol dehydrogenases (ADHs) (Li et al., 2015).

The main ADHs involved in ethanol metabolism are ADH1A, ADH1B, ADH1C, and ADH4. It was reported that ADH5 may also be involved in ethanol metabolism. The other 10 to 20% of ethanol is metabolized through hepatic microsomal ethanol oxidation. The main enzyme involved in this reaction is cytochrome P450 family 2 member E1 (CYP2E1). The expression level of CYP2E1 increases after ethanol consumption. Ethanol can be alternatively metabolized via nonoxidative pathways (Li et al., 2015).

2.1. Mitochondrial damage

Mitochondria have many redox systems, and this redox enzyme system is an early target of ethanol. Ethanol exposure can reduce mitochondrial volume, the ATP synthase levels, and inhibit protein synthesis. Ethanol metabolism can also disrupt the phospholipid bilayer membrane structure of mitochondria, resulting in the release of a large number of oxygen free radicals, lipid peroxidation, unsaturated fatty acid (FA) destruction, and the production of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (Zorov et al., 2014).

Although the destruction of unsaturated FAs damages the integrity of the mitochondrial membrane, the formation of macromolecular complexes due to the condensation of MDA with amino acids or phospholipids markedly decreases the fluidity of the mitochondrial membrane (Cichoż-Lach and Michalak, 2014). The oxidative metabolite of ethanol, acetaldehyde, is highly toxic and can readily undergo crosslinking with DNA or protein macromolecules in mitochondria. After accumulation in cells due to heavy ethanol consumption, acetaldehyde leads to mitochondrial dysfunction by crosslinking to mitochondrial DNA and mitochondrial proteins and inhibiting the electron transport chain (Zorov et al., 2014).

Notably, some nonoxidative metabolites produced by ethanol metabolism can cause damage to the mitochondrial membrane. For instance, fatty acid ethyl esters (FAEEs) can destabilize the mitochondrial membrane, disrupt the flow of electrons in the respiratory chain and inhibit oxidative phosphorylation (Taha et al., 2021).

2.2. Oxidative stress

Ethanol oxidation leads to an increase in free radical production in the liver. Uncontrolled ROS are critical to hepatocyte swelling and apoptosis. ROS can interact with DNA, proteins, and lipids, disrupting numerous metabolic and homeostatic cellular functions. The metabolism of ethanol mediated by CYP2E1 in liver cells is closely related to excessive ROS production and NADPH oxidase subtype NADPH oxidase 4 (NOX4) overexpression. Excessive ROS can alter the function of glutathione (GSH) transporter channels, leading to progressive GSH deficiency in mitochondria and inactivation of oxidative phosphorylase, resulting in an irregular

mitochondrial shape decreases in mitochondrial protein synthesis and altered ATP production in mitochondria. In addition, ROS depletes cellular antioxidant stores, and promotes the formation of lipid peroxidation products, such as MDA and 4-HNE. MDA and 4-HNE can react with DNA bases to form DNA adducts that form outwards-oriented DNA loops. Specifically, MDA reacts with deoxyguanosine residues, and 4-HNE reacts with deoxyadenosine and deoxycytidine. These interactions promote apoptosis and necrotic cell death, contributing to the progression of ethanol-induced liver diseases (Wu and Cederbaum, 2002).

Toll-like receptor 4 (TLR4) signaling cascades are altered by ethanol, leading to the activation of myeloid differentiation primary response 88 (MyD88) and an inflammatory response. The association of MyD88 with interleukin (IL)-1 receptor-associated kinases 1–4 leads to the activation of the tumor necrosis factor receptor-associated factor 6/transforming growth factor β -activated kinase 1 complex, which activates mitogen activated protein kinases (MAPKs), c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated protein kinase (ERK), resulting in inflammation and apoptosis (Jha et al., 2021).

