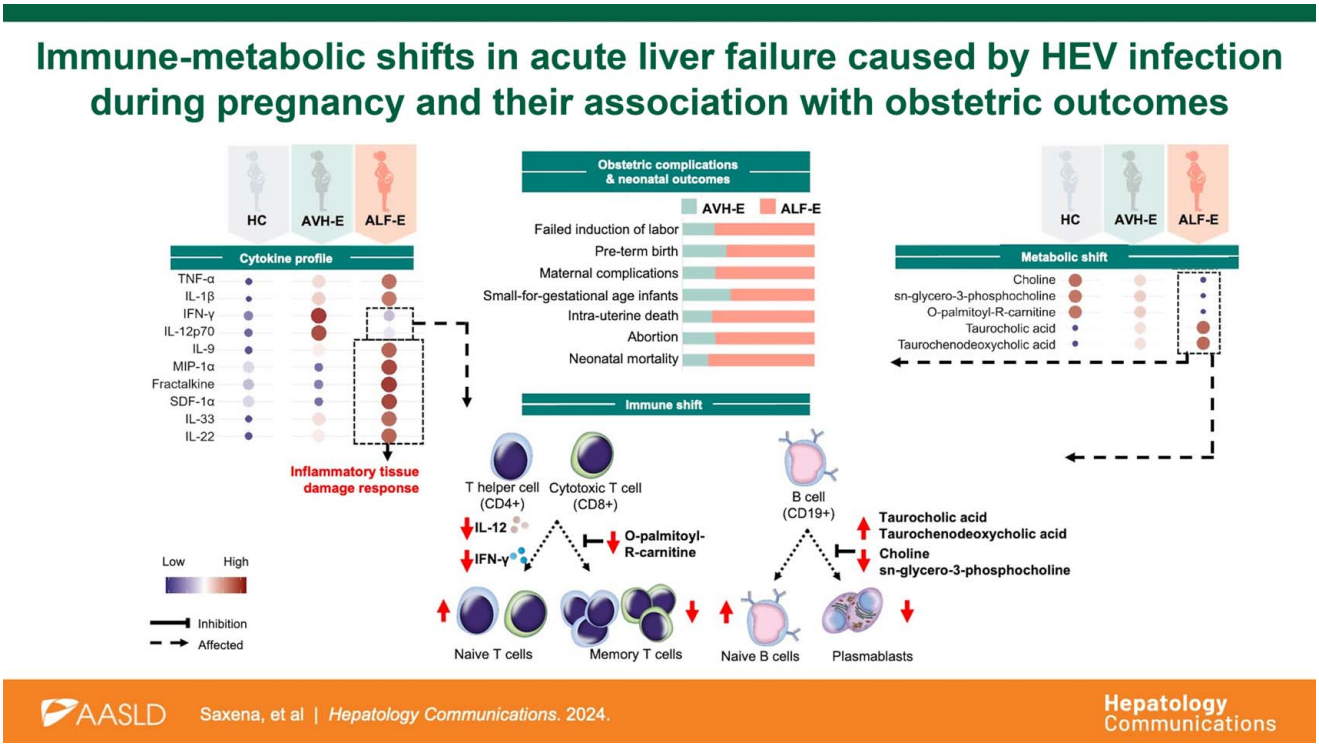
Immune-metabolic shifts in acute liver failure caused by HEV infection during pregnancy and their association with obstetric outcomes

VISUAL ABSTRACT



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Immune-metabolic shifts in acute liver failure caused by HEV infection during pregnancy and their association with obstetric outcomes

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Abstract

Background: Hepatitis-E virus (HEV)-induced liver failure during pregnancy leads to maternal and fetal complications. This study investigates the HEV-associated metabolomic and immunological changes to elucidate the worsening of obstetric outcomes in patients with acute liver failure (ALF) due to HEV.

Methods: Pregnant women with (i) acute viral hepatitis, IgM HEV positive (AVH-E, n = 31, Gr.I), (ii) acute liver failure (ALF-E, n = 15, Gr.II), (iii) acute hepatitis but negative for viral infections (non-HEV, n = 30, Gr.III), and healthy (HC, n = 21, Gr.IV) were evaluated at delivery for plasma untargeted metabolomics, cytokine, and immune profiling.

Results: AVH-E and ALF-E (Gr.I, II) showed elevated TNF-α, IL-1β, IL-9, IL-22, and IL-33 compared to HC. In addition, in ALF-E, IFN-γ and IL-12p70 were decreased, but MIP-1α, fractalkine, SDF-1α, IL-22, and IL-33 were increased compared to AVH-E. Both AVH-E and ALF-E had decreased choline, sn-glycero-3-phosphocholine, *O*-palmitoyl-R-carnitine, and increased taurocholic acid. However, patients with ALF-E had a 2–5-fold decline in these metabolites with raised taurochenodeoxycholic acid. ALF-E showed increased naive T/B cells, decreased CD4, CD8 T_{cm}, T_{em}, and plasmablasts, compared to AVH-E contributing to higher failed inductions, preterm births, maternal complications like eclampsia, disseminated intravascular coagulation, preterm premature rupture of membranes, small-for-gestational-age infants, higher rates of intrauterine death, abortion, and mortality.

Abbreviations: ACLF, acute-on-chronic liver failure; ALF-E, hepatitis E–induced acute liver failure; AVH-E, acute HEV infection; C-section, cesarean section; DIC, disseminated intravascular coagulation; HC, healthy control; IFN, interferon; IUD, intrauterine death; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; PCA, principal component analysis; PLSDA, partial least square discriminant analysis; PT-PROM, preterm premature rupture of membrane; SDF, stromal cell–derived factor; SFH, symphysis fundal height; T_H1, type 1 T helper; T_H2, type 2 T helper; ULN, upper limit of normal; VIP, variable importance in projection.

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Conclusions: HEV infection reduces choline, phosphocholine, and palmitoyl carnitine, enhancing inflammation in ALF-E, while increasing taurocholic and taurochenodeoxycholic acids impairs the immune response. These factors together likely contribute to severe obstetric complications, including higher failed inductions, intrauterine death, and maternal and fetal mortality in ALF-E.

Keywords: cytokines, hepatitis E–induced liver failure, metabolome profiling, obstetric complications, pregnancy

INTRODUCTION

Hepatitis E virus (HEV) is the leading cause of enterically transmitted viral hepatitis worldwide and poses a significant public health threat, particularly in developing countries.[1] While HEV infection generally presents as a self-limiting illness with a low mortality rate of ~1% in the general population, it is associated with significantly higher morbidity and mortality in pregnant women, especially those in the third trimester. Mortality rates among pregnant women with HEV can reach 25%–65%,[2] with the risk of acute liver failure (ALF) and poor obstetric outcomes being markedly elevated. In addition, the incidence of preterm births increases to 65% in HEV-infected pregnancies, contributing to significant fetal morbidity and mortality, with an estimated 70,000 deaths and 3000 stillbirths annually.[1]

The increased severity of HEV infection during pregnancy is linked to altered immune responses. Pregnancy is marked by shifts in the maternal immune system, balancing between proinflammatory T-helper 1 (T_H1) milieu and anti-inflammatory T-helper 2 (T_H2) states at different stages.[3] Although maternal adaptations during pregnancy facilitate fetal tolerance, they also enhance susceptibility to infections, including HEV. Studies have found that HEV-infected pregnant women exhibit impaired immune function, including reduced macrophage activity, reactive oxygen species production, and reduced toll-like receptor signaling, contributing to the development of ALF.[4,5] Increased levels of proinflammatory cytokines such as TNF-α, IL-6, and IL-8, compared to non-pregnant individuals, further worsen pregnancy outcomes.[6] These elevated cytokine levels worsen liver injury, as observed in cytokine storm in patients with acute-on-chronic liver failure (ACLF).[7]

In addition to immunological alterations, pregnancy is accompanied by significant metabolic shifts from an anabolic state during the first and second trimesters to a catabolic state in the third trimester.[8,9] These metabolic alterations are essential for maintenance of tolerogenic immune environment at the feto-maternal interface, supporting fetal growth while preventing rejection of the allogeneic fetus.[9] Key metabolic adaptations, particularly in amino acid and lipid pathways, have been linked to maternal health and fetal development, with some studies even predicting pregnancy complications like gestational diabetes based on early metabolic changes.[10–13] Metabolic disruptions, particularly in bile acid concentrations and lipid metabolism, are also observed in ACLF,[14–17] making it a useful point of comparison for understanding the metabolic consequences of liver failure in HEV-infected pregnant women.

