Review

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Serum hepcidin levels in chronic liver disease: a systematic review and meta-analysis

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Abstract

Objectives: Dysregulation of hepcidin-iron axis is presumed to account for abnormal iron status in patients with chronic liver disease (CLD). Our aim is to determine the effect of specific etiologies of CLD and of cirrhosis on serum hepcidin levels.
Methods: PubMed, Embase, Web of Science were searched for studies comparing serum hepcidin levels in patients with CLD to that in controls using enzyme-linked immunosorbent assay. The study was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Guidelines. Statistical analysis was carried out with STATA using random effects model to calculate the mean difference (MD) between two groups.
Results: Hepcidin levels were significantly lower in subjects with hepatitis C virus (16 studies) [MD −1.6 (95 % CI: −2.66 to −0.54), p<0.01] and alcoholic liver disease (3 studies) [MD −0.84 (95 % CI: −1.6 to −0.07), p=0.03] than controls. Serum hepcidin was significantly higher in subjects with non-alcoholic fatty liver disease (12 studies) [MD 0.62 (95 % CI: 0.21 to 1.03), p<0.01], but did not differ in subjects with hepatitis B and controls (eight studies) [MD −0.65 (95 % CI: −1.47 to 0.16), p=0.12]. Hepcidin levels were significantly lower in patients with cirrhosis of any etiology (four studies) [MD −1.02 (CI: −1.59 to −0.45), p<0.01] vs. controls (CI: confidence interval).
Conclusions: Serum hepcidin levels are altered in common forms of CLD albeit not in a consistent direction. Additional study is needed to determine how changes in hepcidin levels are related to dysregulation of iron metabolism in CLD.

Keywords: alcoholic liver disease; cirrhosis; enzyme-linked immunosorbent assay; iron; non-alcoholic fatty liver disease; viral hepatitis

Introduction

Hepcidin is a peptide hormone synthesized primarily in the liver. It was discovered simultaneously as a regulator of iron homeostasis in the body and an antimicrobial peptide that is excreted in the urine [1, 2]. Its discovery established a central role for the liver in the control of iron metabolism. Hepcidin exerts its regulatory effect by controlling the release of iron from macrophages, enterocytes, and hepatocytes. It does so by means of its interaction with ferroportin, a transmembrane protein that is the only known cellular iron exporter in the body. A number of mechanisms account for hepcidin's effects on iron transit, including its ability to cause internalization and degradation of ferroportin, as well as binding to and occluding the central cavity of ferroportin, thereby preventing iron export [3, 4]. Elevated iron levels, blood transfusions, and iron supplements [5, 6] all stimulate increased hepcidin production, reducing levels of ferroportin, leading to sequestration of iron derived from erythrophagocytosis in macrophages and reduced transfer of dietary iron into the plasma [7]. Patients with TMPRSS6 variants exhibit elevated hepcidin levels [8]. Under conditions of high iron demand such as anemia or expanded erythropoiesis or erythropoiesis stimulating agents, or in patients with hemochromatosis, the opposite occurs: hepcidin levels decrease, ferroportin is stabilized and release of iron from macrophages and enterocytes to the systemic circulation is enhanced (Figure 1) [9, 10].

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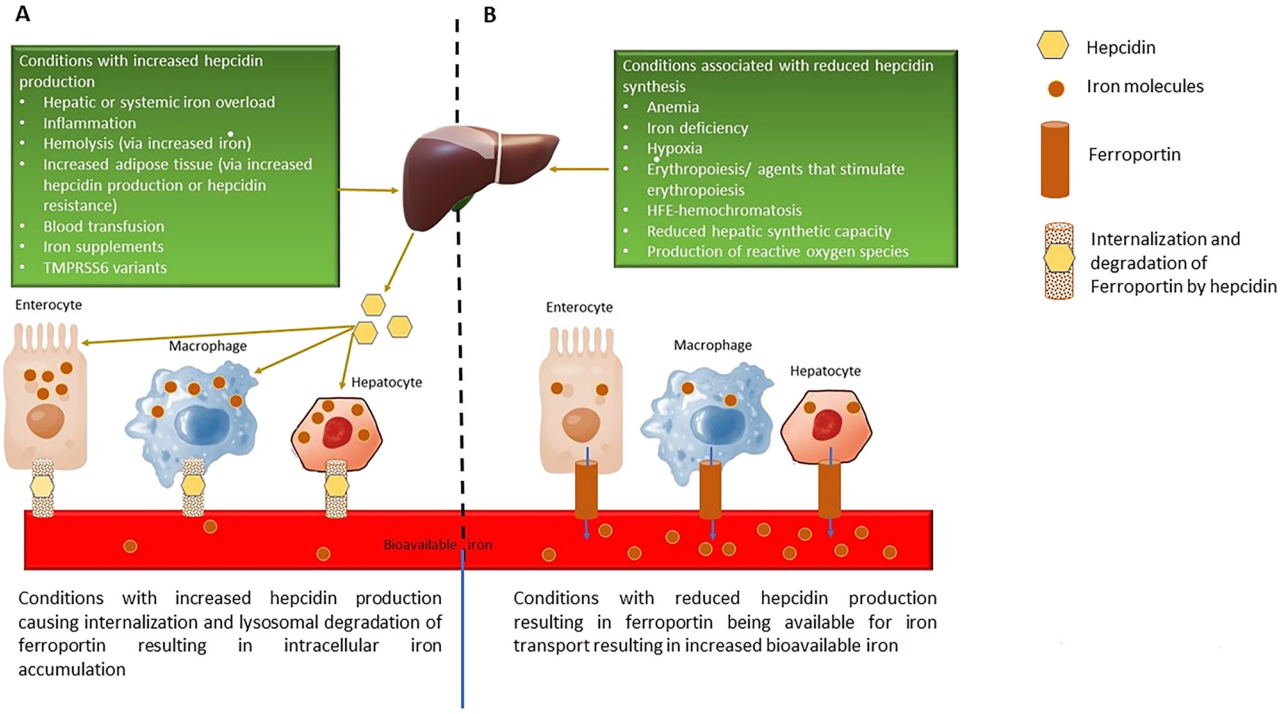

Figure 1: Overview of role of hepcidin in maintaining iron homeostasis. (A) Conditions with increased hepcidin resulting in degradation of ferroportin and intra-cellular sequestration of iron. (B) Conditions with reduced hepcidin resulting increased bioavailable iron.

