

Interrelationships of Alcohol and Iron in Liver Disease with Particular Reference to Ferritin and Transferrin

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Abstract

Alcohol-related liver disease (ALD) remains a major cause of chronic liver injury worldwide and is increasingly recognized to involve significant disturbances in systemic and hepatic iron metabolism. This review aims to examine the interrelationship between chronic alcohol consumption, iron dysregulation, and liver injury, with particular emphasis on the roles of ferritin and transferrin as both biomarkers and mechanistic mediators of disease progression. Current evidence indicates that chronic alcohol exposure suppresses hepcidin, enhances intestinal iron absorption, and promotes hepatic iron accumulation, resulting in elevated ferritin levels, increased transferrin saturation, and generation of non-transferrin-bound iron (NTBI). These changes contribute to oxidative stress, lipid peroxidation, mitochondrial dysfunction, and ferroptotic cell death, accelerating fibrosis and cirrhosis. Ferritin and transferrin are therefore central not only for iron storage and transport but also for disease monitoring, differential diagnosis, and prognostic evaluation. Despite these advances, important knowledge gaps remain regarding the precise contribution of iron-regulatory pathways, interindividual susceptibility, and the therapeutic potential of iron-targeted interventions. Improved understanding of ferritin–transferrin dynamics may enhance biomarker-guided diagnosis and support the development of targeted therapies for ALD. This review explores the complex interplay between alcohol, iron metabolism, and liver injury, with particular emphasis on the hepcidin–ferroportin axis, ferritin–transferrin dynamics, and emerging mechanisms such as ferroptosis. Understanding these interactions may improve diagnostic precision and support the development of targeted therapeutic strategies in ALD. It highlights how alcohol-induced alterations in iron homeostasis contribute to hepatic injury, oxidative stress, and disease progression, while evaluating the clinical significance of these biomarkers in diagnosis, prognosis, and potential therapeutic targeting.

Key words: Alcohol related liver disease, iron binding protein – ferritin and transferrin, divalent metal transporter 1, iron-responsive elements, iron-regulatory proteins

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1. Introduction

1.1 Overview of Alcohol-Related Liver Disease (ALD)

Alcohol-related liver disease (ALD) refers to a spectrum of liver symptoms, ranging from fatty liver to steatohepatitis to liver fibrosis/cirrhosis with chronic inflammation, mainly caused by excessive alcohol consumption. ALD is considered one of the most common causes of chronic liver disease-related mortality worldwide and is a major indication for liver transplantation. [1] Although research to identify new therapeutic targets for the treatment of ALD has continued for decades, the FDA has not approved a single drug for the treatment of ALD. ALD is a spectrum of liver damage caused by chronic excessive

alcohol consumption and is thus a leading cause of liver disease and death in Western countries.[2] Due to alcohol's interference with the body's fat metabolism, more than 90% of heavy drinkers develop reversible fatty liver disease (steatosis), in which fat accumulates, and progress through stages to alcoholic hepatitis, which causes inflammation, oxidative stress due to acetaldehyde, and the release of cytokines such as TNF- α , leading to cell death in approximately 25% of cases. In later stages, irreversible scarring fibrotic cirrhosis can occur, leading to complications such as liver failure, portal hypertension, ascites, jaundice, and predisposition to hepatocellular carcinoma, but early abstinence can prevent or reverse mild damage. Risk factors include female gender, genetics, obesity,

chronic alcohol consumption, etc., and the liver's regenerative activity is overloaded by continuous exposure to toxins. There is no specific treatment for advanced forms of the disease, so complete abstinence, diet and treatment of complications can be considered. [3,4]

1.2 Iron Metabolism in Normal Physiology

Iron is used appropriately by the body as it does not have the ability to actively remove excess iron. It uses iron from dead red blood cells. Absorption of dietary iron occurs in the duodenum. Before entering the enterocytes, dietary iron is converted to ferrous Fe^{2+} by Dcytb (duodenal cytochrome b) and DMT1 (divalent metal transporter 1). Once in enterocytes, ferrous iron exits the basolateral membrane via ferroportin, is oxidized by hephaestin to ferric Fe^{3+} , binds to transferrin, and travels through the bloodstream.[5] Transferrin then transports the iron to the destination cell where it is endocytosed by the cell surface transferrin receptor (TfR1). Transferrin is then cleaved within endosomes and iron is released as ferric iron, which is reduced to ferrous iron by Six-Transmembrane Epithelial Antigen of the Prostate 3 (STEAP3) before being transported into the cell via DMT1. When cells no longer need transferrin, they return it to the surface. Macrophages process up to 20-25 mg of iron per day in heme, which is degraded by heme oxygenase. [6] This iron is exported by the ferroportin, and in cases of excess iron or inflammation, its export is controlled by hepcidin, a peptide produced by the liver that binds to the ferroportin for degradation. Iron regulatory proteins (IRP1 and IRP2) function post-transcriptionally to control both ferritin (storage), TfR1 (absorption), and ferroportin (export) through their respective iron-sensing elements, and excess iron is stored as ferritin or hemosiderin. The daily absorption corresponds to a daily loss (1-2 mg), but a decrease (deficiency) in hepcidin may increase the absorption. Alcohol is soluble in water and lipids and readily diffuses across cell membranes. After alcohol is absorbed from the gastrointestinal tract into the bloodstream, it is primarily metabolized in the liver. Furthermore only a small amount (<10%) is directly excreted in intact form via the lungs, kidneys, and sweat. The enzymatic metabolism of alcohol, including its oxidative biotransformation to acetaldehyde, is primarily mediated by alcohol dehydrogenase (ADH). Systemic iron homeostasis is tightly regulated through the coordinated interaction of intestinal absorption, plasma transport, cellular uptake, storage, and recycling pathways. Hepcidin serves as the master regulator by controlling ferroportin-mediated iron export from enterocytes, hepatocytes, and macrophages. Transferrin ensures the safe transport of ferric iron in circulation, while transferrin receptors

(TfR1 and TfR2) mediate cellular iron uptake and contribute to hepcidin regulation. Ferritin functions as the principal intracellular iron storage protein, protecting cells from free iron toxicity while maintaining iron availability for metabolic demands. Disruption of this tightly balanced network, particularly through alcohol-induced suppression of hepcidin, creates a pathological environment favoring iron overload, oxidative stress, and progressive liver injury. [7,8]

1.3 Importance of Ferritin and Transferrin in Hepatic Iron Homeostasis

Ferritin and transferrin protect against excessive iron toxicity and maintain hepatic iron homeostasis by providing appropriate means for hepatic iron storage, transport, and regulation. The liver is the main store of iron, where ferritin is produced. Ferritin is a globular protein complex that can store up to 4,500 iron atoms in a non-toxic form. The main form of ferritin found in hepatocytes is a light chain variant that provides a buffer against fluctuations in iron levels, and iron regulatory proteins control the response of ferritin to intracellular iron levels.[9] Transferrin is primarily produced by hepatocytes and safely transports ferric iron (Fe^{3+}) to other tissues via the circulatory system. Hepatic transferrin receptor 2 (TfR2) is required to control blood transferrin saturation to regulate hepcidin. Hepcidin is the only hormone that inhibits iron transport from liver, intestine, and macrophage cells via ferroportin when systemic iron levels are high, thereby maintaining systemic iron balance. [10,11] Additionally, ferritin and hepcidin play an important protective role in preventing parenchymal iron accumulation that can lead to oxidative stress, fibrosis, and ferroptosis. Transferrin deficiency in the liver can lead to excessive iron retention, decreased TfR1 receptors, increased ferritin levels, and increased risk of ferroptosis. The liver has three major functions in maintaining systemic iron homeostasis.[12]

- 1) the liver is the primary production site for proteins that maintain systemic iron balance,
- 2) the liver is the storage site for excess iron, and
- 3) the liver is essential for mobilizing iron from hepatocytes into the bloodstream to meet metabolic needs.

Dysregulation of the liver's ability to maintain a balance between these three parameters leads to iron-related disorders. This review will focus on normal liver function in iron homeostasis and the role of the liver in iron-related diseases as shown in the figure. (1) [13,14]

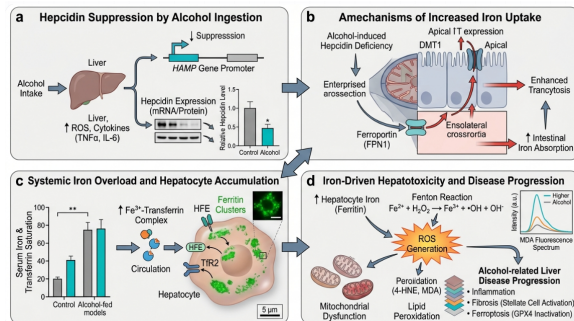


Fig. 1 Interrelationship between alcohol consumption and iron metabolism in liver disease

2. Materials and Methods

2.1 Search Strategy

A structured literature search was performed to identify relevant studies evaluating the interrelationship between alcohol consumption, iron metabolism, ferritin, transferrin, oxidative stress, lipid peroxidation, and ferroptosis in liver disease. Electronic databases including PubMed, Scopus, Web of Science, and Google Scholar were systematically searched for studies published between January 2000 and May 2026.

The search was conducted using combinations of the following Medical Subject Headings (MeSH) terms and keywords: “Alcohol-related liver disease” OR “Alcohol-associated liver disease” AND “Iron metabolism” OR “Iron overload” AND “Ferritin” AND “Transferrin” AND “Hepcidin” AND “Ferroportin” AND “Transferrin saturation” AND “Non-transferrin-bound iron” AND “Oxidative stress” AND “Lipid peroxidation” AND “Ferroptosis” AND “Ferritinophagy” AND “Mitochondrial dysfunction.” Boolean operators (AND/OR) were used to refine the search and improve specificity. Reference lists of selected studies were also manually screened to identify additional relevant publications.

2.2 Inclusion Criteria

Studies were included based on the following criteria:

- Original research articles, clinical studies, animal studies, and review articles.
- Published in English.
- Focused on alcohol-induced disruption of iron metabolism and its pathological consequences.
- Investigated ferritin, transferrin, hepcidin, ferroportin, transferrin receptors, or related iron-regulatory proteins.
- Addressed oxidative stress, lipid peroxidation, ferroptosis, ferritinophagy, or mitochondrial dysfunction in the context of alcohol-related liver disease.
- Published within the selected timeframe (2000–2026).

2.3 Exclusion Criteria

Studies were excluded if they:

- Were duplicate publications.
- Were non-English articles.
- Focused exclusively on non-alcoholic liver diseases without relevant iron metabolism discussion.
- Lacked mechanistic, clinical, or translational relevance to the review objective.
- Included insufficient data or incomplete findings.

2.4 Study Selection and Data Extraction

The study selection process involved three stages: title screening, abstract evaluation, and full-text assessment. Articles were selected based on relevance to the review objective and scientific quality. Priority was given to recent high-impact studies from the last 5–10 years, while foundational landmark studies were retained to provide mechanistic and historical context. Relevant information extracted from the selected studies included:

- Mechanisms of alcohol-induced iron dysregulation
- Ferritin and transferrin alterations
- Hepcidin–ferroportin pathway disruption
- Iron-driven oxidative stress and lipid peroxidation
- Ferroptosis regulatory pathways
- Mitochondrial dysfunction
- Diagnostic biomarkers
- Therapeutic interventions targeting iron overload and ferroptosis

2.5 Data Synthesis

The collected data were qualitatively synthesized and organized into thematic sections, including systemic iron homeostasis, alcohol-mediated iron dysregulation, ferritin and transferrin biology, oxidative stress mechanisms, ferroptosis regulation, clinical implications, and therapeutic perspectives. This approach enabled integrated mechanistic interpretation and identification of current knowledge gaps in alcohol-related liver disease.