Also, ethanol consumption can result in nuclear factor-kappa B (NF- κ B) translocation to the nucleus via the phosphorylation of inhibitory κ B (I κ B) and the release of inflammatory cytokines. Acetaldehyde can induce ROS production and lead to ALD. Specifically, the binding of acetaldehyde to GSH inhibits the scavenging function of hydrogen peroxide, thereby exacerbating oxidative stress and lipid peroxidation. It was also demonstrated that acetaldehyde reduced the ATP content, respiratory control, superoxide dismutase (SOD) activity, and glutathione/oxidized GSH ratio. In addition to aggravating oxidative damage in the liver, FAEE accumulation can lead to pancreatitis. Furthermore, the concentration of FAEEs (20–40 μ M) in the blood has been shown to induce oxidative stress and reduce mitochondrial function in intestinal epithelial cells (Louvet and Mathurin, 2015).

3. Development of fibrosis

3.1. Paracrine effect of hepatocytes

Alcohol is primarily metabolized in hepatocytes to acetaldehyde, a step that can be catalyzed by ADH

or CYP2E1. Alcohol metabolism, especially through CYP2E1, leads to the release of ROS and generation of lipid peroxidation products. ROS are also generated from mitochondria during alcohol metabolism (Liu et al., 2023).

Alcohol metabolism leads to the generation of acetaldehyde and ROS in hepatocytes, both of which can activate hepatic stellate cells (HSCs) through paracrine mechanism. Lipid peroxidation products MDA and 4-HNE, which may be released from alcohol-metabolizing hepatocytes, increase collagen production in cultured HSCs. Furthermore, using a coculture of CYP2E1 transfected HepG2 cells and HSCs, it has been demonstrated that ROS generated in hepatocytes can increase collagen production in HSCs. These results suggest hepatocytes can contribute to the activation of HSCs by generating oxidant stress. Acetaldehyde, an immediate metabolite of ethanol, is mostly produced in hepatocytes and can then enter HSCs in a paracrine manner. It is fibrogenic and induces the expression of both type I collagen genes in cultured HSCs by a transcriptional dependent mechanism (Novitskiy et al., 2006).

3.2. Paracrine effect of Kupffer cells

Kupffer cells have been implicated as mediators of alcoholic liver injury through their release of tumor necrosis factor-alpha (TNF- α), free radicals, and other inflammatory mediators in response to alcohol and lipopolysaccharide (LPS). TNF- α produced by activated Kupffer cells may contribute to HSC activation by inducing apoptosis of hepatocytes, thereby forming apoptotic bodies that have been implicated in fibrogenesis (Kolios et al., 2006). Kupffer cell derived transforming growth factor-beta1 (TGF- β 1) has been implicated in the activation of stellate cells through paracrine mechanism. The activating role of Kupffer cells has been further demonstrated using cocultures of Kupffer cells and HSCs. The following features were reported in the Kupffer cell activated HSCs: phenotypic changes in HSCs as shown by stretching nuclear and cellular enlargement, cytoplasmic spreading, elongation of processes establishing contacts among cells, loss of lipid droplets and vitamin A, HSC proliferation, increased alpha-smooth muscle actin (α -SMA) expression, increased mRNA levels of collagen I, and upregulation of collagen I protein. Experiments using various antioxidants revealed that the stimulatory effect of Kupffer cells on HSC collagen I production was mediated through xanthine

oxidase, NADPH oxidase, and CYP2E1, which are known source of ROS. These results suggest a role of oxidative stress in Kupffer cell-mediated HSC activation (Meng et al., 2019).

3.3. Role of hepatocyte apoptosis

Apoptosis is a form of cell death characterized by organized nuclear and ultimately cellular fragmentation. Increasing evidence suggests that apoptosis of hepatocytes plays an important role in the initiation of alcoholic liver injury. Furthermore, hepatocyte apoptosis is significantly increased in patients with alcoholic hepatitis and correlates with disease severity and hepatic fibrosis (Wang, 2015). Hepatocyte apoptosis produces chemokines and inflammation, which in turn may activate HSCs. Furthermore, apoptosis of hepatocytes results in generation of apoptotic bodies, which can release lipid signals for their uptake by Kupffer cells and HSCs. Phagocytosis of the apoptotic bodies by HSCs and Kupffer cells enhances their expression of profibrogenic genes, such as TGF- β 1, that may initiate HSC activation. These studies suggest that alcohol-induced apoptosis of hepatocytes maybe a mechanism of liver fibrosis (Guo et al., 2022).