Hepcidin is also a type II acute phase reactant. Consistent with this role, hepcidin gene expression is transcriptionally activated by inflammatory cytokines, the most important of which is interleukin-6 [11]. This results in functional iron deficiency despite adequate body iron stores. Hepcidin is thus the key mediator of anemia of inflammation. In addition to iron status and inflammation, factors such as hypoxia, increased levels of reactive oxygen species (ROS), and endoplasmic reticulum stress have been shown to modulate hepcidin expression in animal and cell culture models [12–15]. Some of these factors are stimulatory while others are inhibitory, and the interplay between them is complex [16, 17]. In the context of a chronic pathological process, there may be multiple, opposing inputs with the potential to modulate hepcidin levels, rendering the ultimate outcome in terms of iron metabolism difficult to predict.

Dysregulation of iron metabolism in chronic liver disease (CLD) has been a subject of intense interest over the past several decades [12]. Abnormal iron status has been linked to accelerated progression of liver fibrosis [18, 19], increased risk of hepatocellular carcinoma (HCC) [20, 21] and was associated with decreased responsiveness to hepatitis C virus (HCV) treatment in the interferon era [22]. Numerous trials of iron reduction reported decreased aminotransferase levels in HCV and non-alcoholic fatty liver disease (NAFLD) [23–27]. A long-term trial of iron reduction in patients with cirrhosis due to HCV was associated with a lowered risk of HCC [28], while a meta-analysis of phlebotomy trials in HCV concluded that this intervention improved responses to interferon treatment [29]. These observations indicate that modulation of iron status may alter the course of CLD, supporting the hypothesis that there is a feedback loop between CLD and dysregulation of iron metabolism, with the latter driving the progression of CLD to cirrhosis and HCC. However, claims that hepcidin levels are affected by CLD remain unconfirmed, as studies that have compared serum hepcidin levels in patients with CLD to controls are fraught with discrepancies.

In an attempt to resolve these conflicting results, we carried out a meta-analysis and systematic review of studies comparing serum hepcidin levels in patients with CLD due to various etiologies including HCV, hepatitis B virus (HBV), NAFLD, and alcoholic liver disease (ALD) to healthy controls. In addition, we analyzed whether the presence of cirrhosis, irrespective of underlying etiology, is associated with altered serum hepcidin levels.

Materials and methods

Literature search

Search strategies were developed by the authors with the assistance of a health sciences librarian with expertise in systematic reviews. Comprehensive strategies, including both index and keyword methods were devised for the following databases: PubMed, Web of Science, and Embase, which were searched from inception to April 2022 using the

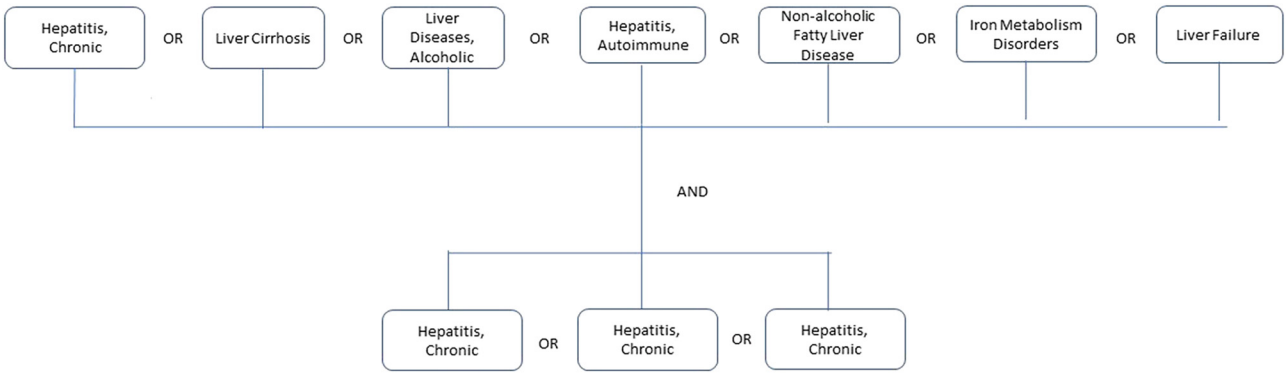

Figure 2: Database search strategies.

terms "Hepatitis, Autoimmune" or "Non-alcoholic Fatty Liver Disease" or "Hepatitis, Chronic" or "Liver Diseases, Alcoholic" or "Liver Failure" or "Iron Metabolism Disorders" or "Liver Cirrhosis" and "Hepcidins" or "Antimicrobial Cationic Peptides" or "HAMP protein, human" (Figure 2). The full PubMed search strategy was adapted for the other databases. In addition, for included studies, a citing paper and reference list search was conducted using SCOPUS to capture any relevant records.