3. Alcohol and Hepatic Iron Accumulation

Alcohol intake disrupts iron metabolism by causing excessive iron accumulation in the liver (hepatic iron accumulation) and worsening liver damage by increasing oxidative stress and promoting cell death (ferroptosis). This is partly because alcohol reduces levels of hepcidin, an important regulator of iron, increasing iron absorption and exacerbating the damage seen in inherited iron overload conditions such as hemochromatosis, creating a detrimental synergistic effect.[15] The development of ALD is highly complex and is thought to involve both genetic and non-genetic factors. Non-genetic factors include reactive oxygen species (ROS) and oxidative stress,

acetaldehyde toxicity, Kupffer cell (KC) activation, cytokines, and the gut-liver axis. Iron is an essential trace element involved in many biological processes such as metabolism, oxygen transport, innate immunity, and DNA synthesis. Although, excessive iron accumulation can cause oxidative stress and iron-dependent cell death, or ferroptosis, leading to liver damage. Ferroptosis, characterized by iron-dependent damage and subsequent plasma membrane disruption, is a new form of controlled cell death. The liver performs three main functions in maintaining systemic iron homeostasis. [16,17]

- 1) The liver is the main producer of proteins that maintain iron balance throughout the body.
- 2) A place to store excess iron,
- 3) The liver plays an important role in mobilizing iron from hepatocytes into the circulation to meet metabolic needs.

Dysregulation of the liver's ability to maintain a balance between these three parameters leads to iron-related disorders. This review will focus on normal liver function in iron homeostasis and the role of the liver in iron-related diseases. [18,19]

3.1 Mechanisms by which Alcohol alters Intestinal Iron Absorption

Alcohol changes the way your body absorbs iron from your intestines in several ways. The main pathway is disruption of homeostasis (i.e., balance), which is normally maintained by hepcidin. Chronic exposure to ethanol increases intestinal permeability, allowing non-heme iron to be absorbed by passive rather than active transport. Additionally, chronic exposure to ethanol reduces hepcidin synthesis. Therefore, increased iron levels do not result in a corresponding increase in hepcidin secretion from the liver, resulting in decreased ferroportin degradation and increased appearance of DMT1 and ferroportin in the duodenum. This allows more Fe^{2+} to be absorbed and enter the bloodstream.[20] Chronic exposure to ethanol also leads to a reduction in the hepcidin feedback mechanism, ultimately resulting in alcoholic patients almost doubling the rate of iron absorption compared to non-alcoholic patients. This is also influenced by alcohol-induced inflammation and endotoxin leakage. In addition to the previous statements, there may be regional differences in uptake. [21] There was no change in absorption in the duodenum, whereas increased absorption was observed in the terminal ileum. In some cases, the use of antioxidants (vitamin C) has been shown to reduce the severity of congestion by inhibiting transporters. Collectively, these changes lead to increased systemic iron content and cause significant oxidative damage in alcoholic patients suffering from alcoholic liver disease. It is a well-established fact that iron and alcohol cause oxidative stress and lipid peroxidation, respectively. Therefore,

alcohol-induced iron overload increases the production of free radicals and pro-inflammatory cytokines, leading to liver damage.[22] Elegant studies using experimental animal models of ALD show that increased iron in Kupffer cells causes activation of nuclear transcription factor kappa (NF- κ B) and increased expression of the proinflammatory cytokine tumor necrosis factor alpha (TNF- α). These effects were reversed by iron chelation, suggesting a role for iron-mediated cell signaling in the pathogenesis of experimental alcoholic liver disease.[23] Chronic alcohol exposure disrupts the normal hepcidin–ferroportin feedback loop by impairing the liver's ability to sense and respond to elevated systemic iron levels. Under physiological conditions, increased iron stores stimulate hepcidin production, which promotes ferroportin degradation and limits further intestinal iron absorption. However, alcohol-induced oxidative stress and acetaldehyde production suppress hepcidin transcription through inhibition of C/EBP α and BMP6/SMAD signaling pathways. This suppression allows ferroportin to remain active on enterocytes and macrophages, sustaining iron export into circulation despite already elevated iron stores. Simultaneously, alcohol upregulates DMT1 expression in the duodenum, further enhancing luminal iron uptake. Together, these mechanisms create a pathological positive feedback loop that promotes progressive hepatic iron accumulation and oxidative injury.

The mechanisms by which chronic alcohol consumption enhances intestinal iron absorption and promotes hepatic iron overload are summarized in Figure 2.

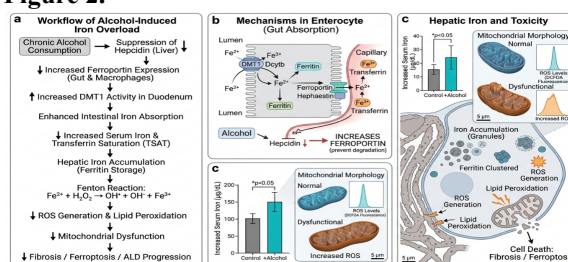


Figure 2 : Alcohol-induced alterations in intestinal iron absorption and hepatic iron overload. Chronic alcohol exposure suppresses hepcidin expression, leading to increased ferroportin and DMT1 activity, enhanced intestinal iron absorption, elevated serum iron levels, and progressive hepatic iron accumulation. Excess iron promotes oxidative stress and ferroptotic liver injury.

3.2 Effects of Alcohol on Hepatic Iron Storage and Release

Alcohol disrupts hepatic iron homeostasis primarily through suppression of hepcidin, resulting in persistent ferroportin activity and increased iron influx into hepatocytes. This promotes excessive ferritin-mediated iron storage and hepatic siderosis,

particularly in chronic alcohol users. In patients with coexisting hereditary hemochromatosis or genetic susceptibility, alcohol further amplifies iron accumulation, oxidative stress, and progression toward fibrosis and cirrhosis. These alterations establish a mechanistic basis for alcohol-induced iron overload and its contribution to liver injury.[24] Interestingly, the rate of hepatic iron accumulation in chronic alcoholics is quite variable, with only about one-third showing any iron storage. Several clinical studies have shown that the amount and length of alcohol consumption, nutritional status, general inflammation and concurrent metabolic diseases have a strong influence on iron loading. Experimental findings also indicate that interindividual differences in hepcidin suppression and expression of intestinal transporters as well as the variation in ferritin turnover can play a role in the variation of iron accumulation among individuals. Polymorphisms in the iron-regulatory pathway, such as HFE mutations and other polymorphisms, could help to exacerbate susceptibility by upregulating iron uptake or by disrupting iron sensing. This variability indicates that AIH is a complex process with several overlapping components: environmental and host-specific.[25]

3.3 Interaction of Alcohol with Iron-Regulatory Hormones (e.g., Hepcidin)

Hepcidin is the central hormone regulating systemic iron balance by controlling ferroportin degradation and limiting iron absorption.[26] Chronic alcohol exposure suppresses hepcidin synthesis through oxidative stress-mediated inhibition of C/EBP α and BMP6/SMAD signaling pathways, resulting in increased intestinal iron absorption and hepatic iron accumulation.[27] This dysregulation contributes to elevated ferritin, increased TSAT, and enhanced susceptibility to oxidative liver injury. The downstream consequences of hepcidin suppression and iron overload are further explored in later sections focusing on ferroptosis and ferritinophagy.[28]

To provide an integrated overview of how chronic alcohol consumption disrupts systemic iron homeostasis and promotes hepatic iron accumulation, Figure 3 summarizes the major regulatory pathways involving hepcidin, ferroportin, intestinal absorption, transferrin saturation, ferritin storage, and labile iron expansion.

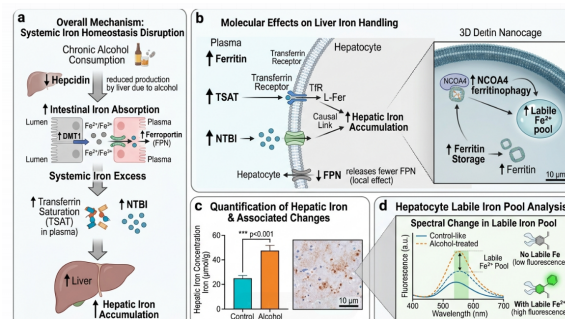


Figure 3. Alcohol-induced disruption of iron homeostasis and hepatic iron overload

4. Ferritin: Structure, Regulation, and Role in Liver Pathology

Ferritin is a 24-subunit protein cage that safely stores iron, which is essential for the prevention of iron toxicity, and the various H (heavy) and L (light) chains form tissue-specific isoforms, which is particularly abundant in the liver. Its synthesis is tightly controlled by iron levels via the IRP/IRE (iron regulatory protein/iron responsive element) system and inflammatory signals. In liver diseases, elevated serum ferritin levels often indicate inflammation, iron overload (hemochromatosis), and liver damage (NAFLD, viral hepatitis), reflecting disruption of iron homeostasis and increased disease severity.[29] The liver is an important organ for iron homeostasis and storage during physiological and acute phases. Within cells, iron is stored primarily in the form of ferritin. Ferritin is composed of L and H subunits, which are highly conserved, genetically separated, and support distinct functions. Iron storage is thought to occur in the cytoplasm. However, iron is also required for nuclear function. The L and H subunits spontaneously assemble into a 24-subunit protein “cage” with a flexible H:L ratio. The H:L ratio varies depending on the cell type. Although there is little tissue-specific regulation of the L gene, several conditions such as cell differentiation, changes in cell proliferation status, oncogenes, cytokines, and heme activate transcription of the H-ferritin gene. Indeed, previous studies have shown an association between ferritin expression and cell proliferation. The role of ferritin in liver pathology involves both iron accumulation and inflammation and serves as an important biomarker and pathogenic factor in liver diseases such as NAFLD/NASH, viral hepatitis (HBV), and iron overload conditions (hemochromatosis). Elevated ferritin often indicates liver damage, inflammation, or iron accumulation and predicts disease severity and progression (fibrosis, cirrhosis), but should be interpreted with caution due to its reactive nature in the acute phase. In excess, iron itself (stored in ferritin and hemosiderin) becomes toxic, causing oxidative stress and damaging liver

cells, making ferritin an important marker and therapeutic target for liver health.[30]

4.1 Ferritin Synthesis and Cellular Localization

Ferritin synthesis is primarily controlled post-transcriptionally by iron regulatory sequences (IREs) located in the 5'UTR region of mRNAs encoding both the heavy chain (FTH1) and light chain (FTL1) isoforms of ferritin. Under conditions of low intracellular iron concentrations, iron regulatory proteins 1 (IRP1) and 2 (IRP2) bind to IREs and are thought to prevent translation initiation by interfering with the ribosome scanning process associated with translation of these ferritin mRNAs. Conversely, under conditions of high intracellular iron concentrations, IRP1 switches to its functional role as a cytosolic aconitase and IRP2 is targeted for degradation, leading to derepression of ferritin mRNA translation and stabilization of ferritin mRNA by slowing down the rate of the deadenylation process associated with degradation of the mRNA molecule. Transcriptional activation of both mRNA isoforms occurs by binding of the transcription factor Nrf2 to antioxidant response elements during oxidative stress responses or by HIF-2 α during hypoxic responses and is mediated primarily by hepatocytes and macrophages. The newly synthesized ferritin heavy and light chains produced in this way form an enzymatically active nanocellular protein complex consisting of 24 protein subunits in the cytosol and are thought to be localized primarily in the cytosol for iron storage, with some residing in the lysosomal and mitochondrial compartments.[31] In polarized epithelial cells (intestinal cells), ferritin accumulates in the basolateral compartment, whereas secretion of ferritin into the serum occurs from hepatocyte and macrophage populations via a non-classical pathway during inflammatory responses and contributes to systemic iron sensing. Plant ferritin is an important iron storage protein with significant structural and functional similarities to animal ferritin. Additionally, plant ferritins are characterized by specific features such as plastid cell localization and transcriptional regulation by iron. Ferritin synthesis is developmentally and environmentally controlled in part by differential expression of different members of a small gene family. The intracellular localization of ferritin and its iron absorbed by rat hepatocytes was studied by ultracentrifugation of cell homogenates in sucrose density gradients. After incubation of hepatocytes with ¹²⁵I-labeled [⁵⁹Fe] ferritin, the cells incorporate most of the label into structures that equilibrate at the density where acid phosphatase and cytochrome c oxidase reside. This suggests a lysosomal or mitochondrial association with ferritin and its iron.[32]