In this study, we investigate the relationship between immune-metabolic shifts and obstetric outcomes in HEV-infected pregnant women, with a focus on how these alterations contribute to the severity of disease.

METHODS

Patients and healthy controls

We recruited a cohort of 76 antenatal patients at the time of delivery (Supplemental Figure S1, http://links.lww.com/HC9/B877) with symptomatic jaundice (visible yellowish discoloration of the sclera and skin along with total serum bilirubin >2 mg/dL) during 2021–2022. Jaundice was used as an inclusion criterion to ensure the selection of patients with significant liver involvement. Blood samples were collected from participants and screened for anti-HEV IgG, anti-HEV IgM, HBsAg, anti-HAV IgM, anti-HCV IgG, anti-HCV IgM, and cytomegalovirus. Pregnant females enrolled were segregated on the basis of HEV IgM/IgG positivity: (i) acute viral hepatitis, IgM HEV positive (group I, AVH-E, $n = 31$); (ii) HEV-induced acute liver failure (group II, ALF-E, $n = 15$); and (iii) acute hepatitis but negative for viral infections (group III, non-HEV, $n = 30$). In addition, a control group of age-matched healthy pregnant females (group IV, HC, $n = 21$) was included. Obstetrical complications and fetal outcomes were noted.

The primary outcome evaluated in this study is gestational age at delivery with annotation of preterm birth classification, along with assessment of intrauterine death (IUD), birth weight, symphysis-fundal height (SFH), common pregnancy-related complications like eclampsia and preterm premature rupture of membrane (PT-PROM). Neonatal complications like neonatal

jaundice, fetal distress, and fetal mortality were also recorded. The secondary outcome evaluated in the study is the correlation of clinical parameters with metabolites, cytokines, and immune cells. Written informed consent was taken from all the participants, and the study was conducted in accordance with the 1975 Declaration of Helsinki. The study was approved by the Institutional Ethics Committee of Maulana Azad Medical College (F.1/IEC/MAMC/(73)/01/2020/), and the Institute of Liver and Biliary Sciences (F.25/5/107/ILBS/AC/2016/11252/DOA/55), New Delhi, India.

Sample collection and processing

Peripheral blood (8–10 mL) was collected from each patient and subject in EDTA vials. The detailed assay is mentioned in Supplemental Information, http://links.lww.com/HC9/B877. Plasma was separated for biochemical, serological, metabolomic analysis, and cytokine profiling.

Plasma metabolomics

Metabolites were extracted from 100 μL of plasma using organic phase extraction protocol, followed by ultra-high performance liquid chromatography coupled to mass spectrometry. Metabolites were extracted using compound discoverer 3.0 (Thermo Scientific). Annotation of features was performed using mass list searches, mzCloud (www.mzcloud.org), and mummichog. The detailed assay is mentioned in Supplemental Information, http://links.lww.com/HC9/B877.

Cytokine profiling

Plasma (25–30 μl) was used for measuring concentrations of 29 cytokines and chemokines (Supplemental Table S1, http://links.lww.com/HC9/B877) using human custom procartaplex (Thermo Fisher Scientific, Cat. No. PPX-29-MX9HKGW). The detailed assay is mentioned in Supplemental Information, http://links.lww.com/HC9/B877.

Immune phenotyping and flow cytometric analysis

Multiparametric flow cytometry was used for phenotypic analysis of T- and B-cell subsets. A cocktail of flow cytometry monoclonal antibodies was used. Processing of samples, complete information of each antibody (Supplemental Table S2, http://links.lww.com/HC9/B877), analysis, and gating strategy for immune cells are detailed in the Supplemental Information, http://links.lww.com/HC9/B877.

Statistical analysis

Statistical analysis was performed using Prism (GraphPad, version 9.0) and R package 4.3.0. The variables between the 2 groups were compared using the unpaired (2-tailed) Student *t* test and Mann-Whitney *U* test. For comparison among more than 2 groups, 1-way ANOVA, the Kruskal-Wallis test followed by probability adjustment by the Mann-Whitney test, or by Bonferroni post-hoc test comparison as appropriate was performed. For categorical comparisons of proportions, the Fisher exact test was used to calculate *p* values, and Cohen's *h* was used to assess effect sizes, indicating the magnitude of observed differences. Statistical significance was defined as p value < 0.05. Cohen's *h* values near 0.2 indicate a small effect, 0.5 a medium effect, and 0.8 a large effect. Data for metabolomics analysis were log normalized and subjected to pareto-scaling using metaboanalyst 6.0 (http://metaboanalyst.ca) for multivariate analyses. Pearson correlation and regression analysis was performed with R package 4.3.0.

RESULTS

Demographics and clinical characteristics

In this study, 76 pregnant females with symptomatic jaundice were enrolled. These females were: AVH-E (median bilirubin 5.2 mg/dL [IQR: 3.49–13], age 25.1 ± 4.2 y, and mean gestational age 31 ± 3 wk); ALF-E (median bilirubin 20.8 mg/dL [IQR: 16.5–22], mean age 25 ± 5 y, and mean gestational age 34 ± 3 wk); and non-HEV (median bilirubin 3.35 mg/dL [IQR 2.3–5.1], age 25.9 ± 4.4 y, and mean gestational age 36 ± 3 wk). In addition to these, HC pregnant females (mean age 24.2 ± 3.2 y, median bilirubin 0.3 mg/dL [IQR: 0.1–0.7], mean gestational age 38 ± 0.9 wk) were also included. All HEV cases were HEV-1. There was no significant difference in age (y) and gestational age among all groups. However, ALT/AST, total serum bilirubin, and urea were significantly increased in non-HEV, AVH-E, and ALF-E compared to healthy controls. Along with the above parameters, prothrombin time was also significantly increased in AVH-E compared to non-HEV. Furthermore, there was a significant increase in ALT/AST, total serum bilirubin, urea, and international normalized ratio in ALF-E compared to AVH-E (Table 1).