3.4. Role of leptin

Leptin plays an important role in the development of hepatic fibrosis. Leptin increases type I collagen production in human stellate cell line, LX-1, and in cultured rat HSCs. This effect of leptin can be mediated through upregulation of TGF- β 1, enhancement of the TGF- β 1 type II receptor, or increased production of tissue inhibitor of metalloproteinase-1 (TIMP-1) in activated HSCs (Pérez-Pérez et al., 2020).

3.5. Role of innate immunity and alcohol

The liver immune system has predominant innate immunity (nonspecific immunity) comprised of Kupffer cells, natural killer (NK) cells and NKT cells, and interferon alpha (IFN- α) and interferon gamma (IFN- γ) cytokines (Wu et al., 2023). Increasing evidence suggests that these innate immune cells and cytokines play important roles in regulating the development and progression of liver fibrosis. Macrophages have been shown to inhibit liver fibrosis through killing HSCs and enhancing matrix degradation during recovery. Innate cytokines IFN- α and IFN- γ inhibit liver fibrosis by blocking TGF- β 1 signaling and HSC activation, and

NK cells have been shown to kill activated HSCs and attenuate the severity of liver fibrosis (Khan et al., 2023).

Alcohol consumption-mediated suppression of the innate immunity has been reported in both animal experiments and clinical studies. Chronic alcohol consumption has been shown to decrease NK cell activity and numbers. Decreased NK activity has also been reported in human alcoholics. Because these innate immune cells and cytokines play an important role in suppressing liver fibrosis as discussed previously, alcohol suppression of innate immunity may be a mechanism whereby alcohol accelerates liver fibrosis in HCV patients (Abraham et al., 2017).

3.6. Genetics of fibrosis

Genetic factors that are involved in the production or elimination of acetaldehyde may make individuals susceptible to ALD, as acetaldehyde has been implicated in the pathogenesis of ALD. Indeed, the presence of super active alcohol dehydrogenase (ADH2) and inactive aldehyde dehydrogenase (ALDH2) alleles has been linked to increased risk for ALD in Asian populations (Wynn and Ramalingam, 2012).

Genetic factors that promote oxidative stress can also make individuals susceptible to alcoholic liver injury. For example, the mutant c2 allele of CYP2E1 that is more transcriptionally active increases the risk of ALD at a given level of cumulative alcohol consumption. The risk appears to be due to increased metabolism of ethanol by CYP2E1 that produces ROS (Forcina et al., 2022).

Genetic factors that modulate the production of pro- and anti-inflammatory cytokines can also influence the susceptibility to ALD. There was a significant excess of the rare allele (TNFA-A) at position 238 in patients with steatohepatitis compared with controls or patients without this lesion. In addition, among heavy drinkers, the presence of the A allele at position 627 in the IL-10 (an anti-inflammatory cytokine) promoter is associated with an increased risk of advanced liver disease (Meunier et al., 2017). This result is probably due to the fact that the 627*A allele is associated with low IL-10 expression which favors inflammation and fibrosis. These examples suggest that genetic polymorphisms of alcohol metabolizing enzymes, oxidative stress, and cytokine production may contribute to the

susceptibility of certain individuals to the development of alcoholic liver fibrosis (Na and Lee, 2017).

4. Heparin production in liver

Hepatocytes express hepcidin 15-1500 times more than other cells in the body, thus making them the primary source of hepcidin (Ginzburg, 2019). This role is perfectly suited for hepatocytes, since they are exposed to the iron absorbed from enterocytes and iron released from macrophages through portal circulation. In basal conditions, hepcidin expression is controlled through iron-load. Iron-load stimulates production of bone morphogenetic protein 6 (BMP6). BMP6 creates a complex with BMP receptor (BMPR) which in turn increases hepcidin expression through intracellular s-mothers against decapentaplegic (SMAD) pathway (Cusi et al., 2020).