4.2 Impact of Alcohol on Ferritin Expression and Function

Alcohol has a profound effect on ferritin by increasing its expression and release, leading to elevated serum levels, responses to alcohol-induced inflammation and cell damage, and iron overload. Because alcohol directly increases ferritin synthesis, inhibits hepcidin (an iron regulator), impairs liver health, and mimics iron overload disorders such as hemochromatosis, ferritin is a marker of both inflammation and iron problems associated with alcohol abuse. Hepatocyte ferritin production increases in a dose-dependent manner after a direct response to alcohol, regardless of whether hepatocyte iron stores are low or high.[33] Alcohol increased the production of H and L ferritin subunits in HepG2 (a human liver cell line) and primary rat hepatocyte cultures. Even at concentrations as low as 10 mM, alcohol significantly increased ferritin levels in both cell types, significantly increasing ferritin mRNA in HepG2 and increasing translation rates in primary hepatocytes. Alcoholic hyperferritinemia is observed in alcoholic patients after chronic alcohol consumption and is caused by decreased hepcidin levels in the liver. Therefore, increased iron intake coincides with increased production of ferritin, which binds excess Fe²⁺. [34] Desferrioxamine treatment decreased both hepcidin suppression and ferritin- and macrophage-associated iron delivery. Therefore, part of alcoholic hyperferritinemia occurs via the iron pathway. Furthermore, alcohol appears to interfere with the protective effects of ferritin, causing ferritinophagy by decreasing levels of the transcription factor Nrf2 and increasing levels of the receptor NCOA4. [35] The subsequent release of labile iron from ferritin stimulates ROS generation through the Fenton reaction, and there is strong evidence that the effects of increased oxidative stress are involved in the development of alcoholic liver disease. The role of alcohol in regulating hepcidin expression in the liver is well established. Alcohol-induced oxidative stress inhibits the DNA-binding activity of C/EBP- α and suppresses hepcidin transcription in the liver. [36] Increased suppression of hepcidin expression by alcohol may be involved in disease severity in patients with hereditary hemochromatosis associated with alcohol consumption. Decreased hepcidin expression in the liver increases iron transporter expression in the intestine. Additionally, alcohol impairs the protective regulatory role of hepcidin against iron overload. As a result, hepatic hepcidin synthesis becomes less responsive to systemic iron levels, promoting excessive iron accumulation. Dysregulation of hepcidin synthesis in the liver may be one of the underlying mechanisms by which alcohol intake causes iron overload. Ultimately, iron acts as a secondary risk factor for alcoholic liver disease.[37]

4.3 Serum Ferritin as a Biomarker in Alcohol-Induced Liver Disease

Serum ferritin is a key diagnostic biomarker for alcoholic liver disease (ALD), indicating iron overload, inflammation, and oxidative damage due to the inhibitory effect of alcohol on hepcidin secretion and the stimulatory effect of alcohol on ferritin expression. It often exceeds 200 µg/L in more than 50% of patients with liver damage due to alcohol dependence, and there is a positive correlation between disease severity, AST/ALT ratio, GGT, and progression from steatosis to cirrhosis and HCC, with a cutoff value of >1000 µg/L indicating the presence of established disease.[38] Intermediate serum ferritin values ranging from 200–1000 µg/L in patients with alcohol-related liver disease require careful clinical interpretation. In individuals with ongoing alcohol consumption, ferritin levels within this range may reflect a combination of hepatic inflammation, oxidative stress, and early iron accumulation rather than severe iron overload alone. Persistent elevation despite continued alcohol use often indicates progressive hepatic injury and increasing risk of fibrosis. In contrast, in patients undergoing alcohol abstinence or recovery, a gradual decline in ferritin levels within weeks to months generally suggests reversal of inflammatory stress and improvement in iron homeostasis. However, persistently elevated ferritin (>500–800 µg/L) despite sustained abstinence should prompt further investigation for coexisting iron overload disorders such as hereditary hemochromatosis or advanced liver fibrosis.

The rapid decline from the normal range in response to abstinence indicates its importance in disease regression, differentiation, and differentiation between ALD and other nonalcoholic liver diseases and hemochromatosis, although their properties as acute-phase reactants have a potential tendency to confound the disease, and saturating levels of transferrin and CRP are required for differentiation and accurate measurement. Ferritin levels and CRP are also prognostic markers of the disease.[39] The liver contains most of the body's iron stores, and processes that damage liver cells release ferritin. Liver damage may also prevent the removal of ferritin from the circulation. Liver damage caused by oxidative stress causes hepatic endothelial cells to release ferritin into the circulation. Ferritin may increase the toxic effects of alcohol on liver fibrosis and cirrhosis. Leakage of tissue ferritin, an intracellular iron storage protein, leads to increased serum ferritin levels. Serum gamma glutamyl transferase (GGT) is often the first marker to be elevated in alcoholic patients. Increases with dose in case of alcohol abuse. Women are less sensitive than men. This enzyme can be released as a result of liver cell damage or as a result of induction after alcohol

exposure. Hepatocyte cholestasis and hepatocyte damage in alcoholic liver disease lead to increased GGT. It increases if you take 50g or more per day for 5 weeks.[40]

The Clinical Interpretation Of Serum Ferritin in Alcohol related liver Diseases is Shown in the Table:1

Table 1: Clinical Interpretation of Serum Ferritin in Alcohol-Related Liver Disease [41]

Parameter	Normal Range	Mild ALD	Severe ALD / Cirrhosis	Hereditary Hemochromatosis	Clinical Significance
Ferritin	200 – 1000 µg/L	Moderate ALD	active inflammation	Intermediate iron overload;	monitor abstinence response
AST/ALT Ratio	<1	>1	>2	Variable	Suggests alcoholic injury
GGT	Normal	Elevated	Markedly Elevated	Mild ↑	Alcohol biomarker
CRP	Normal	Elevated	Elevated	Normal/slightly ↑	Indicates inflammation
Transferrin Saturation	20–45%	40–60%	>60%	>60–70%	Differentiates ALD vs HH
Hepcidin	Normal	Decrease due to alcohol	Markedly suppressed	Inappropriately low due to genetic defect	Reduced hepcidin increases intestinal iron absorption and hepatic iron accumulation.
Liver Iron Stores	Normal	Mild to moderate hepatic iron accumulation	Moderate to severe hepatic iron accumulation	Severe systemic iron overload	Contributes to oxidative stress, fibrosis, cirrhosis, and HCC risk.
Response to Alcohol Absti	Normal	Ferritin and GGT decline within	Ferritin decreases if liver function	No significant improvement without therapeutic	Helps differentiate ALD from hereditary

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4.4 Ferritin and Oxidative Stress in Hepatocellular Injury

Excess free iron serves as a substrate for ROS formation and increases oxidative stress. Oxidative stress is recognized as a cause of organic damage to the liver, the main site of iron storage. Ferroptosis, a mechanism of iron-dependent regulated cell death, has recently attracted attention in the development of organ damage and progression of liver disease. Ferritin is a mediator of oxidative stress in HCC injury, simultaneously protecting against iron dysregulation damage by reducing lipid peroxidation and, paradoxically, enhancing iron dysregulation damage by increasing lipid peroxidation. Ferritin is a major intracellular iron storage protein that binds free Fe^{2+} and prevents Fenton oxidation of H_2O_2 to hydroxyl radicals ($-\text{OH}$) in lipids, protecting against secondary injury, alcohol injury, and iron-mediated lipid peroxidation during ischemia, but in cases of overwhelming oxidative damage, it is destroyed by ferritinophagy (autophagy mediated by NCOA4) or direct oxidation. Increases labile iron available for catalysis.[42] Ferroptosis, a type of iron-mediated cell death due to inhibition of glutathione peroxidoreductase (GPX4) activity and accumulation of phospholipid hydroperoxides. In alcohol-induced liver injury, inhibition of hepcidin by alcohol-induced ROS causes protective iron overload, suppressing hepcidin and causing mitochondrial damage, iron dysregulation, and serum iron chelation, where serum iron concentrations correlate with injury. Deferoxamine, an iron chelator, reverses this.[43] Tightly balanced iron homeostasis is physiologically necessary because unbound free iron is a major contributor to toxic potential as a source of oxidative stress via the Fenton reaction, and because of its role in ferroptosis, a form of iron-dependent regulated cell death. In metabolic-associated fatty liver disease (MASLD), biochemical evidence of excess iron, evidenced by elevated serum ferritin concentrations, is common in approximately one-third of patients. Beyond serving as a passive iron storage protein, ferritin actively regulates intracellular iron availability and oxidative vulnerability. Under conditions of chronic alcohol exposure, increased ferritin synthesis initially acts as a protective mechanism by sequestering excess ferrous iron and limiting Fenton-mediated radical generation. However, dysregulated ferritin turnover through NCOA4-mediated ferritinophagy can release large amounts of labile iron, amplifying oxidative stress, lipid peroxidation, and ferroptotic cell death. Thus, ferritin functions both as a protective buffer and a pathogenic mediator in alcohol-

associated liver injury, depending on the balance between iron sequestration and iron release.[44]

5. Transferrin and Transferrin Saturation in Alcohol-Related Liver Disease

In alcohol-related liver disease (ALD), transferrin saturation (TSAT) and iron levels are often elevated, indicating iron overload and exacerbating liver damage and fibrosis. Although increased intake correlates with increased TSAT, some ALD patients may have fluctuating or low levels of transferrin (the protein itself), a sign of poor prognosis. Elevated TSAT (often >50%) indicates possible iron overload, but in ALD it may also reflect inflammation, mimicking hereditary hemochromatosis, and requires careful differentiation to avoid unnecessary treatment. In alcohol-related liver disease (ALD), alcohol inhibits iron metabolism, increasing iron stores in the body and increasing TSAT and ferritin even in mild cases.[45] High TSAT levels (often >50%) indicate possible iron overload, which is a risk factor for poor liver outcomes such as liver fibrosis and cirrhosis. Elevated levels of TSAT and ferritin mimic hereditary hemochromatosis, but in ALD they are a signal of liver damage, and a decrease in serum transferrin (a protein) itself is a strong predictor of mortality by standard measurements, especially in severe alcoholic hepatitis. The withdrawal phase presents many physical and psychological risks, including anxiety, tremors, insomnia, nausea, seizures, and delirium tremens. The degree to which people experience these symptoms varies greatly from person to person.[46] Approximately 50% of people with a history of heavy drinking will experience withdrawal symptoms, and approximately 2-8% will experience delirium tremens. This is a more severe form of alcohol withdrawal and has a high mortality rate if left untreated. Several risk factors have been pointed out. Withdrawal symptoms have been confirmed to become more severe. These include psychiatric history, biomarkers such as elevated blood urea nitrogen and plasma lipids, and genetic vulnerability.[47]

To provide a concise overview of the major proteins involved in systemic iron regulation and their pathological alterations in alcohol-related liver disease, **Table:2** summarizes their physiological roles, alcohol-induced dysregulation, and downstream consequences in hepatic injury.