Obstetric complications and neonatal outcomes in AVH-E and ALF-E

AVH-E females experienced higher rates of failed induction (25.8%) compared to healthy controls (HC: 4.7%, $p = 0.03$) and non-HEV women (10%, p = NS). Similarly, the incidence of preterm birth was significantly

TABLE 1 Clinical and demographic profile of pregnant females

Variables (median and range)	Healthy control (HC, n = 21)	Total serum bilirubin >2 mg/dL			*p*		
		Without HEV (non-HEV, n = 30)	Acute HEV (AVH-E, n = 31)	HEV-ALF (ALF-E, n = 15)	Non-HEV vs. HC	AVH-E vs. non-HEV	ALF-E vs. AVH-E
Demographics							
Age (y)	24 (19–30)	25 (23–28)	25 (21–28)	25 (21–27)	0.69	0.53	0.66
Gestational age (wk)	38 (37–40)	37 (34–38)	35 (30–38)	34 (27–35)	0.9	0.09	0.77
Parity, n (%)							
Nulliparity	15 (71.4)	18 (60)	14 (45.1)	11 (73.3)			
Primiparity	4 (19)	6 (20)	12 (38.7)	1 (6.7)			
Multiparity	2 (9.5)	6 (20)	5 (16)	3 (20)			
Gravidity, n (%)							
Nulligravidity	11 (52.3)	—	—	14 (93.3)			
Primigravidity	7 (33.3)	15 (50)	2 (6.4)	1 (6.7)			
Multigravidity	3 (14.3)	14 (46.6)	27 (87)	—			
Hematology							
Hemoglobin (g/dL)	13.5 (13–14.5)	11.3 (9.4–12.5)	10.9 (10–13.1)	10.2 (9.2–10.2)	0.2	0.9	0.15
Platelets (10^9/L)	2.31 (19.5–2.8)	1.5 (1.2–2.2)	1.4 (1.2–1.8)	0.73 (0.65–1.27)	0.8	0.88	0.24
Leukocytes (10^9/L)	6.7 (5.4–7.5)	9.8 (7.3–11.6)	8.31 (5.8–11.6)	10.5 (10.3–11.2)	0.78	0.53	0.18
Liver function test							
ALT/SGPT (IU/L)	28 (20–32)	111 (56.7–306)	254 (103–432)	924 (879–1100)	**<0.001**	**0.05**	**0.04**
AST/SGOT (IU/L)	24.5 (20–28)	117 (56.7–317.5)	217 (89–463)	1040 (902–1074)	**<0.001**	**0.04**	**0.01**
S. bilirubin total (mg/dL)	0.3 (0.1–0.7)	3.35 (2.3–5.1)	5.2 (3.49–13)	20.8 (16.5–22)	**0.05**	**0.003**	0.07
S. alkaline phosphatase (IU/L)	98 (50–145)	381 (236.2–528)	328 (234–467)	324 (278.5–362)	0.06	0.73	0.26
Kidney function test							
S. creatinine (mg/dL)	0.9 (0.8–1.3)	1.44 (1.12–1.82)	1.3 (0.85–2.1)	1.7 (1.65–2.05)	0.3	0.95	0.28
Urea (mg/dL)	7 (5–9)	16 (11.25–20.25)	10 (6.5–12)	24 (17–28.5)	**0.009**	**0.01**	0.23
Coagulopathy test							
INR	1 (0.9–1.3)	0.8 (0.76–1.09)	0.9 (0.8–1.08)	3.75 (3.27–3.82)	0.7	0.84	**0.02**
PT (s)	12.5 (12–13)	10.7 (10.1–12.6)	13 (12–14)	14 (12.25–18.5)	0.07	**0.001**	0.8

Note: Data represented in median and interquartile range. Bold values are Statistical significance of p value < 0.05.
Abbreviations: ALF-E, hepatitis E–induced acute liver failure; AVH-E, acute HEV infection; HC, healthy control; INR, International normalized ratio; PT, prothrombin time, S., serum; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvate transaminase.

greater in AVH-E females (60.9%) compared to HC (15%, $p = 0.002$) and non-HEV (33.3%, $p = 0.04$) (Table 2). Poor obstetric outcomes included increased rates of eclampsia, disseminated intravascular coagulation (DIC), PT-PROM, and maternal mortality compared to healthy and non-HEV women. Infants born to AVH-E mothers had a lower incidence of being appropriate-for-gestational age (39.1%, $p = 0.008$ vs. HC: 80%), fewer live births, higher rates of IUD (16.1%, $p = 0.03$ vs. HC: 0%, $p = 0.06$ vs. non-HEV: 6.7%), and abortions. These infants also had a higher incidence of low birth weight (65.2%, $p < 0.001$ vs. HC: 0%) and complications such as fetal distress (39.1%; $p < 0.001$ vs. HC: 0%, p = NS vs. non-HEV: 22.2%), neonatal jaundice, and infant mortality (Table 3).

ALF-E females experienced a significantly higher rate of failed inductions (80%, $p < 0.001$ vs. AVH-E:

TABLE 2 Obstetric characteristics in pregnant females

Variables, n (%)	Healthy control (HC, n = 21)	Total serum bilirubin >2 mg/dL			Effect sizes (Cohen's *h*) and *p*			
		Without HEV (non-HEV,n = 30)	Acute HEV (AVH-E, n = 31)	HEV-ALF (ALF-E, n = 15)	AVH-E vs. HC	AVH-E vs. non-HEV	ALF-E vs. AVH-E	ALF-E vs. non-HEV
Labor								
Spontaneous	18 (85.7)	11 (40.7)	17 (73.9)	1 (33.3)	0.297, 0.48	**0.685, 0.018**	**0.839, 0.008**	0.153, 0.75
Induced	2 (9.5)	16 (59.3)	6 (26.1)	2 (66.7)	0.446, 0.06	**0.685, 0.018**	**0.839, 0.008**	0.153, 0.51
Failed induction	1 (4.7)	3 (10)	8 (25.8)	12 (80)	**0.629, 0.03**	0.422, 0.3	**1.149, <0.001**	**1.571, 0.04**
Mode of delivery								
Vaginal	13 (65)	17 (63)	19 (82.7)	1 (33.3)	0.408, 0.18	0.450, 0.08	**1.053, <0.001**	**0.604, 0.055**
C-section	7 (35)	10 (37)	4 (17.4)	2 (66.7)	0.406, 0.18	0.447, 0.08	**1.051, <0.001**	**0.604, 0.055**
Time of delivery								
Preterm birth	3 (15)	9 (33.3)	14 (60.9)	2 (66.7)	**0.995, 0.002**	**0.560, 0.04**	0.121, 0.52	**0.681, 0.021**
Term birth	17 (85)	18 (66.7)	9 (39.1)	1 (33.3)	**0.995, 0.002**	**0.560, 0.03**	0.121, 0.5	**0.681, 0.02**
Maternal complications								
Eclampsia	—	2 (7.4)	3 (13)	2 (13.3)	0.738, 0.13	0.187, 0.67	0.009, 1	0.196, 0.59
DIC	—	—	2 (8.7)	3 (20)	0.599, 0.5	0.599, 0.49	**0.328, 0.05**	0.927, 0.1
PT-PROM	—	—	2 (8.7)	1 (6.7)	0.599, 0.5	0.599, 0.49	0.075, 0.3	**0.524, 0.032**
Maternal mortality	—	—	2 (8.7)	7 (46.7)	0.599, 0.5	0.599, 0.49	**0.906, <0.001**	**1.505, <0.001**
Symphysis-fundal height (SFH)								
SGA	—	19 (70.4)	12 (52.1)	2 (66.7)	**1.613, <0.001**	0.378, 0.18	0.299, 0.33	0.080, 1
AGA	16 (80)	6 (22.2)	9 (39.1)	1 (33.3)	**0.863, 0.008**	0.370, 0.16	0.121, 0.52	0.249, 0.7
LGA	4 (20)	2 (7.4)	2 (8.7)	—	0.328, 0.2	0.048, 1	0.599, 0.55	0.551, 0.54

Note: Data are represented in numbers and percentages. Effect sizes calculated using Cohen's *h* are mentioned with associated *p* values. Bold values are Statistical significance of *p* value < 0.05.
Abbreviations: AGA, appropriate-for-gestational age; ALF-E, hepatitis E–induced acute liver failure; AVH-E, acute HEV infection; DIC, disseminated intravascular coagulation; LGA, large for gestational age; PT-PROM, preterm premature rupture of membrane; SGA, small for gestational age.