The source of BMP6 in liver are liver sinusoidal endothelial cells (LSEC). This role suits LSEC because of their direct contact with plasma and their intimate relationship with hepatocytes. LSEC are known for their high endocytic activity, which makes them ideal cells for “extracting” plasma iron transporters such as transferrin or serum ferritin. Experimental inactivation of BMP6 causes serious iron-overload. Recent evidence suggests that BMP6 mutations could be the source of a mild but still unrecognized form of hereditary hemochromatosis (HH) (Pomari et al., 2019). Ferritin has been proposed as a potential sensor of iron, but more studies should explore this possibility, as well as other potential molecules (Ananthapadmanabhan et al., 2022).

Acute and chronic iron-load exert their control on hepcidin expression by partially independent mechanisms. This is enforced by observations in BMP6 and hemojuvelin (HJV) knockout models in mice, where chronic iron-load does still induce hepcidin expression (Sastre-Heres et al., 2019). Other factors that control hepcidin expression include erythropoietic drive, hypoxia and inflammation (Kanamori et al., 2011). In specific situations, these factors increase iron availability or reduce iron-load depending on the needs of our cells. Inflammation induces hepcidin expression through janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, though SMAD pathway has been shown to be affected during inflammation as well. Recent evidence suggests that SMAD pathway activation during inflammation could occur in nonparenchymal liver

cells and it is not correlated to hepcidin expression (Kumar et al., 2021).

4.1. Mechanisms of low hepcidin in liver disease

As previously mentioned, hepcidin is a peptide which is under strict control of different regulatory mechanisms. These mechanisms can become dysregulated and cause inappropriate levels of hepcidin. In this respect, chronic low levels of hepcidin are of interest for researchers, because low hepcidin can cause iron overload and increased oxidative stress in liver (Joachim and Mehta, 2022). Increased oxidative stress in combination with other factors as genetic, viruses, alcohol, autoimmune process, hepatotoxins, etc can result in liver fibrosis. Liver fibrosis is the consequence of chronic liver damage, characterized by increased deposition of extracellular matrix induced by activated HSCs, which promotes creation of fibrous scars in the liver. This fibrotic tissue can eventually reorganize and disrupt liver architecture, by creating regenerative nodules, which is the main feature of the end-damage caused by the scarring process, that is, liver cirrhosis (Eming et al., 2017). In liver disease, low hepcidin is linked with many conditions, but the mechanisms behind low levels of hepcidin are still elusive and remain to be fully explained.

4.2. Low hepcidin in alcoholic liver disease (ALD)

Alcohol is an already established inducer of hepatocyte damage, which can progress to overt liver fibrosis. Suspected mechanisms of alcohol-induced liver fibrosis include increased levels of LPS, activation of HSCs and inhibition of antifibrotic actions (Vela, 2018). Alcohol is also linked with disturbances in levels of hepcidin. It is interesting to notice that, in alcoholic patients, low levels of hepcidin are observed even with preserved liver function (Nahon et al., 2016). This would suggest that alcohol is a primary cause of low levels of hepcidin, and not a consequence of alcohol induced liver damage. The rationale behind this observation is the direct effect of alcohol on hepcidin expression. Alcohol can inhibit hepcidin expression through its suppressive effects on CCAAT- enhancer-binding protein (C/EBP) in hepatocytes, at the same time counteracting iron-induced activity of this transcription factor, thus rendering iron-induced hepcidin expression

ineffective (Lyberopoulou et al., 2015). The upregulation of divalent metal transporter 1 (DMT1) and ferroprotein in enterocytes increases serum iron levels and cellular iron load, which is linked with liver fibrosis (Vela, 2018). This effect of alcohol can be reversed with treatment by antioxidants, which is not surprising since alcohol induces oxidative stress. This is the reason why progression rate of fibrosis is twice as high in steatotic drinkers compared to steatotic nondrinkers (Chachami et al., 2004).