Table 2. Major iron metabolism proteins, their physiological functions, alcohol-induced alterations, and pathological contributions in alcohol-related liver disease (ALD). [48]

Protein	Physiological Role	Alteration in ALD	Pathological Consequence
Hepcidin	Master iron regulator	Suppressed	Increased iron absorption
Ferroportin	Iron export	Persistent activity	Elevated serum iron
Ferritin	Iron storage	Increased	Iron sequestration and ferritinophagy
Transferrin	Iron transport	Increased saturation	NTBI formation
TfR1	Cellular iron uptake	Upregulated	Increased hepatocyte iron entry
TfR2	Iron sensing	Dysregulated	Impaired hepcidin feedback
DMT1	Intestinal iron uptake	Upregulated	Enhanced absorption
STEAP3	Ferric to ferrous reduction	Increased activity	Enlarged labile Fe^{2+} pool

5.1 Physiology of Transferrin and Transferrin Receptors

Iron is essential for cellular metabolism and is transported into the blood with transferrin. Transferrin receptors deliver iron to cells through transferrin receptors. Transferrin receptor expression reflects iron demand and proliferative activity. Iron deficiency or excess impairs brain function, so the brain must fine-tune iron homeostasis. This regulation is primarily mediated by interactions between transferrin and transferrin receptors located on the blood-brain barrier, neuronal cell membranes and glial cell membranes. Transferrin is present in brain plasma and extracellular fluid, and transferrin receptors are expressed on brain endothelial cells, choroid plexus epithelial cells,

neurons, and glial cells. Iron influx into the brain is regulated during development, with maximum transport occurring early postnatally and decreasing markedly thereafter.[49] Evidence suggests that iron crosses the blood-brain barrier primarily through receptor-mediated endocytosis of transferrin by endothelial cells, intracellular release of iron, recycling of transferrin into the circulation, and subsequent transport of iron to brain tissues, rather than transcytosis of intact transferrin. Further absorption of iron occurs via the blood-cerebrospinal fluid pathway, and receptor-mediated processes in the choroid plexus make transferrin transport relatively efficient. Once in the brain interstitium, iron binds to brain-derived transferrin and is taken up by neurons and glial cells via a regulated transferrin receptor-dependent mechanism that varies with development and iron status. Transferrin, a glycoprotein produced primarily in the liver, is present in plasma with two iron-rich sites that have a high affinity for ferric iron (Fe^{3+}), forming holotransferrin. Holotransferrin can safely transport approximately 4 mg of total iron into the body while maintaining low levels of potentially toxic iron. Iron is taken up by transferrin cells via receptor-mediated endocytosis. Here, diferric transferrin binds to transferrin receptor 1 (TfR1). Transferrin receptor 1 (TfR1) is a dimeric transmembrane glycoprotein whose levels increase in response to iron regulatory proteins (IRPs) during iron deficiency. IRP binds to the IRE, which is specifically located in the 3'UTR of this receptor mRNA.[50]

5.2 Alcohol-induced changes in Transferrin synthesis

Further absorption of iron occurs via the blood-cerebrospinal fluid pathway, and receptor-mediated processes in the choroid plexus make transferrin transport relatively efficient. Once in the brain interstitium, iron binds to brain-derived transferrin and is taken up by neurons and glial cells via a regulated transferrin receptor-dependent mechanism that varies with development and iron status. Transferrin, a glycoprotein produced primarily in the liver, is present in plasma with two iron-rich sites that have a high affinity for ferric iron (Fe^{3+}), forming holotransferrin.[51] Holotransferrin can safely transport approximately 4 mg of total iron into the body while maintaining low levels of potentially toxic iron. Iron is taken up by transferrin cells via receptor-mediated endocytosis. Here, diferric transferrin binds to transferrin receptor 1 (TfR1). Transferrin receptor 1 (TfR1) is a dimeric transmembrane glycoprotein whose levels increase in response to iron regulatory proteins (IRPs) during iron deficiency. IRP binds to the IRE, which is specifically located in the 3'UTR of this receptor mRNA.[52] The resulting iron receptor complexes are transported from

the cell surface to clathrin-coated endosomes, where acidification mediated by the proton pump V-ATPase sets the pH to approximately 5.5 and Fe^{3+} is released, which is then reduced to Fe^{2+} catalyzed by the ferrireductase STEAP3 and transported into the cell, or rather into the cytosol, by DMT1. Chronic use of ethanol will further promote increased desialylation of glycoproteins in liver endothelial cells, resulting in higher-than-normal levels of CDT in plasma (up to a maximum increase of 10-fold) irrespective of iron status while levels of total transferrin may or may not change in advanced liver disease. Both of these processes are considered diagnostic markers for alcohol use and, when coupled with other factors, are obstacles to the efficient transport of iron by alcoholics.[53] Alcohol affects transferrin synthesis by directly impairing the liver's ability to properly glycosylate (add sugar chains) the protein, leading to more carbohydrate-deficient transferrin (CDT) isoforms, a key biomarker for heavy drinking, and can also suppress overall transferrin production, especially with liver damage, causing iron regulation issues, though the exact mechanisms involve complex changes in glycosylation enzymes like sialyltransferase and sialidase.[54]

5.3 Carbohydrate-Deficient Transferrin (CDT) as an Alcohol Biomarker

Carbohydrate-deficient transferrin (CDT) is an ideal indicator of excessive alcohol consumption. This is because it has a lower false-positive rate and potentially more accurate results than other commonly used markers of excessive alcohol consumption, such as gamma glutamyltransferase (GGT) and mean corpuscular volume (MCV). Furthermore, chronic alcohol consumption impairs hepatic glycosylation (through inhibition of sialyltransferase enzymes) and increases the amount of sialic acid removed from transferrin glycan chains (through induction of sialidase enzymes), resulting in the proportion of asialotransferrin and disialotransferrin isoforms in the total transferrin concentration in heavy drinkers exceeding 1.7–2%.[55] A population's CDT concentrations increase with the amount of ethanol consumed each day and return to pre-ingestion levels within 2 to 4 weeks after drinking cessation, and can be used as a means to monitor the success of both relapse prevention programs and license restoration efforts. However, genetic variations and liver disease can complicate the interpretation of CDT levels and should always be considered in conjunction with other clinical and laboratory findings from the same individual.[56]

5.4 Transferrin Saturation and Implications for Hepatic Iron Loading

Transferrin saturation (TSAT), the ratio of serum iron to total iron-binding capacity (usually around 20-

45%), is the amount of iron available in the plasma and is a means of detecting iron accumulated in the liver (liver) via signaling cells (hepatocytes) that increase the amount of hepcidin (a peptide hormone) released through transferrin receptor 2 (TfR2). When TSAT exceeds the iron utilization capacity of transferrin (defined as >45-50%), the excess serum iron generates non-transferrin-bound iron (NTBI), which accumulates in hepatocytes via PATHS (proton-coupled transporters) such as ZIP14.[57] Further accumulation of NTBI results in higher than normal amounts of iron accumulating in the liver parenchyma, leading to ferroptosis, increased fibrosis, decreased TfR1 expression, and increased ferritin levels. In alcoholic liver disease, the alcohol-induced decrease in hepcidin levels increases TSAT levels, further worsening liver siderosis and liver damage. Additionally, TSAT >60% may increase the risk of developing progressive liver fibrosis and may serve as an indicator of hemochromatosis and the need for therapeutic phlebotomy or chelation. Impaired transferrin transcytosis in the liver is another factor that contributes to NTBI damage, as loss of the feedback system for transferrin transport reduces the amount of transferrin that hepatocytes can produce.

[58]

From a clinical point of view, the distinction between alcohol-related liver disease (ALD) and hereditary hemochromatosis (HH) is made through a step-by-step approach to iron work-up in the context of alcohol use. Alcohol history and assessment of the transferrin carbohydrate content (CDT) serve as important background information in patients who have elevated ferritin and TSAT. The normalization of serum ferritin level, liver enzyme levels, and TSAT after cessation of drinking is strong evidence of secondary iron overload due to ALD. Conversely, the presence of an elevated TSAT index >60-70%, an elevated ferritin level despite abstaining from alcohol, and positive testing of the inherited mutation in HFE support a diagnosis of hereditary hemochromatosis. Thus a longitudinal study of iron indices with genetic testing is still needed to diagnose and make a correct therapeutic decision. Transferrin contributes mechanistically to disease progression by regulating the threshold at which circulating iron exceeds safe transport capacity. Once transferrin saturation TSAT becomes elevated, excess iron appears as NTBI, which is highly reactive and readily taken up by hepatocytes through alternative transporters such as ZIP14. This bypasses normal transferrin receptor-mediated regulation and accelerates intracellular iron accumulation, oxidative injury, mitochondrial dysfunction, and inflammatory signaling. Therefore, transferrin is not only a transport protein but also a key determinant of iron toxicity in alcohol-related liver disease.

The Comparison of Iron Parameters is Shown in Table:3

Table 3: Comparison of Iron Parameters in Alcoholic Liver Disease vs Hereditary Hemochromatosis [58]

Parameter	Alcoholic Liver Disease (ALD)	Hereditary Hemochromatosis (HH)
Ferritin	Elevated (inflammation + iron overload)	Markedly elevated (iron overload primary)
Transferrin	May decrease in severe ALD	Usually normal
Transferrin Saturation (TSAT)	>50% (variable)	>60–70% consistently
Hepcidin	Suppressed by alcohol	Genetically low
CDT	Elevated	Normal
NTBI	Present	Present (high levels)
Mechanism	Alcohol-induced hepcidin suppression + oxidative stress	HFE mutation → impaired iron sensing
Reversibility	Improves with abstinence	Requires phlebotomy
FTH1 expression	Generally increased as a protective response to oxidative stress and excess hepatic iron	Increased to store excess intracellular iron, but may become overwhelmed with progressive iron accumulation
Major regulation of FTH1	Predominantly regulated by oxidative stress, inflammatory signaling, and iron-responsive translational control (IRP/IRE)	Mainly regulated by intracellular iron levels through the IRP/IRE system and iron overload
Ferritinophagy (NCOA4-mediated)	May be dysregulated by alcohol-	Active ferritinophagy releases stored

	induced impairment of autophagy; altered ferritin degradation contributes to liver injury	iron from ferritin, increasing the labile iron pool and oxidative damage
Role of NCOA4	NCOA4-mediated ferritin degradation may be altered during chronic alcohol exposure, although the mechanism is less well established	NCOA4 promotes ferritin degradation and iron mobilization, contributing to iron toxicity
Tryptophan regulation of FTH1	No established evidence that intracellular tryptophan regulates FTH1 stability in ALD	No established evidence that tryptophan regulates ferritinophagy in HH

6. Iron -Alcohol interactions in the pathogenesis of liver injury.

Acute liver injury often progresses to liver failure, and the immune response plays an important role in controlling the inflammatory process. Although serum iron is known to be associated with increased risk of infectious diseases, hepcidin, a key regulator of iron metabolism, has never been systematically studied in this context. The immune response to acute liver injury is dynamic and involves activation of multiple immune cells, including neutrophils, macrophages, dendritic cells (DCs), and T lymphocytes. These cells are involved in both the inflammatory and injury recovery stages. In acetaminophen (APAP)-induced liver injury, neutrophils initially cause inflammation [3] but play a restorative role as the injury resolves.[59] Natural killer T cells protect against liver injury, and mice deficient in natural killer T cells are highly susceptible to APAP-induced liver injury. The liver is an important site of iron and lipid metabolism and a major site of interaction between these two metabolic pathways. Although conflicting results have been reported, most studies support the hypothesis that iron plays a role in hepatic adipogenesis. Iron is an essential

component of several enzymes and transporters involved in lipid metabolism and, as such, can directly influence hepatic lipid load, intrahepatic metabolic pathways, and hepatic lipid secretion. On the other hand, iron in the ferrous form may indirectly affect lipid metabolism through its ability to induce oxidative stress and inflammation, and this hypothesis is currently the subject of much research in the field of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH).[60]