Study selection and data extraction

Studies that compared serum hepcidin levels in patients with CLD due to etiologies other than hemochromatosis to those in healthy controls were included. For consistency, we included only studies which used enzyme-linked immunosorbent assay (ELISA) methodology to measure serum hepcidin levels. We also restricted our meta-analysis to studies that reported serum hepcidin values as nanogram/deciliter or units that could be converted to nanogram/deciliter (Figure 2). Most of the studies did not specify the form of hepcidin measured, i.e., bioactive form, hepcidin-25, or total serum hepcidin. We therefore included all studies that otherwise met our selection criteria. We excluded studies that used methods other than ELISA to measure serum hepcidin levels [30], studies of patients on hemodialysis, animal studies, studies with incomplete data points, non-English language articles, abstracts, review articles, letters to the editor, comments, and opinion papers (Figure 2). The study was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines.

R.S. and Y.Z. independently reviewed the database and agreed on final articles to be included in the meta-analysis. Data were extracted using a standardized template and included the number of patients/controls and serum hepcidin values. Conflicts regarding study selection or data extraction were resolved by mutual discussion and final database was approved by both authors. Data were expressed as mean and standard deviations after being converted if necessary. In addition, study year, patient characteristics, and inclusion/exclusion criteria for individual studies were recorded.

Statistical analysis

Meta-analysis was performed to determine the pooled weighted mean difference of serum hepcidin in patients with CLD vs. controls. Meta-analysis was carried out and forest plots were generated using Stata Corp. Stat version 17 (College Station, TX) using the random effects model. p-Value<0.05 was considered statistically significant. Heterogeneity was calculated by means of a χ^2 test (Cochran Q statistic) and quantified with the I^2 statistic. If I^2>50 %, then there was significant heterogeneity. Funnel plots were obtained to assess the risk of bias. Sensitivity analysis was done using the leave-one-out method on Stata.

Results

After removing duplicates, a total of 2,467 records were identified by the search strategy. Thirty-four studies met inclusion criteria comprising a total of 3,839 subjects which included 2,221 patients and 1,618 controls (Figure 3).

Hepatitis C virus

Meta-analysis of 16 studies (1,322 subjects) concerning patients with HCV showed that serum hepcidin levels were significantly lower in HCV compared with controls with a mean difference of −1.6 (95 % CI: −2.66 to −0.54), p<0.01; I^2=98.51 % (Figure 4) [31–46] (CI: confidence interval).

Alcoholic liver disease

Meta-analysis of three studies (150 subjects) for ALD showed significantly lower serum hepcidin levels in patients with ALD compared to controls with a mean difference of −0.84 (95 % CI: −1.6 to −0.07), p=0.03; I^2=76.87 % (Figure 5) [47–49].

Non-alcoholic fatty liver disease

A total of 12 studies (1,875 subjects) were found for NAFLD and meta-analysis showed higher serum hepcidin levels in patients compared to controls with a mean difference of 0.62 (0.21–1.03), p<0.01; I^2=92.44 % (Figure 6) [35, 38, 50–59].