There is another mechanism of hepcidin suppression by alcohol includes suppression through TLR4 pathway. TLR4 is a trans-membrane protein involved in innate immune responses. In mice with defective TLR4 receptor; alcohol cannot suppress hepcidin expression (Wang et al., 2012). It is interesting to notice that TLR4 deficiency protects from liver fibrosis, making it an interesting candidate to be studied in the context of alcohol-induced hepcidin down-regulation (Zmijewski et al., 2014). The mediator cell of TLR4 signaling remains to be found, and it seems that Kupffer cells are not involved in alcohol-induced hepcidin expression. Hepatocytes are unlikely candidates as well, since their expression of TLRs is low, while their reaction to TLR ligands is weak. Alcohol might disrupt canonical hepcidin pathways such as BMPR/SMAD pathway, but also can suppress hepcidin via hypoxic signals, though the importance of these alcohol-induced actions on hepcidin expression remain to be confirmed (Nemeth and Ganz, 2021). It seems that alcohol consumption in the setting of iron-overload can serve as a strong inducer of liver fibrosis.

In HH patients, alcohol consumption of more than 60 g/day increases the risk of cirrhosis by 9-fold. This increase in risk of progressive liver damage in alcoholic HH patients is in line with the so called “multiple hit” scenario, where two or more pathophysiological factors induce hepatocyte damage in a complementary manner, which eventually leads to liver fibrosis (Parola and Pinzani, 2019).

5. Conclusion

Drinking alcohol is a main cause of hepatic fibrosis. Acetaldehyde, the first metabolite of ethanol, can upregulate transcription of collagen I directly as well as indirectly by upregulating the synthesis of TGF- β 1. ROS generated in hepatocytes by alcohol metabolism can activate collagen production in HSCs in a paracrine manner. Alcohol-induced

hepatocyte apoptotic bodies can be phagocytosed by HSCs and Kupffer cells and result in increased expression of TGF- β 1 and subsequent HSC activation. Kupffer cells may contribute to the activation of HSCs by releasing ROS and TGF- β 1. Innate immunity may suppress hepatic fibrosis by killing activated HSCs and blocking TGF- β 1 signaling. The pathological process of ALD is very complex and involves a variety of potential mechanisms. While intensive studies have been devoted to understanding the pathogenesis of ALD and possible therapeutic ALD treatments, the molecular mechanisms of ALD are still incompletely understood, resulting in a limitation to the effectiveness of ALD treatment. To date, the clinical treatments of ALD have been focused on drug-promoted abstinence and behavioral interventions that reduce alcohol intake.

This review has addressed the importance of low levels of hepcidin in liver fibrosis. The main mechanisms of this disturbance are realized through alcohol-induced injury. Low levels of hepcidin can cause iron-overload, but as recent data suggest, low hepcidin can have additional repercussion to liver architecture because of hepcidin’s ability to control HSC activation, which is one of the main pathophysiological features in liver fibrosis. These mechanisms are in concert with clinical studies that have established hepcidin and hepcidin/ferritin ratio as an important biochemical parameter of liver fibrosis with the ability to predict patient mortality and increased risk of HCC.

References

- Abraham KP, Salinas AG, Lovinger DM. Alcohol and the Brain: Neuronal Molecular Targets, Synapses, and Circuits. *Neuron*. 2017; 96(6): 1223-1238.
- Ananthapadmanabhan S, Soodin D, Sritharan N, Sivapathasingam V. Ramsay Hunt syndrome with multiple cranial neuropathy: a literature review. *Eur Arch Otorhinolaryngol*. 2022; 279(5): 2239-2244.
- Chachami G, Simos G, Hatziefthimiou A, Bonanou S, Molyvdas PA, Paraskeva E. Cobalt induces hypoxia-inducible factor-1 α expression in airway smooth muscle cells by a reactive oxygen species- and PI3K-dependent mechanism. *Am J Respir Cell Mol Biol*. 2004; 31(5): 544-551.

- Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. *World J Gastroenterol.* 2014; 20(25): 8082-8091.
- Cusi K, Isaacs S, Barb D, Basu R, Caprio S, Garvey WT, Kashyap S, Mechanick JI, Mouzaki M, Nadolsky K, Rinella ME, Vos MB, Younossi Z. American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD). *Endocr Pract.* 2020; 28(5): 528-562.
- Eming SA, Wynn TA, Martin P. Inflammation and metabolism in tissue repair and regeneration. *Science.* 2017; 356(6342): 1026-1030.
- Forcina GC, Pope L, Murray M, Dong W, Abu-Remaileh M, Bertozzi CR, Dixon SJ. Ferroptosis regulation by the NGLY1/NFE2L1 pathway. *Proc Natl Acad Sci U S A.* 2022;119(11): 21186-46119.
- Ginzburg YZ. Hepcidin-ferroportin axis in health and disease. *Vitam Horm.* 2019; 110: 17-45.
- Guo R, Jia X, Ding Z, Wang G, Jiang M, Li B, Chen S, Xia B, Zhang Q, Liu J, Zheng R, Gao Z, Xie X. Loss of MLKL ameliorates liver fibrosis by inhibiting hepatocyte necroptosis and hepatic stellate cell activation. *Theranostics.* 2022; 12(11): 5220-5236
- Guo X, Wang Y, Shen Y, Gao Y, Chang Y, Duan X. Gene expression profiles of sodium-dependent vitamin C transporters in mice after alcohol consumption. *Acta Biochim Biophys Sin (Shanghai).* 2013; 45(11): 912-920.
- Jha AK, Gairola S, Kundu S, Doye P, Syed AM, Ram C, Murty US, Naidu VGM, Sahu BD. Toll-like receptor 4: An attractive therapeutic target for acute kidney injury. *Life Sci.* 2021; 271: 119155.
- Joachim JH, Mehta KJ. Hepcidin in hepatocellular carcinoma. *Br J Cancer.* 2022; 127(2): 185-192.
- Kanamori Y, Murakami M, Sugiyama M, Hashimoto O, Matsui T, Funaba M. Hepcidin and IL-1 β . *Vitam Horm.* 2011; 110: 143-156.
- Khan RS, Lalor PF, Thursz M, Newsome PN. The role of neutrophils in alcohol-related hepatitis. *J Hepatol.* 2023; 79(4): 1037-1048.
- Kolios G, Valatas V, Kouroumalis E. Role of Kupffer cells in the pathogenesis of liver disease. *World J Gastroenterol.* 2006;12(46):7413-20.
- Kumar S, Duan Q, Wu R, Harris EN, Su Q. Pathophysiological communication between hepatocytes and non-parenchymal cells in liver injury from NAFLD to liver fibrosis. *Adv Drug Deliv Rev.* 2021; 176: 113869.
- Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y. The Role of Oxidative Stress and Antioxidants in Liver Diseases. *Int J Mol Sci.* 2015; 6(11): 26087-26124.
- Liu P, Qian Y, Liu X, Zhu X, Zhang X, Lv Y, Xiang J. Immunomodulatory role of mesenchymal stem cell therapy in liver fibrosis. *Front Immunol.* 2023; 13: 1096402.
- Louvet A, Mathurin P. Alcoholic liver disease: mechanisms of injury and targeted treatment. *Nat Rev Gastroenterol Hepatol.* 2015; 12(4): 231-242.
- Lyberopoulou A, Chachami G, Gatselis NK, Kyrtzopoulou E, Saitis A, Gabeta S, Eliades P, Paraskeva E, Zachou K, Koukoulis GK, Mamalaki A, Dalekos GN, Simos G. Low Serum Hepcidin in Patients with Autoimmune Liver Diseases. *PLoS One.* 2015; 10(8): 135-486.
- Meng D, Qin Y, Lu N, Fang K, Hu Y, Tian Z, Zhang C. Kupffer Cells Promote the Differentiation of Adult Liver Hematopoietic Stem and Progenitor Cells into Lymphocytes via ICAM-1 and LFA-1 Interaction. *Stem Cells Int.* 2019; 2019: 4848279.
- Meunier L, Larrey D, Ursic-Bedoya J. Liver Biopsy in Chronic Liver Diseases: Is There a Favorable Benefit: Risk Balance? *Ann Hepatol.* 2017; 16(4): 487-489.
- Na HK, Lee JY. Molecular Basis of Alcohol-Related Gastric and Colon Cancer. *Int J Mol Sci.* 2017;18(6):1116.
- Nahon P, Nuraldeen R, Rufat P, Sutton A, Trautwein C, Strnad P. In alcoholic cirrhosis, low-serum hepcidin levels associate with poor long-term survival. *Liver Int.* 2016; 36(2): 185-188.
- Nemeth E, Ganz T. Hepcidin-Ferroportin Interaction Controls Systemic Iron Homeostasis. *Int J Mol Sci.* 2021; 22(12): 6493.
- Novitskiy G, Traore K, Wang L, Trush MA, Mezey