6.1 Synergistic oxidative stress from alcohol metabolism and iron.

Oxidative stress is recognized to play an important role in initiating and promoting carcinogenesis. Alcoholic liver disease is associated with significant oxidative stress and the accumulation of iron in the liver, a transition element that also causes oxidative stress. The combined prooxidant effects of ethanol and iron are at least additive and possibly synergistic in inducing oxidative stress and antioxidant depletion in hepatocytes. One of the cellular consequences of sustained oxidative stress and redox imbalance resulting from the combined effects of alcohol and iron is lipid peroxidation, which leads to the production of aldehyde products such as 4-hydroxy-2-nonenal, which is associated with site-specific mutations in the p53 gene.[61] Alcohol metabolism generates reactive oxygen species (ROS) through alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1), generates acetaldehyde, and changes in the NAD⁺/NADH ratio cause increased production of superoxide ions (O₂⁻) and hydrogen peroxide (H₂O₂). Iron facilitates this process through the Fenton reaction. In the Fenton reaction, reactive (unstable) Fe²⁺ causes the formation of OH⁻ from H₂O₂. In addition to increasing TSAT and NTBI, hepcidin suppression (alcohol-induced) results in increased hepatocyte uptake of excess iron produced by ethanol consumption, which further enhances ethanol-induced ROS, leading to lipid peroxidation, protein carbonyl (i.e., hydroxyethyl radical) formation, and decreased mitochondrial integrity/function. Changed. The combination of these factors inhibits the effectiveness of certain antioxidants, particularly GSH and superoxide dismutase (SOD), promoting ferroptosis, NF-κB-mediated inflammation, and fibrosis resulting from the “second round” of ethanol toxicity. Acetaldehyde, a toxic byproduct of ethanol metabolism, contributes significantly to iron overload in alcohol-associated liver disease by increasing the production of reactive oxygen species (ROS). The resulting oxidative stress suppresses hepcidin synthesis through disruption of two major regulatory pathways: C/EBPα and BMP6/SMAD. ROS impair the transcriptional activity of C/EBPα, a key factor required for hepcidin gene expression, while also

inhibiting BMP6-induced SMAD signaling, which normally promotes hepcidin production. Reduced hepcidin levels enhance ferroportin-mediated iron export and intestinal iron absorption, leading to excessive iron accumulation in the liver. This excess iron further amplifies oxidative damage, lipid peroxidation, inflammation, and hepatocellular injury, creating a vicious cycle that accelerates the progression of alcohol-associated liver disease. [62]

6.2 Iron-driven lipid peroxidation and mitochondrial dysfunction.

The most important biochemical pathway that connects iron overload to hepatocellular injury in alcohol-related liver disease (ALD) is called lipid peroxidation (LPO). It goes through three key phases: initiation phase, propagation phase, and termination phase. In the initiation step, ferrous iron (Fe²⁺) reacts with hydrogen peroxide (H₂O₂) in the presence of hydrogen peroxide to produce highly reactive hydroxyl radicals (•OH).[63] These radicals remove hydrogen atoms from the membrane phospholipids containing polyunsaturated fatty acids (PUFAs) and form lipid radicals. These lipid radicals can rapidly react with molecular oxygen to produce lipid peroxy radicals that can react with nearby PUFAs to produce lipid hydroperoxides and propagate the chain reaction. Iron and lipoxygenase (LOX) activity also promote this process. The termination involves antioxidant systems like glutathione (GSH), glutathione peroxidase 4 (GPX4), vitamin E and ferroptosis suppressor protein 1 (FSP1) that neutralize lipid radicals or convert lipid hydroperoxides into non-toxic products. In chronic alcohol exposure, the levels of GSH and GPX4 are depleted, reducing protection, and permitting unchecked lipid peroxidation. The biological effects involve destabilisation of membranes, impairment of mitochondria, release of pro-inflammatory mediators, triggering of ferroptosis, and continuous hepatocellular damage and fibrosis and cirrhosis.[64]

Among iron species, ferrous iron (Fe²⁺) plays the most critical pathogenic role because of its high redox reactivity. Unlike ferric iron (Fe³⁺), which is relatively stable and safely transported by transferrin or stored in ferritin, Fe²⁺ exists within the labile iron pool and readily participates in redox cycling. In the Fenton reaction, Fe²⁺ reacts directly with hydrogen peroxide (H₂O₂) to generate hydroxyl radicals (•OH), one of the most reactive and damaging oxygen species in biological systems. These hydroxyl radicals rapidly attack membrane lipids, proteins, and nucleic acids, initiating widespread oxidative injury. Fe³⁺ generated during this reaction can be reduced back to Fe²⁺ by superoxide anions (O₂^{•-}) through the Haber–Weiss cycle, sustaining continuous radical production. In alcohol-related liver disease, chronic ethanol

metabolism increases both hydrogen peroxide production and the labile Fe^{2+} pool, greatly amplifying iron-catalyzed oxidative damage and ferroptotic susceptibility.

Mitochondria have multiple interconnected mechanisms of amplifying ferroptotic injury. Iron accumulation could be an intracellular problem, where it may enhance the formation of specific reactive oxygen species (ROS) by Fenton chemistry and impair the function of the electron transport chain (ETC). This results in bioenergetic failure, ATP depletion and increased oxidative stress. Moreover, iron-induced damage to mitochondrial iron–sulfur (Fe–S) clusters leads to the disruption of the activity of important respiratory enzymes and to the destabilization of mitochondrial metabolism. Structural changes like mitochondrial shrinkage, increased membrane density, decreased cristae and loss of mitochondrial membrane potential ($\Delta\Psi$) are known hallmarks of ferroptosis. Chronic ethanol metabolism, through CYP2E1 and acetaldehyde toxicity, also leads to increased mitochondrial ROS production, causing a synergistic effect to enhance lipid peroxidation, ferroptosis, and chronic hepatocyte injury in the context of alcohol-associated liver disease.[65]

To improve mechanistic clarity, **Table 4** outlines the sequential phases of lipid peroxidation, the major reactive intermediates involved, and their biological consequences in the progression of alcohol-related liver disease.

Table 4. Sequential phases of lipid peroxidation are associated molecular mediators, and pathological consequences in alcohol-related liver disease (ALD). [66]

Phase	Key Event	Major Molecules	Biological Outcome
Initiation	Fenton reaction	Fe^{2+} , H_2O_2	Hydroxyl radical generation
Propagation	Radical chain amplification	PUFA, LOX	Lipid hydroperoxides
Amplification	Membrane damage	ACSL4, LPCAT3	Ferroptosis sensitization
Termination	Radical neutralization	GPX4, GSH, FSP1, Vit E	Protection

Failure of defense	Antioxidant depletion	GSH depletion	Uncontrolled LPO
Consequences	Cell death	ROS, MDA, 4-HNE	Fibrosis/cirrhosis

6.3 Role of Kupffer cells and inflammatory pathways

In the liver, resident macrophages, Kupffer cells, are a key player in the pathogenesis of alcohol-associated liver disease (ALD). Alcohol consumption, over an extended period of time, leads to increased permeability of the gut and uptake of bacterial endotoxins (e.g., LPS) into the portal circulation, which stimulates Kupffer cells. Activated Kupffer cells generate Reactive Oxygen Species (ROS), nitric oxide, and pro-inflammatory cytokines such as Tumor Necrosis Factor- α (TNF- α), Interleukin-1 β (IL-1 β) and Interleukin-6 (IL-6) which further contribute to hepatocellular injury and inflammation. Microbial factors, such as iron overload, serve to further activate the Kupffer cells, by increasing oxidative stress and by inducing inflammatory signaling pathways. These processes trigger transcription factors like nuclear factor-kappa B (NF- κ B) and activation of inflammasome complexes, resulting in ongoing production of cytokines and recruitment of inflammatory cells. Chronic inflammation increases the activity of hepatic stellate cells, deposition of extracellular matrix, fibrosis and cirrhosis. Therefore, Kupffer cells play a pivotal role between alcohol-induced liver injury, iron-mediated oxidative damage and chronic hepatic inflammation.[67]

6.4 Interaction between alcohol-induced inflammation and iron dysregulation

Chronic alcohol drinking induces a chronic state of inflammation, which has a very strong link to iron metabolism and plays an important role in the development of alcohol-related liver disease (ALD). Alcohol increases intestinal permeability and increases the entry of bacterial endotoxins such as lipopolysaccharide (LPS) into portal circulation which is a process that activates the Kupffer cells. This activation triggers the release of pro-inflammatory cytokines, including tumour necrosis factor α (TNF- α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6), which further exacerbates hepatocellular injury and oxidative stress. There are also complex iron regulatory effects of inflammation. Physiological levels of IL-6 activate the JAK/STAT3 pathway, which promotes the production of hepcidin to prevent excess iron from being available. At the same time, chronic alcohol exposure causes reduced hepcidin expression via oxidative stress and inhibiting C/EBP α and BMP6/SMAD signaling by acetaldehyde. This

dysregulated state results in competition between inflammatory and alcohol dependent signals, resulting in an imbalance of iron homeostasis, increased iron absorption, and hepatic iron accumulation. This creates a vicious cycle of increased oxidative stress and inflammatory signaling which perpetuates iron overload, leading to increased fibrosis, cirrhosis, and ferroptotic liver injury.

Given the increasing recognition of ferroptosis as a critical mechanism of alcohol-induced liver injury, **Table:5** summarizes the major molecular regulators of ferroptosis, their biological functions, and their relevance in ALD progression and therapeutic targeting.

Table 5. Key molecular regulators of ferroptosis, their functional roles, pathological alterations, and therapeutic implications in alcohol-related liver disease.[68]

Regulator	Function	Effect in ALD	Therapeutic Potential
GPX4	Detoxifies lipid peroxides	Decreased	Ferroptosis suppression
SLC7A11	Cystine uptake	Downregulated	GSH restoration
System Xc ⁻	Maintains antioxidant defense	Impaired	Targetable
ACSL4	PUFA incorporation	Upregulated	Promotes LPO
NCOA4	Ferritinophagy	Increased	Releases labile iron
FSP1	CoQ10 reduction	Reduced protection	Alternative anti-ferroptosis pathway
Nrf2	Antioxidant regulator	Dysfunctional	Cytoprotective target
p53	Metabolic regulator	Context-dependent	Ferroptosis modulation

6.5 Genetic factors influencing susceptibility (e.g., HFE mutations)

The genetic susceptibility plays a major role in the susceptibility and severity of iron-overload in alcoholic liver disease. The most studied genetic factors are mutations in the HFE gene, specifically the C282Y, H63D, and S65C (rare) mutations, which are linked to hereditary hemochromatosis. These mutations are involved in the regulation of iron absorption via hepcidin signalling, leading to intestinal iron over-absorption and a progressive accumulation of iron in the liver. The effects of HFE mutations can

be further enhanced in individuals who drink alcohol on a regular basis, as the effects of alcohol also decrease hepcidin expression and increase iron absorption. Alcohol exposure and genetic susceptibility can result in higher levels of liver iron deposition, greater production of reactive oxygen species, lipid peroxidation, inflammation and fibrogenesis. Therefore, carriers of an HFE mutation may have increased risk for advanced liver disease such as alcoholic hepatitis, cirrhosis and hepatocellular carcinoma.[69] In addition to HFE, mutations in other genes that regulate iron metabolism, including transferrin receptor 2 (TFR2), hemojuvelin (HJV), hepcidin antimicrobial peptide (HAMP), and ferroportin (SLC40A1), may also affect iron metabolism and disease susceptibility. These genetic factors will facilitate recognition of high-risk individuals, prognosis of the disease, and personalized treatment of alcohol-induced liver diseases. Hepcidin regulation in alcohol-associated liver disease is governed by the opposing effects of inflammation and alcohol exposure. Inflammatory cytokines, particularly interleukin-6 (IL-6), stimulate hepcidin expression through activation of the JAK/STAT3 signaling pathway, serving as a protective mechanism to limit iron availability during tissue injury and infection. In contrast, chronic alcohol consumption suppresses hepcidin synthesis by promoting oxidative stress and acetaldehyde production, which inhibit key regulatory pathways such as C/EBP α and BMP6/SMAD. As a result, alcohol enhances intestinal iron absorption and iron release from macrophages, leading to hepatic iron accumulation. The balance between inflammation-induced hepcidin upregulation and alcohol-mediated hepcidin suppression is dynamic and may vary according to the stage and severity of liver disease. This bidirectional regulation creates a complex interplay between immune responses, iron metabolism, and oxidative stress, contributing to the progression of liver injury, fibrosis, and cirrhosis in alcohol-associated liver disease. [70]

6.6 The Regulatory Network of Ferroptosis in ALD

Ferroptosis is a distinct form of regulated cell death characterized by iron-dependent accumulation of lipid peroxides and loss of membrane integrity. Unlike apoptosis, ferroptosis does not involve caspase activation, DNA fragmentation, or apoptotic body formation. Unlike necrosis, it is a regulated and metabolically controlled process rather than passive cell rupture. Although ferritinophagy contributes to ferroptosis by releasing intracellular iron, ferroptosis is mechanistically distinct from autophagy, as its central event is iron-catalyzed lipid peroxidation. It also differs from pyroptosis, which is mediated by inflammasome activation and gasdermin pore formation.