TABLE 3 Birth characteristics in pregnant females

Variables, n (%)	Healthy control (HC, n = 21)	Total serum bilirubin >2 mg/dL			Effect sizes (Cohen's *h*) and *p*			
		Without HEV (non-HEV, n = 30)	Acute HEV (AVH-E, n = 31)	HEV-ALF (ALF-E, n = 15)	AVH-E vs. HC	AVH-E vs. non-HEV	ALF-E vs. AVH-E	ALF-E vs. non-HEV
Fetal outcome								
Live birth	20 (95.2)	27 (90)	23 (74.2)	3 (20)	0.624, 0.12	0.422, 0.14	**1.149, <0.001**	**1.571, <0.001**
IUD	—	2 (6.7)	5 (16.1)	8 (53.3)	**0.826, 0.03**	0.302, 0.06	**0.811 <0.001**	**1.113, <0.001**
Abortion	1 (4.8)	1 (3.3)	3 (9.7)	4 (26.7)	0.192, 0.6	0.268, 0.23	**0.453, 0.02**	**0.721, 0.002**
Birth weight (g)								
Normal	21 (100)	9 (33.3)	8 (34.8)	1 (33.3)	**1.880, <0.001**	0.032, 1	0.032, 1	0, 1
Low	—	18 (66.7)	15 (65.2)	2 (66.7)	**1.880, <0.001**	0.032, 1	0.032, 1	0, 1
Neonatal complications								
Nuchal cord	—	1 (3.7)	—	—	0, 1	0.387, 0.48	0, 1	0.387, 1
Fetal distress	—	6 (22.2)	9 (39.1)	1 (33.3)	**1.351, <0.001**	0.370, 0.16	0.121, 0.5	0.249, 0.7
Neonatal jaundice	2 (10)	6 (22.2)	4 (17.4)	—	0.217, 0.68	0.121, 0.74	0.861, 0.15	0.981, 0.08
Neonatal mortality	—	—	2 (8.7)	1 (33.3)	0.599, 0.5	0.599, 0.49	0.631, 0.07	**1.230, 0.007**

Note: Data are represented in numbers and percentages. Effect sizes calculated using Cohen's *h* are mentioned with associated *p* values. Bold values are Statistical significance of *p* value < 0.05.
Abbreviations: ALF-E, hepatitis E–induced acute liver failure; AVH-E, acute HEV infection; IUD, intrauterine death.

25.8%, $p = 0.04$ vs. non-HEV: 10%), high incidence of IUD (53.3%, $p < 0.001$ vs. AVH-E: 16.1%, $p < 0.001$ vs. non-HEV: 6.7%), abortions (26.7%, $p = 0.02$ vs. AVH-E: 9.7%, $p = 0.002$ vs. non-HEV: 3.3%), eclampsia, DIC (20%, $p = 0.05$ vs. AVH-E: 8.7%, p = NS vs. non-HEV: 0%), PT-PROM, and maternal mortality (46.7%, $p < 0.001$ vs. AVH-E: 8.7%, $p < 0.001$ vs. non-HEV: 0%) compared to AVH-E and non-HEV pregnant women (Table 2). Only 3 females underwent delivery; 1 with spontaneous labor had term delivery, and the other 2 preterm deliveries responded to induced labor with cesarean section. The remaining women experienced spontaneous pregnancy losses, including abortions and IUDs. All infants had low birth weight, and the infants born after induced delivery showed signs of small for gestational age, fetal distress, and neonatal mortality on the fourth day after birth (Table 3).

AVH-E pregnant females show T_H1 cytokine profile and metabolic shift compared to non-HEV and healthy females

In general, during the third trimester, T_H2 immunity predominates. However, nearing and at the time of delivery, there is a shift from T_H2 to T_H1 immunity to facilitate parturition in healthy females. The principal component analysis plot showed partially overlapping cytokine profiles of AVH-E, non-HEV, and HC (Figure 1A). In healthy pregnant females, we observed a T_H1 profile characterized by IL-1β, IL-8, and TNF-α (Supplemental Table S3, http://links.lww.com/HC9/B877). However, the level of each T_H1 cytokine was significantly increased by 2–4.5 fold in AVH-E compared to healthy and non-HEV females (Figures 1B–E). In addition, IL-18, IL-27, TNF-α, and immunoregulatory IL-7 and IL-29 were found to be elevated in AVH-E compared to non-HEV (Figures 1C, D, Supplemental Table S4, http://links.lww.com/HC9/B877).

On the contrary, Supplemental Figure S4, http://links.lww.com/HC9/B877 depicted that non-HEV females had a predominant T_H2 cytokine profile, with decreased levels of IL-18, IL-27 and IFN-γ, and elevated levels of IL-2 and IL-21 compared to HC (Supplemental Figure S4A, http://links.lww.com/HC9/B877, Supplemental Table S4, http://links.lww.com/HC9/B877). Interestingly, antiviral response cytokines, specifically IFN-γ, MCP-3, and IL-12p70, were increased in AVH-E patients compared to healthy controls, reflecting their role in immune cell recruitment (Figure 1F). However, AVH-E showed reduced levels of IL-12p70 in comparison to non-HEV. In AVH-E, there was a decrease in IL-4 compared to non-HEV (Figure 1F). Overall, these results suggest a more robust T_H1 cytokine profile in AVH-E pregnant females compared to healthy and non-HEV females at delivery.

Aligned with cytokine profile shift, our comparison of metabolic profile in AVH-E with non-HEV and healthy revealed a notable decline in sn-glycero-3-phosphocholine, choline, D-proline, and *O*-palmitoyl-R-carnitine levels, while taurocholic acid levels were increased in AVH-E compared to non-HEV and HC (Figures 2A–E). Reduction in these key metabolites, along with increased plasma taurocholic acid levels in AVH-E pregnant females, is associated with polarization toward T_H1 immune response.[18–23] Indeed, our supplemental data (Supplemental Figures S4B, C, http://links.lww.com/HC9/B877) depicted that in non-HEV, there was a decline in *N*-palmitoyl-D-erythro-sphingosine, sphinganine, and dimethyl benzimidazole compared to HC, which was associated with T_H2 immune phenotype.[24–26]

Furthermore, in AVH-E, there were increased maternal complications, including eclampsia, DIC, PT-PROM, and maternal mortality, alongside poor neonatal outcomes like fetal distress syndrome, neonatal jaundice, and increased mortality.