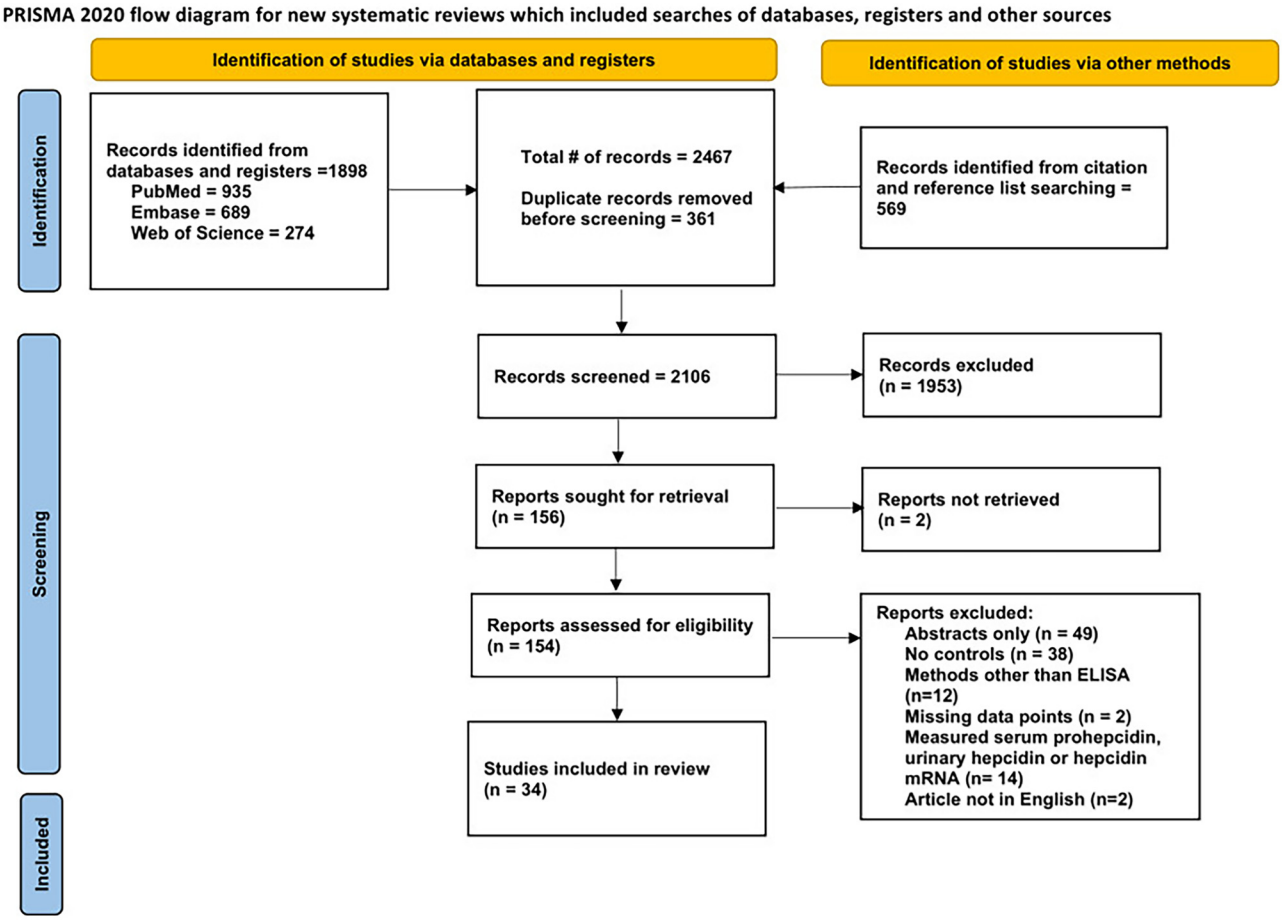

Figure 3: Prisma flow diagram for selection of studies.

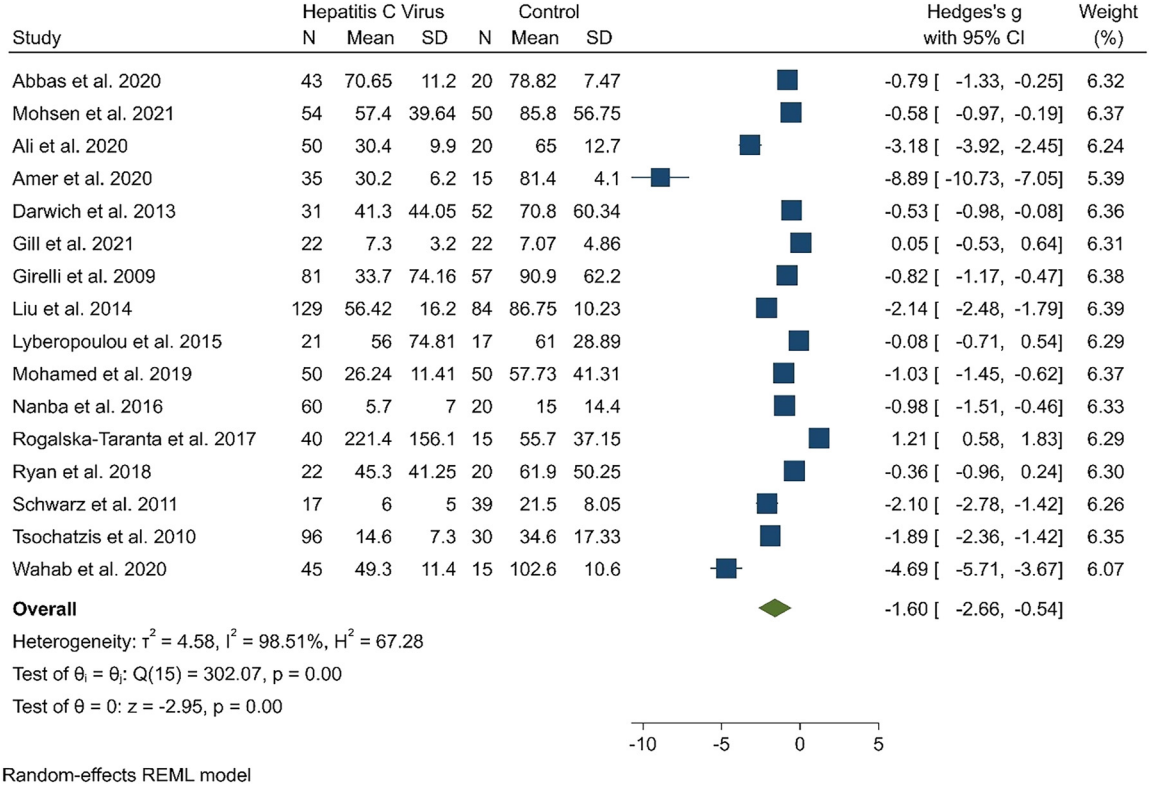

Figure 4: Meta-analysis of studies comparing serum hepcidin levels in patients with hepatitis C virus to controls.

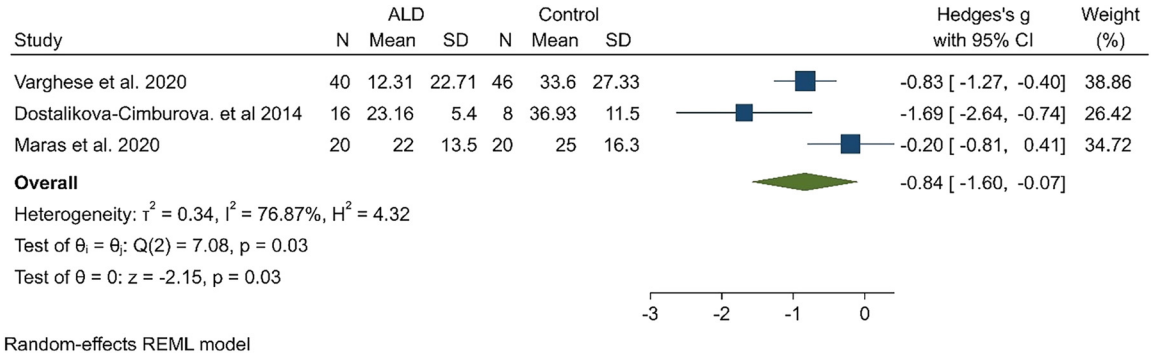

Figure 5: Meta-analysis of studies comparing serum hepcidin levels in patients with alcoholic liver disease to controls.