The molecular hallmarks of ferroptosis include intracellular iron overload, glutathione (GSH) depletion, inactivation of glutathione peroxidase 4 (GPX4), accumulation of phospholipid hydroperoxides, and increased activity of acyl-CoA synthetase long-chain family member 4 (ACSL4), which enriches membranes with polyunsaturated fatty acids (PUFAs) highly susceptible to oxidation. Additional regulatory pathways include system Xc⁻ (SLC7A11/SLC3A2), ferritinophagy mediated by NCOA4, ferroptosis suppressor protein 1 (FSP1), Nrf2 antioxidant signaling, and p53-dependent metabolic regulation. Together, these pathways determine cellular susceptibility to ferroptotic injury in alcohol-associated liver disease.

Ferroptosis is a regulated form of cell death characterized by iron-dependent lipid peroxidation and has emerged as an important mechanism in the pathogenesis of alcohol-associated liver disease (ALD). Chronic alcohol consumption disrupts iron homeostasis by suppressing hepcidin expression and increasing intestinal iron absorption, leading to hepatic iron accumulation. Excess ferrous iron (Fe²⁺) catalyzes the generation of reactive oxygen species through Fenton reactions, promoting lipid peroxidation and ferroptotic cell death.[71]

The ferroptosis regulatory network in ALD involves several interconnected pathways. Iron metabolism regulators, including hepcidin, transferrin receptor 1 (TFR1), ferritin, and ferroportin, control intracellular iron availability. Antioxidant defense systems, particularly the system Xc⁻/glutathione (GSH)/glutathione peroxidase 4 (GPX4) axis, protect cells from lipid peroxidation. Alcohol-induced depletion of GSH and inhibition of GPX4 reduce the liver's ability to neutralize lipid hydroperoxides, thereby enhancing ferroptosis. In addition, mitochondrial dysfunction, oxidative stress, and inflammatory signaling mediated by Kupffer cells further amplify ferroptotic injury. Recent studies also implicate transcription factors such as Nrf2 and p53 in modulating ferroptosis sensitivity. Collectively, these pathways form a complex regulatory network that links iron overload, oxidative stress, inflammation, and hepatocyte death, making ferroptosis a promising therapeutic target in ALD. [72]

Alcohol-associated liver disease (ALD) is ferroptosis sensitive and its sensitivity is regulated by several key molecular regulators. The key antioxidant enzyme to remove lipid hydroperoxide to non-toxic lipid alcohols and to prevent the damage of membranes is glutathione peroxidase 4 (GPX4). It is active in the presence of reduced glutathione (GSH) provided by the cystine/glutamate antiporter system Xc⁻, which consists mainly of the SLC7A11 and SLC3A2 genes. Chronic alcohol exposure depletes GSH and reduces

the expression of SLC7A11, which reduces this protective axis and leads to increased sensitivity to ferroptosis. At the same time, acyl-CoA synthetase long-chain family member 4 (ACSL4) catalyzes the incorporation of polyunsaturated fatty acids (PUFAs) in membrane phospholipids producing substrates that are highly susceptible to peroxidation. LPCAT3 also supports phospholipid remodeling and lipoxygenases (LOXs) promote the production of lipid peroxide. Ferroptosis suppressor protein 1 (FSP1) is a pathway that is independent of GPX4 and which involves reduction of coenzyme Q10 and inhibition of lipid radical propagation. In ALD, these pathways become dysregulated leading to an iron overload, increased lipid peroxidation, impairment of mitochondrial function and eventual hepatocellular injury.

6.7 Recent studies on NCOA4-mediated ferritinophagy and the role of ACSL4.

have expanded our understanding of the ferroptosis regulatory network in alcohol-associated liver disease (ALD), highlighting the critical roles of NCOA4-mediated ferritinophagy and ACSL4 (Acyl-CoA Synthetase Long-Chain Family Member 4). NCOA4 functions as a selective cargo receptor that delivers ferritin to lysosomes for degradation, a process known as ferritinophagy. This mechanism releases stored iron into the intracellular labile iron pool, thereby increasing iron availability for Fenton reactions and promoting lipid peroxidation and ferroptosis. Recent evidence indicates that alcohol exposure enhances NCOA4 expression and ferritinophagy, leading to hepatic iron accumulation and ferroptotic hepatocyte death. Experimental inhibition or knockdown of NCOA4 has been shown to attenuate iron overload, reduce lipid peroxidation, and protect against alcohol-induced liver injury, identifying ferritinophagy as a promising therapeutic target.[73]

Another major advancement involves ACSL4, a key enzyme that catalyzes the incorporation of polyunsaturated fatty acids (PUFAs) into membrane phospholipids. ACSL4 is now recognized as a central determinant of ferroptosis sensitivity because it generates PUFA-containing phospholipids that serve as substrates for lipid peroxidation. Increased ACSL4 expression enhances membrane susceptibility to oxidative damage and accelerates ferroptotic cell death.[74] Recent ferroptosis research has identified ACSL4 as a critical downstream effector linking iron overload, lipid remodelling, and hepatocellular injury. In ALD, alcohol-induced oxidative stress and iron dysregulation may promote ACSL4-dependent lipid peroxidation, thereby amplifying ferroptotic liver damage. Consequently, ACSL4 inhibitors and strategies that modulate lipid metabolism are being explored as potential therapeutic approaches for preventing ferroptosis-driven liver injury. Together,

these findings suggest that the NCOA4–ferritinophagy–iron axis and the ACSL4-mediated lipid peroxidation pathway constitute two interconnected regulatory hubs of ferroptosis in ALD. Targeting these pathways may offer novel opportunities to mitigate hepatic iron overload, oxidative stress, inflammation, and disease progression.[75]

To illustrate the mechanistic continuum linking iron overload to oxidative injury and ferroptotic cell death, **Figure 4** summarizes the sequence of Fe^{2+} -mediated ROS generation, lipid peroxidation, mitochondrial dysfunction, antioxidant system failure, and ferroptosis in alcohol-related liver disease.

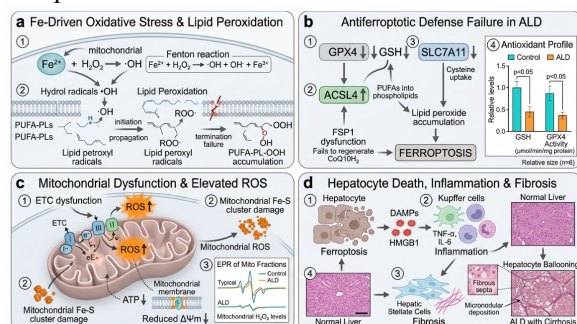


Figure 4. Iron-driven oxidative injury, lipid peroxidation, mitochondrial dysfunction, and ferroptosis in ALD

7 Clinical Implications

7.1 Diagnostic markers: ferritin, transferrin saturation, CDT, hepcidin, Iron dependent Oxidative stress.

There are several types of useful biomarkers in the diagnosis and evaluation of alcohol associated liver disease (ALD) and iron overload disorders. Serum ferritin is a measure of body ferritin stores, but is also an acute phase reactant that can rise with inflammation, liver damage, and excessive alcohol use. The ratio of serum iron to total iron-binding capacity (TIBC) is one of the most reliable indicators of iron overload, and is usually high in hereditary hemochromatosis. One specific biomarker of chronic heavy drinking is called carbohydrate-deficient transferrin (CDT), and it is used to identify and monitor excessive levels of alcohol consumption. The major regulator of systemic iron homeostasis is hepcidin, which regulates the uptake of iron in the gut and the release of iron from macrophages. Chronic alcohol intake results in a decrease in hepcidin levels, favoring iron absorption and hepatic iron deposition. These markers can be used together to help distinguish alcohol-related liver injury from genetic iron overload diseases and can help gauge the severity and progression of the disease. Under lactating conditions of ABCF1, the protein undergoes a structural change that exposes a C-terminal nuclear localization signal

(NLS2). This enables the protein to enter the nucleus. Transcriptional Activation of KDM3A: ABCF1-K430la is a transcription factor once in the nucleus. It directly interacts with the promoter region of KDM3A, thereby increasing its expression. KDM3A: A demethylase that removes H3K9me2 marks, which are generally linked to repression of transcription and chromatin compaction. KDM3A's action is to demethylate H3K9 to "open" chromatin and thus make the HIF1A gene more accessible for transcription. This results in much higher HIF1A mRNA and protein expression levels, Lactate Feedback Loop: HIF1A then upregulates the production of lactate dehydrogenase (LDHA), which allows pyruvate to be turned into lactate. This further boosts lactate levels, which in turn leads to even more lactylation of ABCF1 and continues the cycle, favoring tumor growth and metastasis.[76]

7.2 Distinguishing ALD from hereditary hemochromatosis.

It is important to distinguish alcohol-associated liver disease (ALD) from hereditary hemochromatosis (HH), as both may have elevated serum ferritin, elevated TSAT, and hepatic iron accumulation. The iron overload is typically associated with chronic alcohol consumption in ALD, which increases the absorption of iron from the gut, decreases production of hepcidin and increases deposition of iron in the liver. By comparison, HH is a genetically transmitted disorder; the most frequent cause of HH is mutations in the HFE gene which leads to an uncontrolled uptake of iron and a progressive systemic iron overload. There are a number of clinical and laboratory criteria that help differentiate between the two conditions. Patients with HH tend to have a family history of iron overload, very high ferritin saturation (HS>45-50%) and a pathological history of HFE mutations. In HH, iron deposition is mainly found in the hepatocytes, while in ALD, it is located both in hepatocytes and in the Kupffer cells; in addition, there are characteristic histological features that include steatosis, alcoholic hepatitis and fibrosis. The importance of genetic testing, iron studies, liver imaging, and histopathological examination is therefore important to help confirm the proper diagnosis and move onto the proper management pathway. Genetic variations in the HFE gene, particularly C282Y and H63D, can significantly influence the impact of alcohol consumption on iron metabolism and liver disease progression. Individuals carrying these variants often exhibit increased intestinal iron absorption and greater hepatic iron accumulation, and chronic alcohol intake can further exacerbate these effects by suppressing hepcidin expression and enhancing oxidative stress. Consequently, the coexistence of HFE mutations and excessive alcohol consumption is associated with an

elevated risk of fibrosis, cirrhosis, and hepatocellular carcinoma. In contrast, non-HFE hereditary hemochromatosis, caused by mutations in genes such as HJV, HAMP, TFR2, or SLC40A1, typically presents with earlier onset, more severe systemic iron overload, markedly elevated TSAT, and progressive iron deposition even in the absence of alcohol exposure. Alcohol-associated liver disease (ALD), also is characterized by secondary iron overload driven primarily by alcohol-induced alterations in hepcidin regulation, inflammation, and oxidative stress, with iron accumulation often being less pronounced and more variable than in hereditary forms of hemochromatosis [77]

7.3 Clinical consequences of combined alcohol abuse and iron overload

Chronic alcohol abuse and iron overload has synergistic harmful effects on liver health. Lipid peroxidation is caused by excess iron which causes the formation of ROS by Fenton reactions, and by alcohol metabolism which causes an increase in oxidative stress. These two processes compound the hepatocyte damage, inflammation and mitochondrial dysfunction. Therefore, people with both diseases are at a much greater risk for alcoholic hepatitis, progressive fibrosis, cirrhosis and liver failure. [78] In addition to liver damage, excessive alcohol and iron raises the risk of potential complications like portal hypertension, hepatic decompensation and hepatocellular carcinoma. The deposition of iron can also be responsible for extrahepatic effects, including impaired immune responses, diabetes mellitus, endocrine dysfunction, and cardiomyopathy. As a result, there is evidence that concurrent alcohol misuse and iron overload are linked to disease progression, worse clinical outcomes, and higher mortality. [79]

7.4 Alcohol/Iron-induced tumor microenvironments to vascular proliferation

The Lactate-Rich Microenvironment In the tumor microenvironment, lactate is formed during glycolysis, and is elevated in tumor cells as a result of the Warburg effect. This lactate is a substrate for post-translational modification called protein lactylation.