Increased inflammatory cytokines cause tissue damage in ALF-E

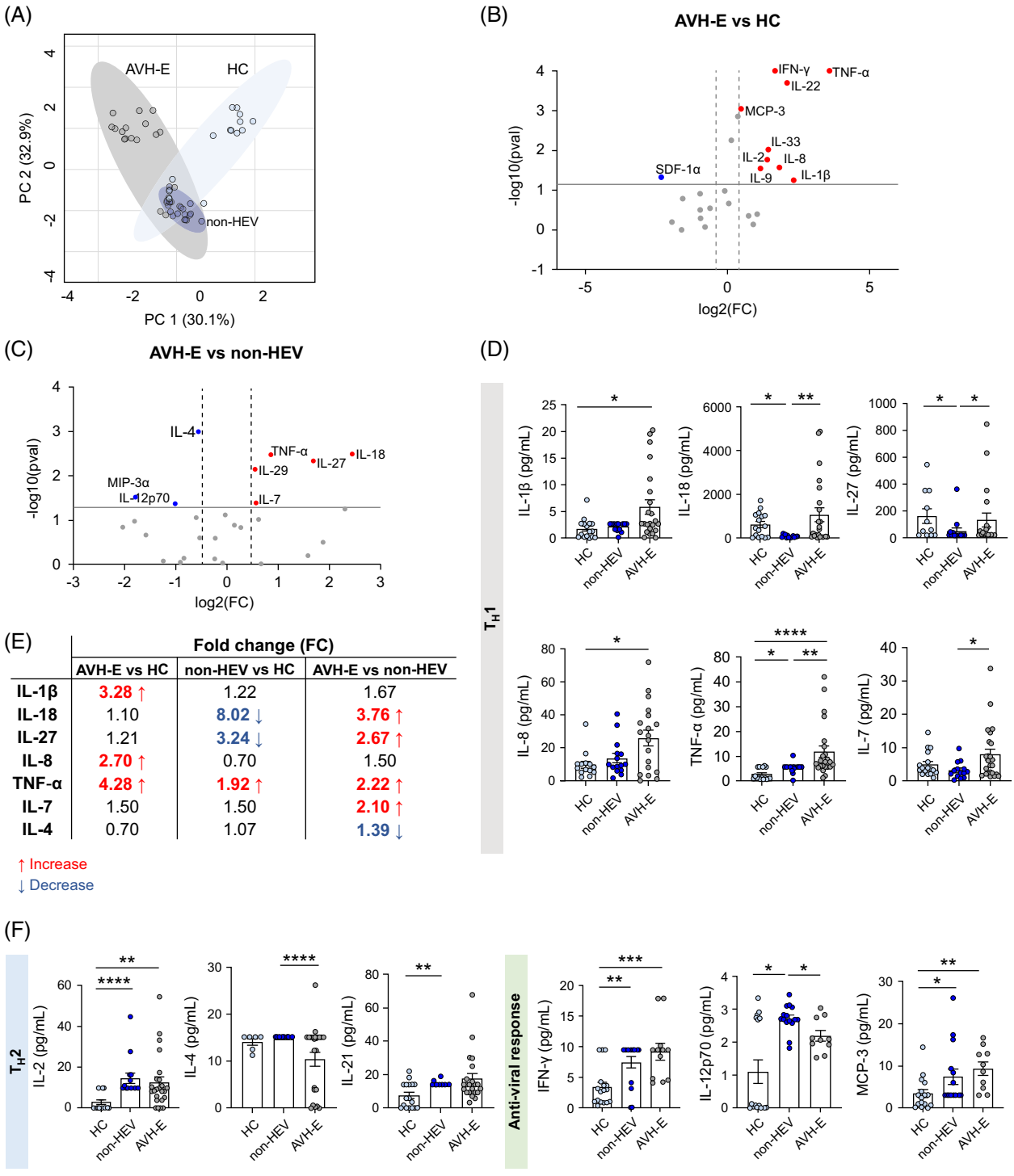
Principal component analysis plot showed the distinctly separated cytokine profile of ALF-E compared to overlapping profiles of HC and AVH-E (Figure 3A). There was no significant difference in concentrations of IL-1β, IL-18, IL-27, IL-8, TNF-α, and IL-7 in AVH-E and ALF-E (Supplemental Figure S4D, http://links.lww.com/HC9/B877). However, in ALF-E, levels of IL-9, MIP-1α, fractalkine, SDF-1α, IL-33, and IL-22 were significantly increased compared to AVH-E (Figures 3B–D). In addition, immune recruitment and antiviral responsive cytokines IFN-γ, IL-12p70, MCP-3, and MIP-3α also significantly declined in ALF-E compared to AVH-E (Figures 3B–E, Supplemental Table S5, http://links.lww.com/HC9/B877). These findings highlight that the severe impact of aggressive inflammatory response in ALF-E may contribute to liver tissue damage.

Furthermore, the correlation matrix elucidated the associations between cytokines and clinical outcomes in HEV-infected pregnant women (Figure 3F). Linear regression analysis demonstrated that plasma concentrations of MCP-3 and MIP-3α were positively correlated, while MIP-1α, fractalkine, and SDF-1α were negatively correlated with live births and term births (Supplemental Figure S5, http://links.lww.com/HC9/B877).

Severe metabolomic alterations in ALF-E and their contribution to clinical outcomes

Given the significant role of metabolites in clinical outcomes, a comparison of the metabolome profiles of

CYTOKINES



(E)

	Fold change (FC)		
	AVH-E vs HC	non-HEV vs HC	AVH-E vs non-HEV
IL-1β	3.28 ↑	1.22	1.67
IL-18	1.10	8.02 ↓	3.76 ↑
IL-27	1.21	3.24 ↓	2.67 ↑
IL-8	2.70 ↑	0.70	1.50
TNF-α	4.28 ↑	1.92 ↑	2.22 ↑
IL-7	1.50	1.50	2.10 ↑
IL-4	0.70	1.07	1.39 ↓

↑ Increase
↓ Decrease

FIGURE 1 Differential cytokine expression profiling in pregnant women with and without acute hepatitis E. (A) PCA differentiating HC, AVH-E, and non-HEV based on cytokine profiles. (B) Volcano plot illustrating the differential cytokine expression between AVH-E and HC groups, highlighting significant changes based on log2 fold change and −log10 (*p* value). (C) Volcano plot showing significant cytokine variations between AVH-E and non-HEV, displayed by log2 FC and −log10 (*p* value). (D) Bar plots showing the concentrations of T_H1 cytokines in HC, non-HEV, and AVH-E. (E) Table depicting fold change in concentration of cytokines among the groups. (F) Bar plots detailing the levels of T_H2 cytokines and antiviral response cytokines in the same cohorts as (D). Statistical significance is indicated by asterisks: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. Data are presented as mean ± SEM, with the Kruskal-Wallis test conducted for group comparisons. Abbreviations: AVH-E, acute HEV infection; FC, fold change; HC, healthy control; PCA, principal component analysis.