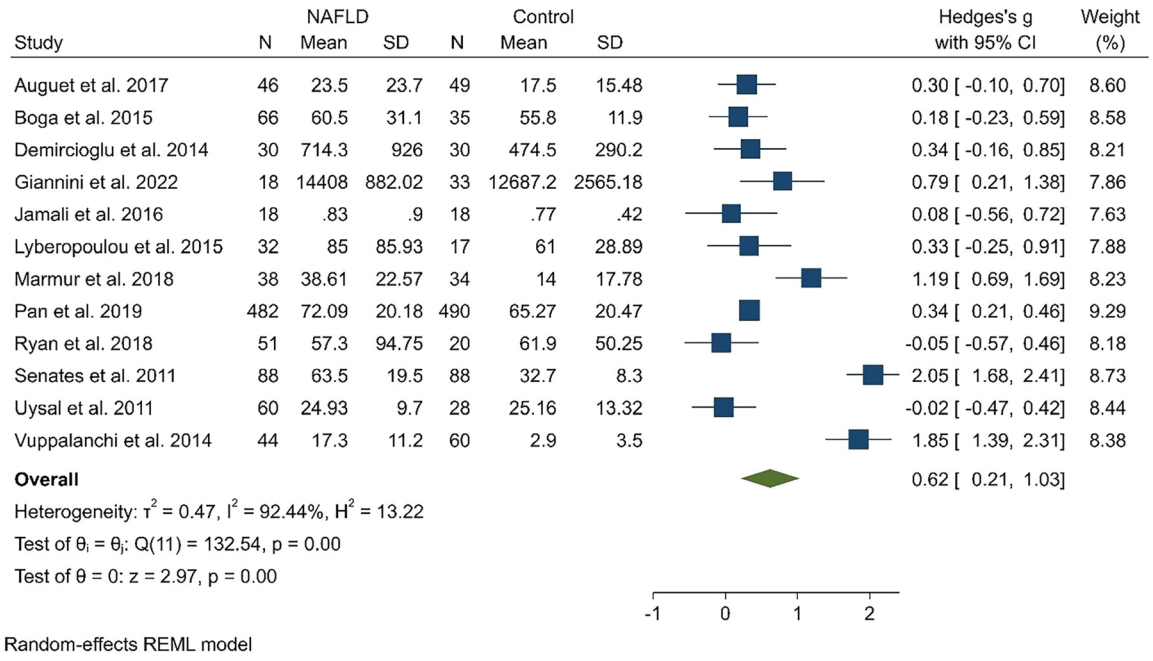

Figure 6: Meta-analysis of studies comparing serum hepcidin levels in patients with non-alcoholic fatty liver disease to controls.

Hepatitis B virus

For HBV, meta-analysis of eight studies (626 subjects) revealed that there was no difference in serum hepcidin levels between patients and controls with a mean difference of −0.65 (95 % CI: −1.47 to 0.16), p=0.12; I^2=94.1 % (Figure 7) [35, 38, 48, 60–64].

Cirrhosis irrespective of underlying etiology

We found four studies (287 subjects) that compared hepcidin levels in patients with cirrhosis to levels in controls. Three of these studies included patients with cirrhosis due to HBV and one with cirrhosis due to ALD. Serum hepcidin levels were significantly reduced in patients with cirrhosis compared to controls, mean difference of −1.02 (95 % CI: −1.59 to −0.45), p<0.01, I^2=76.45 % (Figure 8) [49, 60–62].

The results are summarized in Table 1. Data for study characteristics and quality assessment of studies are presented in Tables 2 and 3, respectively. Funnel plots were obtained to assess the risk of publication bias (Supplementary Figure 1). Sensitivity analysis confirmed the findings for all groups except for ALD, where exclusion of certain studies from the meta-analysis resulted in no significant difference in serum hepcidin levels between patients and healthy controls (Supplementary Figure 2).

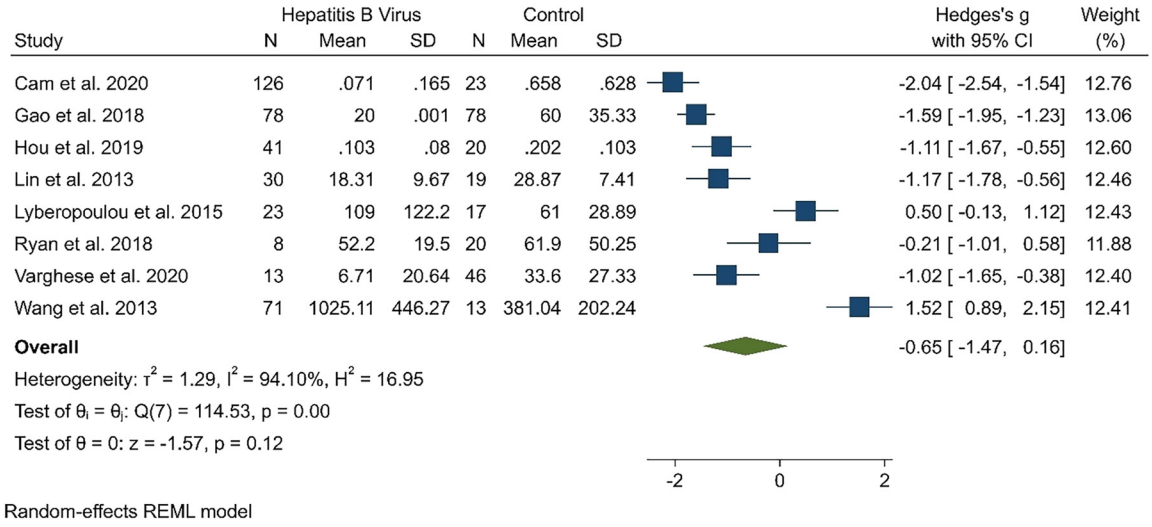

Figure 7: Meta-analysis of studies comparing serum hepcidin levels in patients with hepatitis B virus to controls.