ASH2L Lactylation A protein called ASH2L is lactylated at the K312 residue in hepatocellular carcinoma (HCC). This process is controlled by certain enzymes: The enzyme AARS1 leads to the lactylation of ASH2L. HDAC1 is responsible for its delactylation in Eraser. However, in tumour cells, this balance is disrupted, resulting in high levels of ASH2L-K312-lac. VEGFA is epigenetically activated. ASH2L is a key component of the COMPASS complex, which regulates gene expression by modifying histones.

Lactylation of ASH2L alters its function: ASH2L-K312-lac has greater binding affinity to the genomic

region of Vascular Endothelial Growth Factor A (VEGFA). MLL Complex Recruitment: It recruits the MLL1 methyltransferase complex to the VEGFA promoter and enhancer regions. H3K4 Methylation: This complex methylates H3K4 (H3K4me1 and H3K4me3) which are epigenetic marks and are known to "open" chromatin and activate gene transcription.

Vascular Proliferation (Angiogenesis) The epigenetic activation results in much higher levels of VEGFA protein expression and secretion. Direct Endothelial Cell Stimulation: VEGFA directly stimulates the proliferation, migration and tube formation of vascular endothelial cells. ASH2L-K312-lac expression is positively associated with increased microvessel density (MVD) in tumors: Clinical data has revealed that high expression of ASH2L-K312-lac is correlated with increased microvessel density (MVD) in tumors. Tumor Progression: This massive neovascularization supplies the tumor with oxygen and nourishment, which allows it to grow rapidly and spread to other parts of the body. [80]

8. Therapeutic Considerations

8.1 Alcohol abstinence and its effects on iron parameters

Abstinence from alcohol is strongly recommended and may adversely affect the iron parameters. Alcohol abstinence is recommended and may affect iron parameters. Abstaining from alcohol makes a significant difference in iron metabolism and liver function in people who have alcohol-associated liver disease. Alcohol abuse causes a decrease in hepcidin expression, increased iron absorption from the gut and increased iron storage in the liver, with increased serum ferritin and TSAT. After stopping drinking, the regulation of hepcidin slowly resumes, which leads to a decrease in iron absorption and release of excess iron stores.[81] Treatment with sustained abstinence has been demonstrated to be associated with an improvement in liver enzyme profiles, normalization of TSAT, and a decrease in serum ferritin levels in clinical studies. When alcohol is no longer in the body, the withdrawal effects of alcohol lead to decreased oxidative stress and inflammation which also help to restore iron homeostasis. Therefore, keeping alcohol consumption to a minimum is a fundamental part of the treatment of ALD and may be helpful in preventing fibrosis, cirrhosis, and other iron-overload related complications.[82] Current prognostic scores such as the Model for End-Stage Liver Disease (MELD), Glasgow Alcoholic Hepatitis Score (GAHS), and Fibrosis-4 (FIB-4) index reflect only liver function, renal function, coagulation parameters, inflammation, and fibrosis, without considering disturbances in iron metabolism. Elevated ferritin, TSAT, and changes in transferrin levels are associated with hepatic inflammation, oxidative stress, and disease

progression, suggesting that these are potentially useful complementary prognostic markers. A possible solution would be to add ferritin or TSAT to the current algorithms used to predict prognosis in patients with chronic liver disease, especially in those with alcohol-associated liver disease (ALD) or advanced chronic liver disease. For instance, if two patients had the same MELD score, high levels of ferritin or TSAT could identify those with more inflammatory activity or iron-mediated hepatocellular damage who have a higher risk of liver decompensation, acute-on-chronic liver failure, or short-term mortality. Similarly, the inclusion of iron indices in the GAHS could help to identify those patients with severe alcoholic hepatitis who are not likely to benefit from medical therapy and who require close monitoring, early referral to tertiary liver centres or assessment for liver transplantation. Ferritin or transferrin could provide increased discrimination when added to FIB-4 in patients with chronic liver disease between those who have stable fibrosis and have a higher risk of progression to cirrhosis or liver-related complications. The clinical appeal of iron indices is their wide availability in most clinical laboratories, their low cost, and their routine measurement. From a clinical point of view, the serum ferritin, transferrin, serum iron, and TSAT all offer the advantages of being readily available, inexpensive, and routinely measured in the majority of clinical laboratories. The use of these biomarkers in the routine assessment could aid in more personalized follow-up measures. Elevated iron indices in patients with similar conventional prognosis factors may necessitate closer laboratory monitoring, earlier screening for portal hypertension and hepatocellular carcinoma, optimization of alcohol cessation interventions, nutritional assessment, and timely referral to specialists. However, patients with low-risk iron profiles and stable conventional scores might not need as rigorous surveillance. Further research is needed to develop and validate iron indices, which are incorporated with existing scoring systems, to better predict mortality. To evaluate whether iron biomarkers offer clinically useful improvements in prognostic accuracy, statistical methods must be used such as multivariable regression, machine learning algorithms, and incremental measures of predictive performance (including area under the receiver operating characteristic curve (AUROC), net reclassification improvement (NRI), and integrated discrimination improvement (IDI)). These models will need to be validated externally to ensure their application to a wide range of patient populations and liver disease causes before their use in clinical practice. Until validated, iron indices should be used as adjunctive markers to existing prognostic scores. This can enhance the identification of high-risk

patients, clinical decision-making, and develop more personalized approaches in chronic liver disease. [83]

8.2 Phlebotomy and iron chelation in ALD

Therapy for excess body iron includes phlebotomy and iron chelators, although the role of these therapies in alcohol-associated liver disease (ALD) is less well-established than in hereditary hemochromatosis. Therapeutic phlebotomy is an effective method to decrease the iron stores by bleeding at regular intervals which decreases the serum iron, hepatic iron deposition, oxidative stress and risk of iron mediated liver injury. It is especially useful for ALD patients who have a known iron overload or the presence of hereditary hemochromatosis. [84] In certain patients, unable to undergo phlebotomy due to liver disease, poor venous access and/or anemia, deferral iron chelation therapy, such as deferoxamine, deferasirox or deferiprone, may be considered. Chelators bind excess iron and facilitate its elimination which helps to reduce iron catalysed oxidative damage. There is, therefore limited evidence that supports the routine use of phlebotomy and/or iron chelation in ALD, and treatment should be personalized based on the iron status, liver function tests, and the presence of genetic and/or secondary iron overload. The most effective treatment is alcohol abstinence, and iron-reduction treatments are used as ancillary treatments in selected patients.[85]

Despite sharing common pathological traits of liver disease, alcohol-related liver disease (ALD) and hereditary hemochromatosis (HH) are two diseases with different fundamental mechanisms, including hepatic iron overload, oxidative stress, lipid peroxidation, and progressive fibrosis. The accumulation of iron is a secondary phenomenon in ALD and is in part related to the suppression of hepcidin by alcohol, enhanced intestinal iron absorption, inflammatory signaling and oxidative stress. This process is a dynamic one and partially reversible upon alcohol abstinence. HH, in contrast, is a primary iron overload disorder that is a result of mutations in HFE or other iron-regulatory genes, and results in a chronic impairment of hepcidin signaling and ongoing iron accumulation regardless of environmental factors. Both are capable of triggering both the ferroptosis and fibrogenic pathways, but the additional burden of acetaldehyde toxicity, endotoxemia from the gut and inflammatory action mediated by Kupffer cells are hallmarks of ALD. These mechanistic differences have clinical implications, as they affect the course of disease, reversibility and treatment options.

8.3 Antioxidant and anti-inflammatory strategies

Vitamin C and E. Treatment with antioxidants and anti-inflammatory drugs has been a subject of much interest as an adjunctive approach for the treatment of

alcohol-associated liver disease (ALD), especially in patients with iron overload. High levels of iron and alcohol metabolism result in the formation of ROS, which causes oxidative stress, lipid peroxidation, mitochondrial dysfunction and chronic inflammation. Several antioxidants have been studied to suppress free-radical formation, restore liver endogenous antioxidant capacity and decrease oxidative liver damage, including vitamin E, N-acetylcysteine (NAC), S-adenosylmethionine (SAMe) and polyphenolic compounds. [86,87] Anti-inflammatory strategies seek to inhibit the production of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) that lead to hepatocellular damage and fibrosis. Anti-inflammatory signaling pathways, anti-macrophage activation, and anti-ferroptosis-related therapies are emerging therapies that may provide further benefits. While a few compounds have been identified as having possible antioxidant and anti-inflammatory activity in experimental and clinical studies, abstinence from alcohol is currently the best treatment and more research is required to determine the long-term efficacy of these compounds in ALD patients with iron dysregulation. [88,89]

Ferroptosis is a new therapeutic strategy that holds promise for alcohol-induced liver disease (ALD). Ferrostatin-1 and liproxstatin-1 are both ferroptosis inhibitors that have been shown to protect in experimental models through blocking lipid peroxidation and maintaining membrane integrity. Iron chelators such as deferoxamine and deferasirox decrease labile iron pool and iron activation of the oxidative injury, but there is a lack of human experience with their use in ALD. Enhance GPX4-dependent protection against lipid peroxidation through the use of antioxidants including N-acetylcysteine (NAC), vitamin E and glutathione precursors. Besides, new molecular drugs such as hepcidin agonists, Nrf2 activators, and modulators of the system Xc-/SLC7A11 pathway are being investigated as targeted approaches to restore iron homeostasis and inhibit ferroptotic cell death. Such therapies may have great translational potential if combined with abstinence from alcohol and liver-protective conventional therapy.

9. Future Directions

9.1 Need for improved biomarkers

There exist current biomarkers for alcoholic liver disease (ALD), such as ferritin, TSAT, CDT, or hepcidin, that face the challenge of poor specificity attributable to competing inflammation, genetic heterogeneity, or comorbidities, giving rise to high (30% to 40%) false-positive results, as well as poor stratification of early stages of steatosis from advanced fibrotic lesions. [90] Sanger sequencing-derived

traditional biomarkers, such as GGT or AST/ALT ratios, lack sensitivity for recent abstinence or mild drinking patterns, whereas highly successful chronic heavy drinking biomarkers like CDT fail for acute liver damage or renal failure, while proteomic multianalyte assays (B2M, IGFBP3, or IGFBP3) for severe ALD give promising validation results (AUC >0.9), yet require larger validation studies. There remains an urgent demand for noninvasive composite multi overlapping signatures for microbiome, cytokines, or extracellular vesicles for improved early diagnosis, prognosis, and stratified treatments since liver biopsy or patient (alcohol use history) is either invasive or prone to biased validation studies. [91] Alcoholic liver disease encompasses a continuum of hepatic injury, from simple fatty change to alcoholic hepatitis, progressive fibrosis, cirrhosis, and ultimately hepatocellular carcinoma, yet its clinical assessment remains heavily dependent on routine biochemical tests with limited diagnostic and prognostic value. Traditional markers such as aminotransferases, γ -glutamyl transferase, and bilirubin are neither sufficiently sensitive nor specific, often failing to detect early disease or accurately mirror the extent of hepatic damage. [92] Their interpretation is further confounded by factors such as ongoing alcohol consumption, nutritional deficiencies, systemic inflammation, and coexisting metabolic disorders. Consequently, there is a clear unmet need for more robust biomarkers that can reflect the complex biological mechanisms driving ALD, including hepatocyte apoptosis and necrosis, inflammatory and immune responses, oxidative stress, dysregulated iron metabolism, and alterations in the gut-liver axis. [93] Novel biomarker candidates, including apoptosis-associated cytokeratin fragments, circulating non-coding RNAs, extracellular vesicle cargo, immune mediators, iron-related indices, and microbiota-derived metabolites, offer opportunities for earlier detection, improved disease stratification, and more accurate monitoring of therapeutic response. Advancing such disease-specific and mechanism-based biomarkers is critical for enabling timely intervention and moving toward more individualized management of alcoholic liver disease. [94]