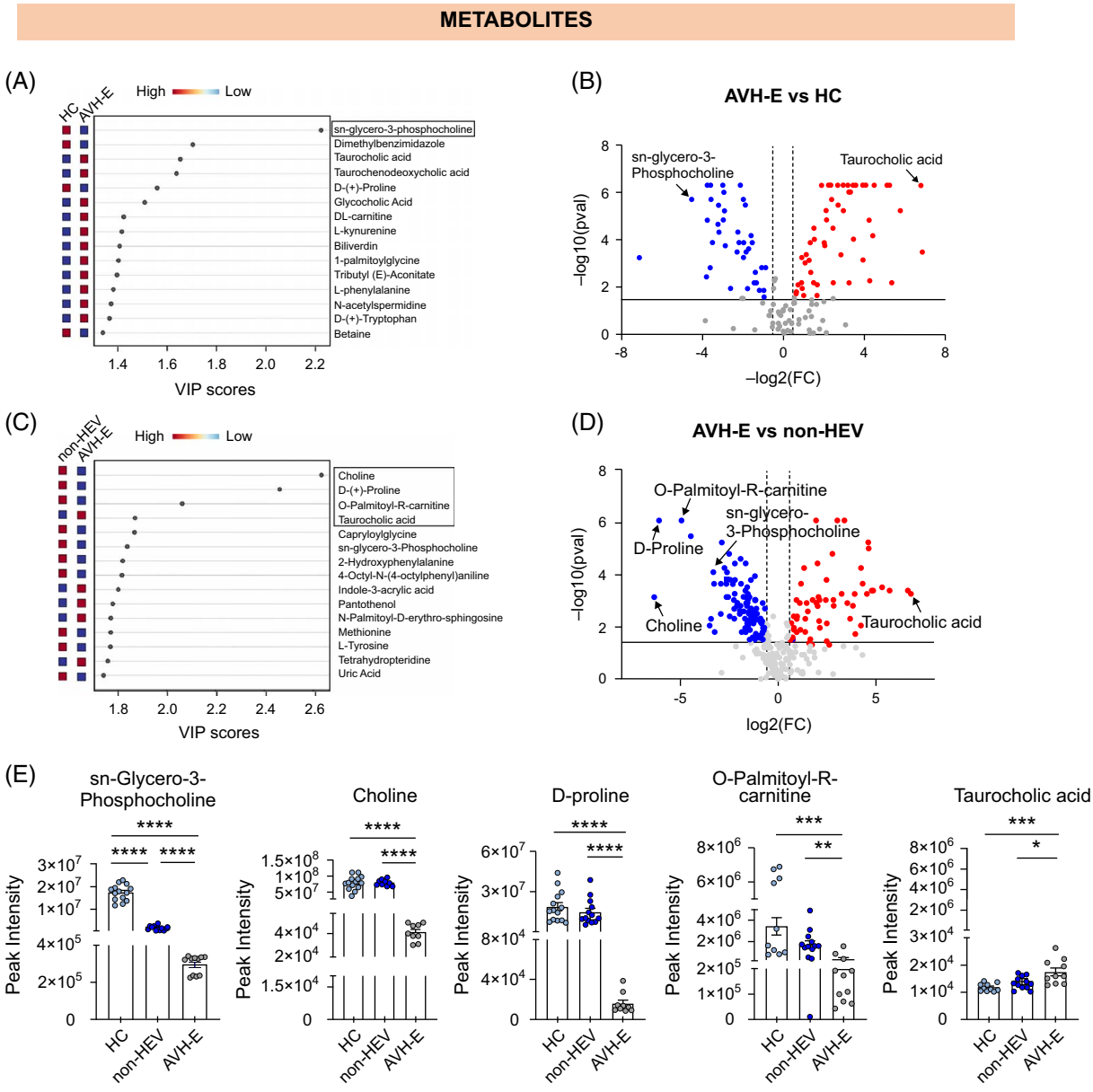


FIGURE 2 Comparative metabolomic analysis in pregnancy with and without acute hepatitis E infection. (A) VIP scores from the PLS-DA model, identifying key metabolites that differentiate between AVH-E and HC (VIP score >2.0 are considered). Colored boxes on the left indicate the relative concentration of the corresponding metabolite. (B) Visualization of VIP scores, highlighting metabolites with significant differential expression between AVH-E and HC groups. (C) Volcano plot depicting significant metabolite variations between AVH-E and non-HEV, represented by log2 FC and −log10 *p* value, to identify metabolites with statistically significant changes in concentration. (D) Volcano plot showing significant metabolite variations between AVH-E and non-HEV, displayed by log2 FC and −log10 (*p* value). (E) Bar plots representing the peak intensity distributions of key metabolites across HC, non-HEV, and AVH-E. Data in box plots are presented as mean ± SEM, and statistical significance was determined using the Kruskal-Wallis test, denoted by asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). Abbreviations: AVH-E, acute HEV infection; FC, fold change; HC, healthy control; PLS-DA, partial least squares-discriminant analysis; VIP, variable importance in projection.

ALF-E, AVH-E, and HC using a principal component analysis plot showed clear segregation of the 3 groups, with AVH-E and ALF-E closely aligned (Figure 4A). ALF-E females showed reduced levels of sn-glycero-3-phosphocholine, and *O*-palmitoyl-R-carnitine in comparison to AVH-E and HC (Figures 4B–D). In addition to these metabolites, ALF-E also showed a significant decrease in choline, while there was a marked elevation of taurocholic acid, taurochenodeoxycholic acid, and sphinganine, compared to AVH-E.

Enrichment and pathway analysis revealed substantial alterations in primary bile acid biosynthesis, glycerophospholipid metabolism, and taurine/hypotaurine metabolism, suggesting that these pathways play pivotal roles in the metabolic response to HEV infection during pregnancy. These metabolites are shown to

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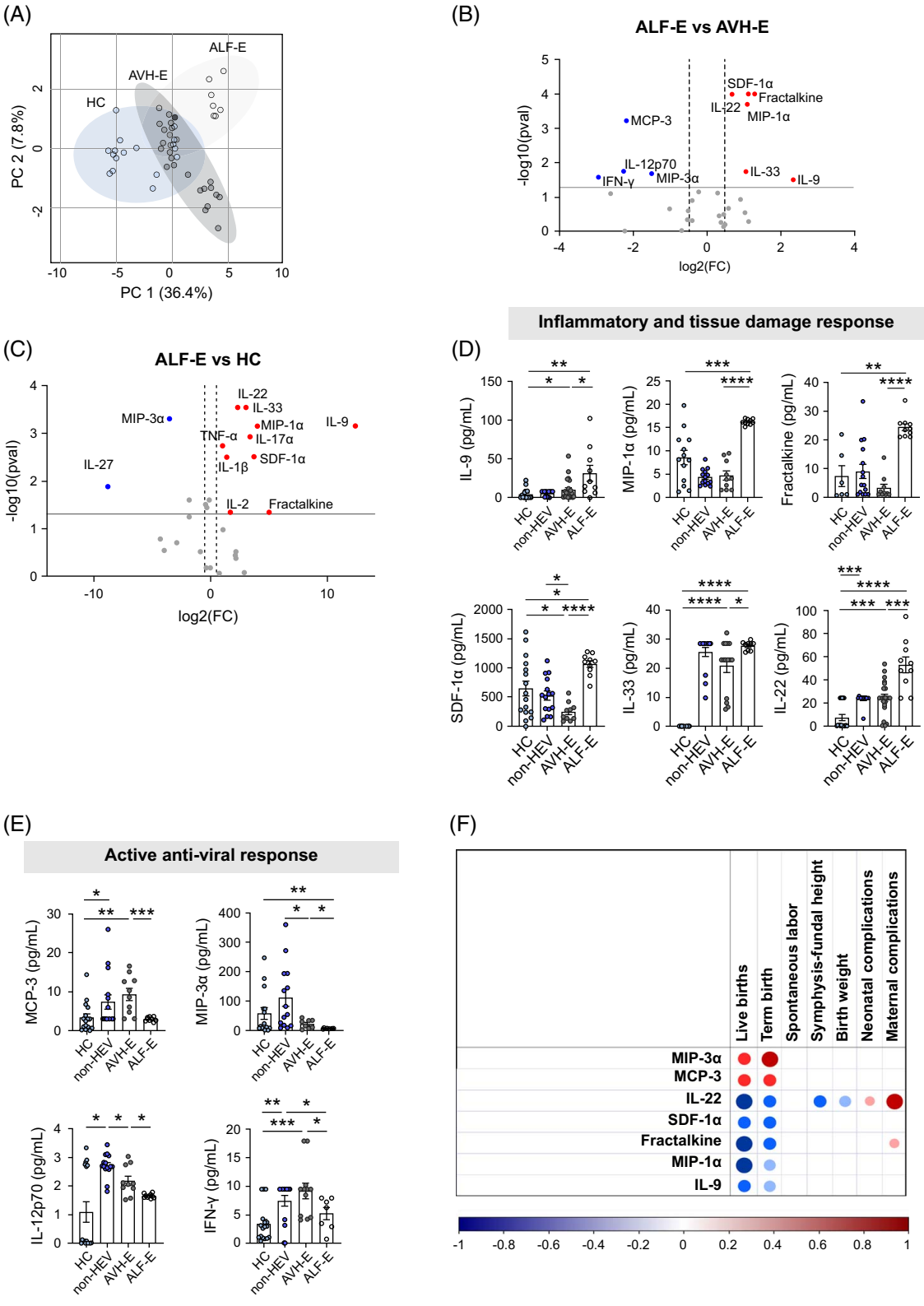