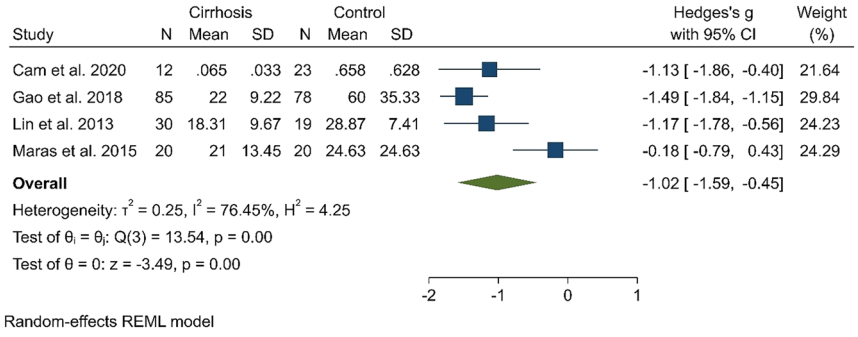

Figure 8: Meta-analysis of studies comparing serum hepcidin levels in patients with cirrhosis of any etiology to controls.

Table 1: Summary of results comparing serum hepcidin level in patients with liver disease with controls.

Comparison	Number of studies	Mean difference	Confidence interval	p-Value	Heterogeneity
Hepatitis C virus vs. controls	16	−1.6	−2.66 to −0.54	<0.01	98.5 %
Alcoholic liver disease vs. controls	3	−0.84	−1.6 to −0.07	0.03	76.87 %
Non-alcoholic fatty liver disease vs. controls	12	0.62	0.21 to 1.03	<0.01	92.44 %
Hepatitis B virus vs. controls	8	−0.65	−1.47 to 0.16	0.12	94.1 %
Cirrhosis from any etiology vs. controls	4	−1.02	−1.59 to −0.45	<0.01	76.45 %

Table 2: Overall characteristics of studies included for meta-analysis.

Study	Year	Country	Subgroup	n		%Female		Age, years	
				Study	Control	Study	Control	Study	Control
Abbas et al. [31]	2020	Egypt	HCV	20	20	55.00	35	48.45	49.4
Mohsen et al. [41]	2021	Iraq	HCV	54	50				
Ali et al. [42]	2020	Egypt	HCV	50	20	40			
Amer et al. [43]	2020	Egypt	HCV	35	15			41–76	33–63
Auguet et al. [50]	2017	Spain	NAFLD	46	49	100	100	48.3	43.9
Boga et al. [51]	2015	Turkey	NAFLD	66	35	65.2	57.1	44.4	43
Cam et al. [62]	2020	Turkey	HBV, cirrhosis	126	23	35.7	47.8	39	33
Darwich et al. [32]	2013	Spain	HCV	31	52	32.2	36.4	56.3	49.8
Demircioglu et al. [58]	2014	Turkey	NAFLD	30	30	36.7	56.7	12.1	12.7

Table 2: (continued)

Study	Year	Country	Subgroup	n		%Female		Age, years	
				Study	Control	Study	Control	Study	Control
Dostalikova-Cimburova et al. [47]	2014	Czech Republic	ALD	16	8			57.38	57.4
Gao et al. [60]	2018	China	HBV, cirrhosis	163	78	32.1	38.5	46	44
Giannini et al. [59]	2022	Italy	NAFLD	18	33	55.6	36.4	9.7	6.3
Gill et al. [44]	2021	Pakistan	HCV	22	22	0	0	50.35	50.35
Girelli et al. [33]	2009	Italy	HCV	81	57	35.8	36.8	42.2	35
Hou et al. [63]	2019	China	HBV	41	20	22	50	36.83	35.7
Jamali et al. [52]	2016	Iran	NAFLD, cirrhosis	18	18	27.8	27.8	34.5	30.44
Lin et al. [61]	2013	China	HBV, cirrhosis	30	19	5	10.5	50.37	49.74
Liu et al. [34]	2014	China	HCV	129	84				
Lyberopoulou et al. [35]	2015	Greece	HCV, HBV, NAFLD	21	17	52.4	41.2	40.6	45.2
Maras et al. [49]	2015	India	Cirrhosis	20	20	25	20	34	33
Marmur et al. [53]	2018	Sweden	NAFLD	38	34	34.2	55.9	54.42	40
Mohamed et al. [36]	2019	Egypt	HCV	50	50	52	54	10	9.5
Nanba et al. [45]	2016	Japan	HCV	60	20	51.7		57	
Pan et al. [54]	2019	China	NAFLD	482	490	32	32.4	48	48
Rogalska-Taranta et al. [37]	2017	Poland	HCV	19	15	21.1	46.7	44	40
Ryan et al. [38]	2018	United Kingdom	HCV, HBV	81	20	30.9	60	50–55	58
Schwarz et al. [39]	2011	Germany	HCV	17	39	0	43.6	38	36
Senates et al. [55]	2011	Turkey	NAFLD	88	88	36.4	21.8	44	43
Tsochatzis et al. [40]	2010	Greece	HCV	96	30	54.2	56.7	44	44
Uysal et al. [56]	2011	Turkey	NAFLD	60	28	33.3	64.3	47.7	48.1
Varghese et al. [48]	2020	India	HBV, ALD, cirrhosis	53	46	0	0	45.5–47.4	46.6
Vuppalanchi et al. [57]	2014	United States of America	NAFLD, cirrhosis	44	60	88.6	23.3	47	51
Wahab et al. [46]	2020	Egypt	HCV	45	15	53.3	46.7	40.7	36.2
Wang et al. [64]	2013	China	HBV	71	13	38	38.5	40.7	36.3

HCV, hepatitis C virus; ALD, alcoholic liver disease; NAFLD, non-alcoholic fatty liver disease; HBV, hepatitis B virus.