9.2 Emerging insights into iron-alcohol molecular interactions

Emerging insights into iron-alcohol interactions highlight their crucial role in biological processes, particularly in Alcoholic Liver Disease (ALD), where alcohol metabolism generates reactive oxygen species (ROS) that damage liver cells, often involving iron overload and NOX4 pathways, while also influencing iron regulatory proteins like hepcidin. Mechanistically, researchers are uncovering how iron centers in enzymes (like Fe-ADHs) catalyze alcohol

reactions via specific binding (e.g., Fe-C bonds), using advanced spectroscopy (ENDOR, EXAFS) and simulations (QM/MM) to understand alcohol oxidation, hydrogen borrowing, and novel thermostable iron enzymes for industrial biocatalysis.[95] These studies reveal complex interplay affecting cell function, from enzyme catalysis to potential drug targets, impacting both disease and biotechnology. Recent advances highlight that alcohol and iron interact at multiple molecular levels to exacerbate cellular injury, especially within the liver. Ethanol metabolism increases oxidative stress and acetaldehyde formation, which destabilize intracellular iron storage proteins and expand the pool of redox-active iron. Concurrently, alcohol suppresses hepcidin signaling and alters iron transport mechanisms, promoting excessive iron uptake and hepatic retention.[96] The resulting iron surplus accelerates reactive oxygen species generation through iron-catalyzed redox reactions, intensifying lipid peroxidation, mitochondrial impairment, and genomic damage. Beyond hepatocytes, these iron-alcohol interactions influence immune and stromal cells, driving inflammatory signaling and fibrotic remodeling. Together, these emerging molecular insights underscore iron as an active mediator that magnifies alcohol-induced toxicity rather than a passive metabolic consequence.[97] The existing biomarkers for ALD, including ferritin, TSAT, CDT, and hepcidin, are prone to challenges such as a lack of specificity influenced by the confounding effects of inflammation, genetic factors, and co-existing conditions, causing a false-positive rate of 30-40% among patients.[98] Sensitivity is low regarding recent abstinence or moderate alcohol consumption for the classical biomarkers GGT or AST/ALT ratios, while CDT performs superbly in the case of chronic heavy drinkers but poorly in acute liver or renal insults; proteomic biomarker cocktails like B2M, IGFBP3, and IGFBAL demonstrate promise with AUROC > 0.9 values in the case of patients having severe ALD. There are non-invasive biomarker requirements regarding the development of omics-based signatures involving the microbiome, cytokines, and exosomes for non-invasive early patient identification, diagnosis, predictions, and personalized medicine due to the invasiveness and inaccuracies associated with alcohol patient histories.[99]

9.3 Potential therapeutic targets involving ferritin and transferrin pathways

Iron serves many fundamental biologic roles such as cell cycle progression, DNA synthesis and repair, mitochondrial function, and regulation of inflammation. However, iron has been implicated in oxidative stress by the virtue of its ability to donate free electrons to oxygen molecule and thus in

generation reactive oxygen species (ROS). Both iron and ROS have been implicated in acute kidney injury (AKI).[100] Approaches concerning iron regulation pathways in conjunction with ferritin and transferrin in alcoholic liver disease include iron overload and oxidative stress modulation in alcoholic liver disease. Suppressing ferritinophagy in NCOA4-knockdown or stabilizers maintains iron sequestration from the pathway of labile iron release and ferroptosis. Overexpression of Nrf2 activators such as sulforaphane maintains ferritin H-chain ferroxidase activity to suppress the Fenton reaction.[101] Modulating transferrin receptor 1 in siRNA or in antibodies restricts the uptake of NTBI iron within hepatocytes. Transferrin expression in the liver reverses deficiency that occurs in alcoholic liver disease conditions to suppress hepcidin feedback. The antiproliferative properties of hepcidin mimetics or "mini-hepcidins" reduce ferroportin in excess conditions of iron overload; in fact, preliminary studies confirm decreased siderosis and reduced inflammation.[102] Use of iron chelators like deferasirox provides synergistic effects in reducing NTBI iron in parallel to Tehzeeb-approved alcohol cessation. Iron is important for critical cellular functions such as in erythropoiesis, hypoxia signaling, mitochondrial function, and DNA synthesis and repair. As free iron is toxic, iron metabolism is highly regulated. In AKI, ferroptosis is a significant contributor to cell death. Ferroptosis inhibition and Hepcidin administration are promising novel strategies to prevent and treat AKI, particularly in conditions such as AKI associated with cardiac surgery where ischemia-reperfusion and heme- and non-heme iron mediated toxicity play prominent pathogenic roles.[103]

10. Limitations of the Current Review

Despite the growing body of evidence linking alcohol consumption, iron dysregulation, and liver injury, several limitations should be acknowledged. First, human studies investigating iron metabolism in alcohol-related liver disease (ALD) remain highly heterogeneous, with variations in patient populations, disease stages, alcohol consumption patterns, and diagnostic criteria. Second, nutritional status, systemic inflammation, and coexisting metabolic disorders often act as important confounding factors that influence serum ferritin, transferrin saturation (TSAT), and other iron-related biomarkers, making interpretation challenging. Third, genetic variability, particularly HFE mutations and other iron-regulatory polymorphisms, is not consistently evaluated across clinical studies, limiting precise risk stratification. Finally, much of the mechanistic understanding of ferroptosis, ferritinophagy, and iron-mediated oxidative injury in ALD is derived from preclinical

and experimental models, with limited translational validation in human subjects. These limitations highlight the need for standardized clinical studies and longitudinal investigations to better define the role of iron dysregulation in ALD progression and therapeutic response.

11. Limitations

- Heterogeneity among clinical studies limits direct comparison.
- Iron biomarkers are influenced by inflammation and nutrition.
- Genetic variability is not consistently evaluated.
- Limited human evidence for ferroptosis mechanisms.
- Heavy reliance on animal and experimental studies.
- Lack of standardized diagnostic criteria across studies.
- Limited longitudinal studies on disease progression.
- Insufficient clinical trials on iron-targeted therapies.

12. Conclusion.

The relationship between alcohol consumption and iron metabolism is an important pathogenetic and progression factor of liver damage, and ferritin and transferrin are central mediators and markers of such a relationship. Chronic alcohol consumption leads to disturbances in iron metabolism by inhibiting the production of hepcidin, promoting increased iron absorption in the intestines, and favoring iron accumulation in the liver. The high level of ferritin indicates iron storage and alcohol-induced inflammation, and abnormalities in transferrin and TSAT reveal high levels of serum iron available for oxidative reactions.

The additive effects of alcohol-induced oxyradical stress and iron-stimulated free radical production contribute synergistically to hepatocellular damage, inflammation, fibrosis, and the risk of cirrhosis and hepatocellular carcinoma. The molecular and clinical linkage between alcohol and iron, especially in terms of ferritin and transferrin, indicates that early diagnosis, strict alcohol abstinence, and iron-directed treatment are critical for affected individuals. Studies on improved biomarkers and/or therapies for such conditions could offer much better treatment to patients with iron and alcohol-related liver injuries. The interplay between alcohol and iron represents a critical pathogenic axis in liver disease, with ferritin and transferrin serving as key molecular indicators of this interaction. Chronic alcohol exposure disrupts iron regulatory pathways, leading to elevated ferritin levels that reflect both iron accumulation and inflammatory

stress, while altered transferrin dynamics contribute to increased iron delivery to hepatocytes. These changes enhance the pool of redox-active iron, intensifying oxidative damage, inflammatory signaling, and fibrogenesis within the liver. Recognition of the dual role of ferritin and transferrin as biomarkers and mediators of disease progression underscores the importance of integrating iron metabolism into the clinical assessment and management of alcohol-related liver disorders. In conclusion, the interplay between alcohol consumption and iron metabolism plays a pivotal role in the pathogenesis and progression of liver disease, with ferritin and transferrin serving as key indicators of this dysregulation. Chronic alcohol intake enhances intestinal iron absorption, impairs hepatic iron handling, and promotes oxidative stress, leading to elevated serum ferritin levels that reflect both iron overload and alcohol-induced inflammation. Concurrently, alterations in transferrin synthesis and saturation compromise iron transport and regulatory balance, further exacerbating hepatocellular injury. The combined disturbance of ferritin and transferrin homeostasis not only amplifies liver damage through iron-driven reactive oxygen species generation but also complicates disease assessment, as these markers are influenced by both iron burden and inflammatory status. Iron indices have the potential to be easily acquired and inexpensive adjunctive markers that can improve the current prognosis for chronic liver disease. They could be combined with existing scores, such as MELD, GAHS and FIB-4, to enhance risk stratification and help guide more individualised clinical management. But prospective studies and external validation are needed to establish their incremental prognostic value before they are put into routine clinical use. Meanwhile, the use of iron indices should be used as an adjunct to existing prognostic scoring systems. A nuanced interpretation of ferritin and transferrin parameters, integrated with clinical and molecular insights, is therefore essential for improving disease stratification, guiding therapeutic decisions, and advancing personalized approaches in alcohol-related liver disease.

12. List of abbreviations:

Abbreviation	Full Form
ADP	Adenosine Diphosphate
AKI	Acute Kidney Injury
ALD	Alcohol-Associated Liver Disease / Alcohol-Related Liver Disease
ALT	Alanine Aminotransferase

AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
AUROC	Area Under the Receiver Operating Characteristic Curve
B2M	Beta-2 Microglobulin
BMP6	Bone Morphogenetic Protein 6
CDT	Carbohydrate-Deficient Transferrin
C/EBP α	CCAAT/Enhancer-Binding Protein Alpha
DMT1	Divalent Metal Transporter 1
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
EGTA	Ethylene Glycol Tetraacetic Acid
FDA	Food and Drug Administration
Fe ²⁺	Ferrous Iron
Fe ³⁺	Ferric Iron
FSP1	Ferroptosis Suppressor Protein 1
GPX4	Glutathione Peroxidase 4
GSH	Glutathione
HAMP	Hepcidin Antimicrobial Peptide Gene
HCC	Hepatocellular Carcinoma
HFE	Hemochromatosis Gene
IL-1 β	Interleukin-1 Beta
IL-6	Interleukin-6
IRP	Iron Regulatory Protein

IRE	Iron Responsive Element
JAK	Janus Kinase
LPO	Lipid Peroxidation
LPS	Lipopolysaccharide
NAC	N-Acetylcysteine
NCOA4	Nuclear Receptor Coactivator 4
NF- κ B	Nuclear Factor Kappa B
Nrf2	Nuclear Factor Erythroid 2-Related Factor 2
NTBI	Non-Transferrin-Bound Iron
PUFA	Polyunsaturated Fatty Acid
ROS	Reactive Oxygen Species
SAMe	S-Adenosylmethionine
SMAD	Sma and Mad-Related Proteins
STAT3	Signal Transducer and Activator of Transcription 3
TFR1	Transferrin Receptor 1
TFR2	Transferrin Receptor 2
TNF- α	Tumor Necrosis Factor Alpha
TSAT	Transferrin Saturation

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14 Declaration:

I hereby declare that the article titled "Interrelationships of Alcohol and Iron in Liver Disease with Particular Reference to Ferritin and Transferrin" is my original work and has been written by me & my Team. I confirm that this article has not been published elsewhere and is not currently under consideration for publication in any other journal, magazine, or platform.

15 Author Contribution:

Author 1: Literature review

Author 2: Data analysis, interpretation of results, and critical revision of the manuscript.

Author 3: Conceptualization, methodology, data collection, and initial drafting of the manuscript.

16 Ethics Approval

Ethical approval was not required for this study because it is a review of previously published literature and did not involve the collection of primary data from human participants or the use of identifiable personal information.

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