(A)
ALF-E
AVH-E
HC
PC 2 (7.8%)
PC 1 (36.4%)
(B)
ALF-E vs AVH-E
SDF-1α
Fractalkine
IL-22
MIP-1α
MCP-3
IL-12p70
IFN-γ
MIP-3α
IL-33
IL-9
-log10(pval)
log2(FC)
(C)
ALF-E vs HC
IL-22
IL-33
MIP-3α
MIP-1α
IL-9
TNF-α
IL-17α
IL-27
SDF-1α
IL-1β
IL-2
Fractalkine
-log10(pval)
log2(FC)
Inflammatory and tissue damage response
(D)
IL-9 (pg/mL)
MIP-1α (pg/mL)
Fractalkine (pg/mL)
SDF-1α (pg/mL)
IL-33 (pg/mL)
IL-22 (pg/mL)
HC
non-HEV
AVH-E
ALF-E
(E)
Active anti-viral response
MCP-3 (pg/mL)
MIP-3α (pg/mL)
IL-12p70 (pg/mL)
IFN-γ (pg/mL)
HC
non-HEV
AVH-E
ALF-E
(F)
Live births
Term birth
Spontaneous labor
Symphysis-fundal height
Birth weight
Neonatal complications
Maternal complications
MIP-3α
MCP-3
IL-22
SDF-1α
Fractalkine
MIP-1α
IL-9
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1

FIGURE 3 Plasma cytokine expression and correlation with pregnancy outcomes in AVH-E and ALF-E. (A) PCA differentiating HC, AVH-E, and ALF-E based on cytokine expression profiles. (B) Volcano plot detailing the metabolites with significant changes in abundance between ALF-E and AVH-E, as indicated by log2 FC and –log10 (p value). (C) Volcano plot showing significant cytokine variations between ALF-E and HC, displayed by log2 FC and –log10 (p value). (D) Bar plots showing levels of cytokines associated with inflammation and tissue damage compared among HC, non-HEV, AVH-E, and ALF-E. (E) Concentrations of key cytokines indicative of antiviral immune response in HC, non-HEV, AVH-E, and ALF-E cohorts, highlighting significant variations. (F) Correlation matrix as a heatmap, presenting the strength and direction of associations between cytokine levels and clinical outcomes, with the size and color of the dots denoting the magnitude and polarity of correlation coefficients ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$). Data are represented as mean ± SEM. Kruskal-Wallis tests were used for comparisons across the 3 groups, with statistical significance considered at $p < 0.05$. Abbreviations: ALF-E, hepatitis E–induced acute liver failure; AVH-E, acute HEV infection; FC, fold change; HC, healthy control; PCA, principal component analysis.

interact through the coenzyme-A and sphingomyelin, highlighting their role in energy production and cell membrane integrity (Figure 4E).

Further, the correlation plot (Figure 4F) and supplementary regression analysis (Supplemental Figure S6, http://links.lww.com/HC9/B877) showed strong predictions of obstetric and neonatal characteristics based on the abundance of specific metabolites in HEV-infected females. Levels of choline were negatively correlated with the occurrence of spontaneous labor. *O*-palmitoyl-R-carnitine showed a negative correlation with spontaneous labor and a positive correlation with live births. Taurocholic acid showed a strong negative correlation with live births, SFH, and birth weight, while it was positively correlated with the occurrence of maternal and neonatal complications. Conversely, sn-glycero-3-phosphocholine was positively correlated with live births, SFH, and birth weight, and negatively correlated with maternal and neonatal complications. These findings highlight the profound metabolic alterations induced by HEV infection in ALF-E females and their association with adverse pregnancy outcomes.

Distinct decline in T- and B-cell subsets in patients with ALF-E

We conducted comprehensive immunophenotyping to assess the distribution of lymphocyte subsets among pregnant HC, AVH-E, and ALF-E. Notably, the percentage of total CD4+ T cells was markedly increased in ALF-E compared to both AVH-E and HC. Detailed subset analysis revealed that naïve CD4+ T cells (T_{naive}) were comparably lower in AVH-E but significantly elevated in ALF-E. In contrast, central memory (T_{cm}) and effector memory (T_{em}) CD4+ T cells exhibited a decrease in AVH-E and were diminished in ALF-E, suggesting a depletion of memory T cells in a more severe disease stage (Figure 5A).

There was a significant increase in total CD8+ T-cell percentages in AVH-E, suggesting an enhanced proliferation as an initial robust immune response aimed at controlling the viral infection. However, in ALF-E, a contrast drop in total CD8+ T, T_{cm}, and T_{em} with increased naïve CD8+ T cells was observed compared to AVH-E and healthy controls (Figures 5A-B). Further, the B-cell compartment also showed significant defects in plasmablasts but an increase in naïve B cells in ALF-E compared to AVH-E and healthy controls (Figure 5C). Mean fluorescence intensity heatmaps for the above cell type markers also indicated alterations in ALF-E (Figures 5A–C, right panels).

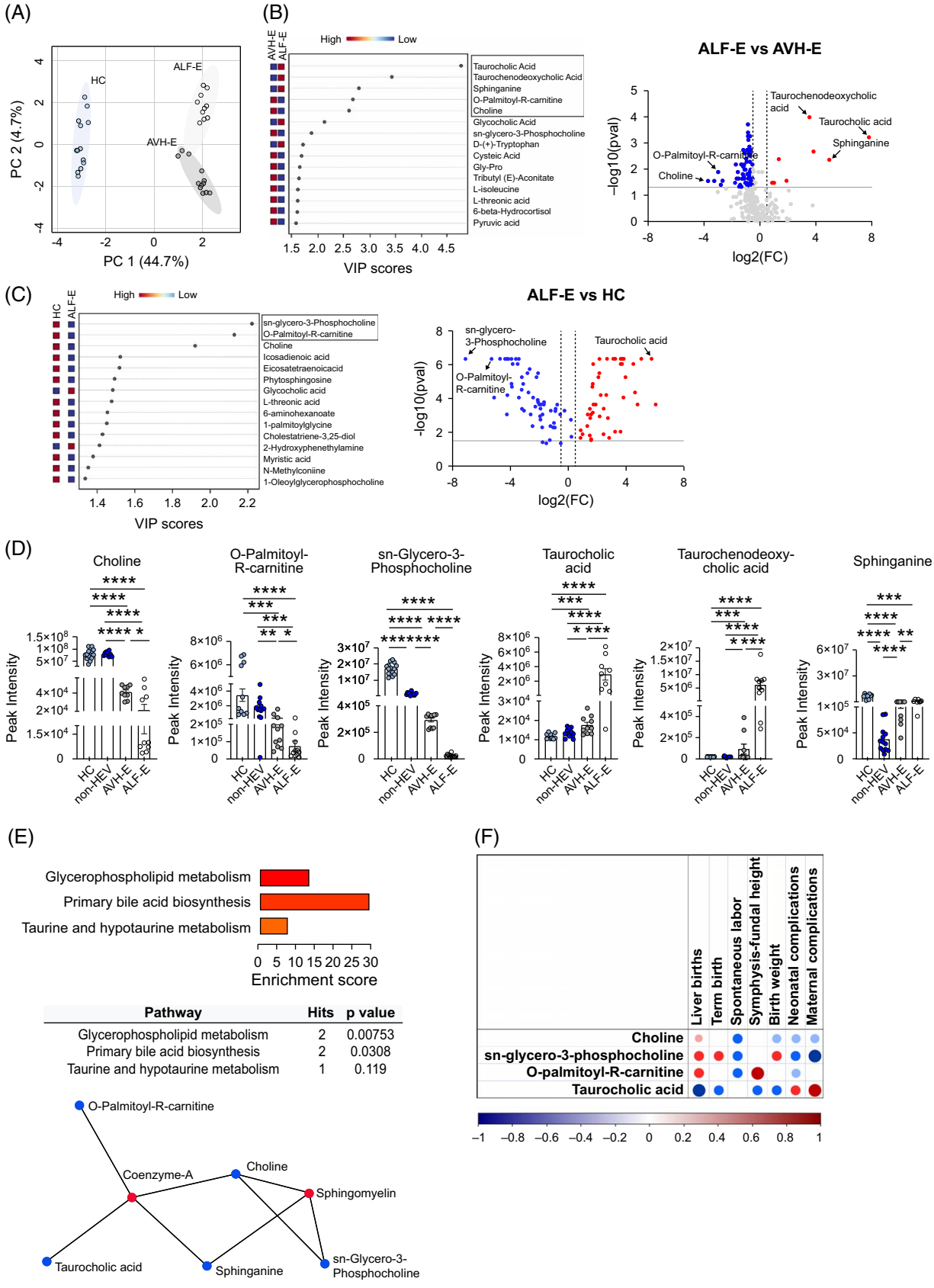
Increase in T_{cm} and T_{em} in AVH-E, potentially reflecting an adaptive shift to enhance the immune capacity to remember and respond to HEV antigens more effectively. However, in ALF-E, a notable decrease in T_{cm}, T_{em}, and plasmablast subsets highlighted the significant memory cell depletion, impacting sustained long-term immune response, contributing to liver damage, and poorer clinical outcomes in these patients. The correlation of immune cells and metabolites in ALF-E revealed the negative correlation of taurocholic acid with CD4/CD8 T_{cm}, T_{em}, and plasmablast and a positive correlation with naïve B, naïve CD4, and CD8 T cells (Figure 5D).