Table 3: The Newcastle-Ottawa scale is used to assess the quality of case control studies. The scale assigns up to four points for comparability, two points for selection, and three points for outcome.

Study	Type of study	Blinding	Selection	Comparability	Outcome	Overall quality score
Abbas et al. [31]	Case control	N/A	3	2	3	8
Mohsen et al. [41]	Case control	N/A	3		3	6
Ali et al. [42]	Case control	N/A	3	1	3	7
Amer et al. [43]	Case control	N/A	2		3	5
Auguet et al. [50]	Case control	N/A	3	1	3	7
Boga et al. [51]	Case control	N/A	3	1	3	7
Cam et al. [62]	Case control	N/A	3	1	3	7
Darwich et al. [32]	Case control	N/A	3	1	3	7
Demircioglu et al. [58]	Case control	N/A	3	1	3	7
Dostalikova-Cimburova et al. [47]	Case control	N/A	3	1	3	7
Gao et al. [60]	Case control	N/A	3	1	3	7
Giannini et al. [59]	Case control	N/A	3	1	3	7
Gill et al. [44]	Case control	N/A	3	1	3	7
Girelli et al. [33]	Case control	N/A	3	1	3	7
Hou et al. [63]	Case control	N/A	3	1	3	7
Jamali et al. [52]	Case control	N/A	3	1	3	7
Lin et al. [61]	Case control	N/A	3	2	3	8
Liu et al. [34]	Case control	N/A	3	1	3	7
Lyberopoulou et al. [35]	Case control	N/A	3	1	3	7
Maras et al. [49]	Case control	N/A	3	1	3	7
Marmur et al. [53]	Case control	N/A	3	1	3	7

Table 3: (continued)

Study	Type of study	Blinding	Selection	Comparability	Outcome	Overall quality score
Mohamed et al. [36]	Case control	N/A	3	1	3	7
Nanba et al. [45]	Case control	N/A	3		3	6
Pan et al. [54]	Case control	N/A	3	2	3	8
Rogalska-Taranta et al. [37]	Case control	N/A	3	1	3	7
Ryan et al. [38]	Case control	N/A	3	1	3	7
Schwarz et al. [39]	Case control	N/A	2		3	5
Senates et al. [55]	Case control	N/A	3	1	3	7
Tsochatzis et al. [40]	Case control	N/A	3	1	3	7
Uysal et al. [56]	Case control	N/A	3	1	3	7
Varghese et al. [48]	Case control	N/A	3	1	3	7
Vuppalanchi et al. [57]	Case control	N/A	3	1	3	7
Wahab et al. [46]	Case control	N/A	3	1	3	7
Wang et al. [64]	Case control	N/A	3	1	3	7

We did not perform a meta-analysis of the two studies that compared hepcidin levels in patients with cirrhosis to patients with liver disease without cirrhosis due to the small number of studies.

Similarly, we did not carry out a meta-analysis on studies of patients with autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) as we found only two studies for patients with these conditions that met our inclusion criteria. Lyberopoulou et al. reported low serum hepcidin levels compared with controls for patients with AIH and PBC/PSC [35]. Radicheva et al. reported combined results for patients with AIH and PBC/PSC and found that serum hepcidin levels in patients were not significantly different from that in controls [65].

Discussion

Some patients with CLD demonstrate evidence of aberrant iron metabolism, which has been implicated in fibrosis progression and increased risk of HCC [18, 22, 66, 67]. Given the primacy of hepcidin in the control of iron metabolism, it is reasonable to hypothesize that the fundamental cause of altered iron metabolism in CLD is dysregulation of hepcidin expression. Many investigations have addressed this question by comparing hepcidin levels in patients with CLD to that in controls. Because of the considerable variability in the results, we performed a meta-analysis in an attempt to bring clarity to this topic.

Our results show that serum hepcidin levels are significantly lower in subjects with HCV compared to controls. This finding is consistent with research in cell culture and in transgenic mice demonstrating that hepcidin gene expression is suppressed by HCV [68, 69]. These approaches implicated virus-mediated increases in ROS production as the main factor driving reductions in hepcidin, with differing downstream mechanisms proposed to account for the downregulation of hepcidin expression by ROS, i.e., increased histone deacetylase activity in one model, and decreased DNA-binding activity of CCAT/enhancer binding proteinα (C/EBPα, the main transcription factor controlling hepcidin gene expression) in another [68, 69]. The concept that abnormalities of serum iron studies in HCV-infected individuals arise as a consequence of viral effects on hepcidin expression is consistent with the finding that those abnormalities normalize promptly with eradication of HCV infection [70].

Our meta-analysis found that serum hepcidin is also significantly reduced in patients with ALD, though sensitivity analysis did not confirm this finding. This result is broadly consistent with studies showing that ethanol downregulates hepcidin expression in rodent models [71]. Similar to HCV, ethanol appears to modulate hepcidin expression via increased levels of ROS that constrain the transcriptional activity of C/EBPα [72].