- E. Effects of ethanol and acetaldehyde on reactive oxygen species production in rat hepatic stellate cells. *Alcohol Clin Exp Res.* 2006; 30(8): 1429-1435.
- Parola M, Pinzani M. Liver fibrosis: Pathophysiology, pathogenetic targets and clinical issues. *Mol Aspects Med.* 2019; 65: 37-55.
- Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T, Sánchez-Margalet V. Role of Leptin in Inflammation and Vice Versa. *Int J Mol Sci.* 2020; 21(16): 5887.
- Pomari E, Piubelli C, Perandin F, Bisoffi Z. Digital PCR: a new technology for diagnosis of parasitic infections. *Clin Microbiol Infect.* 2019; 25(12): 1510-1516.
- Sangkhae V, Nemeth E. Regulation of the Iron Homeostatic Hormone Hepcidin. *Adv Nutr.* 2018; 8(1): 126-136.
- Sastre-Heres AJ, Iglesias I, Alaguero-Calero M, Ruiz-Sánchez D, García-Díaz B, Peña-Díaz J. Comparative Study of Different Classification Models in Renal-Cell Carcinoma. *Pathol Oncol Res.* 2019; 25(4): 1357-1362.
- Singal AK, Bataller R, Ahn J, Kamath PS, Shah VH. ACG Clinical Guideline: Alcoholic Liver Disease. *Am J Gastroenterol.* 2011;(2):175-194.
- Taha MAI, Badawy MEI, Abdel-Razik RK, Younis HM, Abo-El-Saad MM. Mitochondrial dysfunction and oxidative stress in liver of male albino rats after exposing to sub-chronic intoxication of chlorpyrifos, cypermethrin, and imidacloprid. *Pestic Biochem Physiol.* 2021; 178: 104938.
- Vela D. Low hepcidin in liver fibrosis and cirrhosis; a tale of progressive disorder and a case for a new biochemical marker. *Mol Med.* 2018; 24: 5.
- Wang K. Pathophysiological Role of Hepatocyte Nuclear Factor 6 in Negative Regulation of Hepatic Apoptosis: A Novel Hypothesis. *Curr Mol Med.* 2015; 15(5): 412-417.
- Wang L, Trebicka E, Fu Y, Ellenbogen S, Hong CC, Babitt JL, Lin HY, Cherayil BJ. The bone morphogenetic protein-hepcidin axis as a therapeutic target in inflammatory bowel disease. *Inflamm Bowel Dis.* 2012; 18(1): 112-119.
- Wu D, Cederbaum AI. Oxidative stress and alcoholic liver disease. *Semin Liver Dis.* 2002; 29(2): 141-154.
- Wu X, Fan X, Miyata T, Kim A, Cajigas-Du Ross CK, Ray S, Huang E, Taiwo M, Arya R, Wu J, Nagy LE. Recent Advances in Understanding of Pathogenesis of Alcohol-Associated Liver Disease. *Annu Rev Pathol.* 2023; 18: 411-438.
- Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med.* 2012; 18(7): 1028-1040.
- Zmijewski E, Lu S, Harrison-Findik DD. TLR4 signaling and the inhibition of liver hepcidin expression by alcohol. *World J Gastroenterol.* 2014; 20(34): 12161-12170.
- Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev.* 2014;(3): 909-950.