By contrast, our results indicate that serum hepcidin levels in subjects with NAFLD are elevated compared to controls. The tendency for patients with NAFLD to have relatively higher levels of serum hepcidin than other forms of CLD can perhaps be understood in the context of the multiple factors involved in the pathogenesis of NAFLD. Obesity itself is associated with increased hepcidin levels, perhaps augmented by small amounts of hepcidin originating from adipose tissue [73], and the presence of metabolic syndrome and its components engender a chronic inflammatory state, further stimulating hepcidin expression. In contrast to these results, animal models of fatty liver have generally shown downregulation of hepcidin, even in models that are characterized by insulin resistance and

inflammation, apparently due to lowered hepatic iron stores resulting from increased iron utilization or impairment of intestinal iron uptake, depending on the model [71]. However, with few exceptions, these experiments examined hepcidin mRNA, not serum hepcidin levels.

The findings above suggest that some forms of CLD affect hepcidin levels independent of the presence of cirrhosis and impaired hepatic synthetic function. The levels of many proteins synthesized by the liver are diminished in advanced liver disease, so it is reasonable to predict that the same might be true of hepcidin. Our finding of a significant difference between serum hepcidin levels in patients with cirrhosis and controls supports this prediction, but there are reasons to interpret these results cautiously. Three of the four studies examined only patients with cirrhosis due to HBV. Since we did not find a significant difference in serum hepcidin levels between subjects with HBV and controls, this would suggest that HBV patients develop low serum hepcidin levels only after the disease has progressed to cirrhosis. However, two of the studies found no statistically significant difference in serum hepcidin levels in HBV patients with or without cirrhosis [60, 62]. This raises concern that the results may be spurious, i.e., resulting from low study numbers or skewed data. Unfortunately, we did not find any studies on serum hepcidin levels in cirrhosis due to other etiologies, hence we are unable to comment on the effect of cirrhosis on serum hepcidin levels in general.

In extrapolating our results to predicted downstream effects on iron metabolism, it is important to recognize that much of our current knowledge concerning the regulation of hepcidin is based on measurements of hepcidin transcripts derived from animal models or cell culture. There has been relatively little investigation into the existence of post-transcriptional mechanisms that might modulate the abundance or activity of secreted hepcidin. However, there are reports of inconsistencies between hepatic mRNA levels, levels of hepcidin in the circulation or urine, and markers of iron status, suggesting that such mechanisms may exist. For example, in a study of patients with dysmetabolic iron overload, Barisani et al. found no correlation between urinary hepcidin measurements and hepcidin mRNA levels and also noted that hepatic iron concentrations were more strongly correlated with urinary hepcidin levels than with hepatic hepcidin transcripts [74]. More recently, Rametta and colleagues provided evidence for a state of mild hepcidin resistance in patients with this condition, based on comparisons of serum hepcidin concentrations and changes in transferrin saturation following a dose of oral iron [75]. These data suggest that a model of iron homeostasis that presumes the existence of a one-to-one correspondence between the abundance of hepatic hepcidin transcripts and the quantity and biological activity of the resulting peptide, generating predictable effects on iron absorption (and ultimately body iron stores) may be overly simplistic.

A few comments are needed regarding the very high levels of heterogeneity observed in our analysis. Several authors have emphasized the importance of understanding the sources of heterogeneity in meta-analyses [76, 77]. Some portion of the heterogeneity may be the result of clinical heterogeneity due to differences in the baseline characteristics of the patients included in some of these analyses. For example, some studies included patients with cirrhosis, some without, while others included a mix of both. Hepcidin levels are also known to be affected by fasting and circadian rhythms [78], and may be modulated by factors specific to liver disease, such as the proportion of patients with advanced liver disease in a given study population.

Another important source of heterogeneity may be the methodology by which hepcidin was quantitated. In order to facilitate comparisons, we limited our analysis to studies that used ELISA-based assays. However, not all of the studies used the same ELISA. In many cases, a commercially sourced assay was chosen, while others were developed in-house. This is noteworthy because the generation of antibodies to hepcidin poses particular challenges due to its small size, conserved amino acid sequence and intramolecular disulfide bonds [78]. Depending on the antibodies used in an ELISA, a given assay may measure not only the biologically active 25-amino acid peptide, but also the 22- and 20-amino acid isoforms of hepcidin, whose functions, if any, are unknown [79]. A large number of studies did not specify the form of hepcidin measured by their assay. Another concern is the fact that the majority of hepcidin in the circulation is bound to α2-macroglobulin; the extent to which the various ELISAs measure total vs. bound vs. unbound hepcidin is unclear (and the biological significance of the binding to α2-macroglobulin remains undefined) [80]. These factors may explain the fact that hepcidin levels (in both patients and controls) differed by as much as three orders of magnitude across the various studies. The technical challenges involved in hepcidin assays have been highlighted by an international group of experts, who reported that measurements of hepcidin performed on a panel of standard samples varied widely between methods, including different immunochemical methods [79]. Hence, methodological differences likely also contributed to the heterogeneity we observed and are a limitation of our study, despite the presence of healthy controls. The subjects also belonged to different demographics hence factors such as nutritional habits may influence serum hepcidin levels.

In conclusion, our meta-analysis indicates that hepcidin levels are altered in some common forms of CLD. However, the direction of the alteration is inconsistent, with decreased

hepcidin levels observed in HCV and ALD and increased levels in NAFLD. Since disturbances in iron metabolism are frequently observed in patients with these conditions, further study is needed to establish how changes in hepcidin levels are related to dysregulation of iron metabolism in these forms of CLD. Studies using rigorously validated assays for hepcidin in well-characterized groups of patients with and without CLD will be essential to this effort.

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