DISCUSSION

Our findings demonstrate that HEV infection in pregnancy, particularly when complicated by acute liver failure (ALF), is associated with significant immune and metabolic dysregulation, which likely contributes to the observed adverse obstetric outcomes. The immune-metabolic shifts observed in HEV-infected pregnant women, including heightened inflammatory responses and altered metabolism, significantly increase the risk of adverse obstetric outcomes, such as preterm births, eclampsia, and IUD, posing heightened risks to both maternal and fetal health.

Our cytokine profiling shows an altered inflammatory environment with increased IL-22, IL-33, and chemokines such as MIP-1α, SDF-1α, and fractalkine in ALF-E, compared to HC and AVH-E. We also observed significant reductions in plasma choline and sn-glycero-3-phosphocholine levels in ALF-E with obstetric complications, compared to both HC and AVH-E. Choline plays a protective role against inflammatory cytokines;

METABOLITES



Pathway	Hits	p value
Glycerophospholipid metabolism	2	0.00753
Primary bile acid biosynthesis	2	0.0308
Taurine and hypotaurine metabolism	1	0.119

FIGURE 4 Differential plasma metabolomic signatures associated with ALF-E and AVH-E. (A) PCA of metabolites reveals distinct separation among HC (blue), AVH-E (black), and ALF-E (gray) in pregnant females. (B) VIP scores from the PLS-DA model, identifying key metabolites that differentiate between ALF-E and AVH-E. (VIP score >2.0 are considered). Colored boxes on the left indicate the relative concentration of the corresponding metabolite. (Right) Volcano plot showing significant metabolite variations, displayed by log2 FC and −log10 (p value). (C) Scores of VIP derived from the PLS-DA highlight metabolites with significant differential expression in ALF-E and HC. (Right) Volcano plot showing differential metabolites between ALF-E and HC, with log2 FC on the x-axis and −log10 (p value) on the y-axis. (D) Bar plots showing differential abundance of metabolites with noted statistical significance. (E) Enrichment scores and pathway impact analysis reveal the most significantly altered metabolic pathways due to ALF-E during pregnancy. The interaction network of key metabolites within these pathways is illustrated below. (F) Correlation matrix displayed as a heatmap illustrates the association between key metabolite levels and clinical parameters in ALF-E. The color and size of the dots within the matrix indicate the strength and direction of the correlations. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$). Data are represented as mean ± SEM. Kruskal-Wallis tests were used for comparisons across the 3 groups, with statistical significance considered at $p < 0.05$. Abbreviations: ALF-E, hepatitis E–induced acute liver failure; AVH-E, acute HEV infection; FC, fold change; HC, healthy control; PCA, principal component analysis; PLS-DA, partial least squares-discriminant analysis; VIP, variable importance in projection.

however, a decline in choline is associated with increased inflammatory cytokines.[27] Inflammation resulting from increased IL-22 and IL-33, in addition to increased chemokines MIP-1α, SDF-1α, and fractalkine, results in immune infiltration that further worsens liver tissue damage and poses significant risks to maternal and fetal health.

Furthermore, choline and phosphocholine are also vital for B-cell activation and antibody production.[28] Indeed, choline and phosphocholine demand increases in healthy pregnancies due to extensive fetal cell proliferation. A reduction may compromise immune cell integrity, particularly affecting B-cell functions, and hinder fetal development by limiting nutrients necessary for neural development.[29] Choline also supports placental angiogenesis, and its deficiency impairs vascularization, potentially causing restricted fetal growth and pre-eclampsia.[30–32] A similar decline in choline was observed in patients with ACLF with increased mortality, as reported by Bajaj et al.[33] These metabolites are critical for cellular membrane integrity, particularly in immune cells, and their deficiency can disrupt lipid metabolism and cellular functions.[34]

Both ALF-E and ACLF are characterized by significant immune dysregulation and systemic inflammation, which critically influence disease progression and outcomes. In ACLF, this dysregulation is often driven by an exaggerated inflammatory response due to the underlying chronic liver disease and acute insults, leading to organ failures and high short-term mortality.[7] In our study, we observed a similar hyperinflammatory state in ALF-E, which contributes to severe maternal and fetal outcomes. This is further supported by findings from Sooryanarain et al,[35] who identified that estrogen signaling and the modulation of SOCS3 levels through the signal transducer and activator of transcription pathway play a critical role in the immune response during HEV replication in pregnant women.

Metabolically, both conditions exhibit significant disruptions. In ACLF, studies have documented alterations in lipid metabolism, energy production, and bile acid metabolism, which correlate with increased mortality.[14–17] Specific metabolites that show significant dysregulation in both ACLF and ALF-E, as observed in our study, include choline, taurocholic acid, and taurochenodeoxycholic acid, emphasizing the shared metabolic disturbances between ACLF and ALF-E. However, there are key differences in the metabolic profiles of these 2 conditions. In ACLF, the disruptions in bile acid metabolism, such as elevated levels of taurocholic acid, are primarily driven by the interaction between chronic liver disease and acute triggers. In contrast, the metabolic alterations in ALF-E are influenced by the acute viral infection and the additional metabolic demands of pregnancy. For instance, the depletion of choline and carnitine is pronounced in ALF-E due to the heightened requirements for fetal development. These distinctions highlight the unique metabolic challenges in ALF-E during pregnancy compared to ACLF, despite the similarities in metabolic disturbances.

ALF-E females also showed lower plasma levels of palmitoyl carnitine compared to AVH-E and HC, aligning with the findings of decreased acylcarnitine levels in non-pregnant HEV patients by Khan et al.[36] Palmitoyl carnitine is essential for cellular energy production through fatty acid oxidation, which is crucial for T-cell functions.[23] A decrease in palmitoyl carnitine can lead to energy deficiency, impacting T-cell proliferation and function, and causing systemic energy deficits that contribute to both maternal health and fetal energy supply.[37] In our study, pregnant females with ALF-E experienced IUDs in 16.1% of cases and abortions in 9.7%. Among the infants born to these mothers, 52.1% were small for gestational age, and 65.2% had low birth weight. It is evident that carnitine deficiency is associated with preterm birth, low birth weight, intrauterine growth retardation, and IUD.[38,39]

Acute HEV infection in pregnant women progressing to ALF-E leads to compromised T- and B-cell activation, marked by reduced memory T cells and plasmablasts due to deficits in choline, phosphocholine, and palmitoyl carnitine. Furthermore, reduced IL-12p70 and IFN-γ in

IMMUNE CELLS

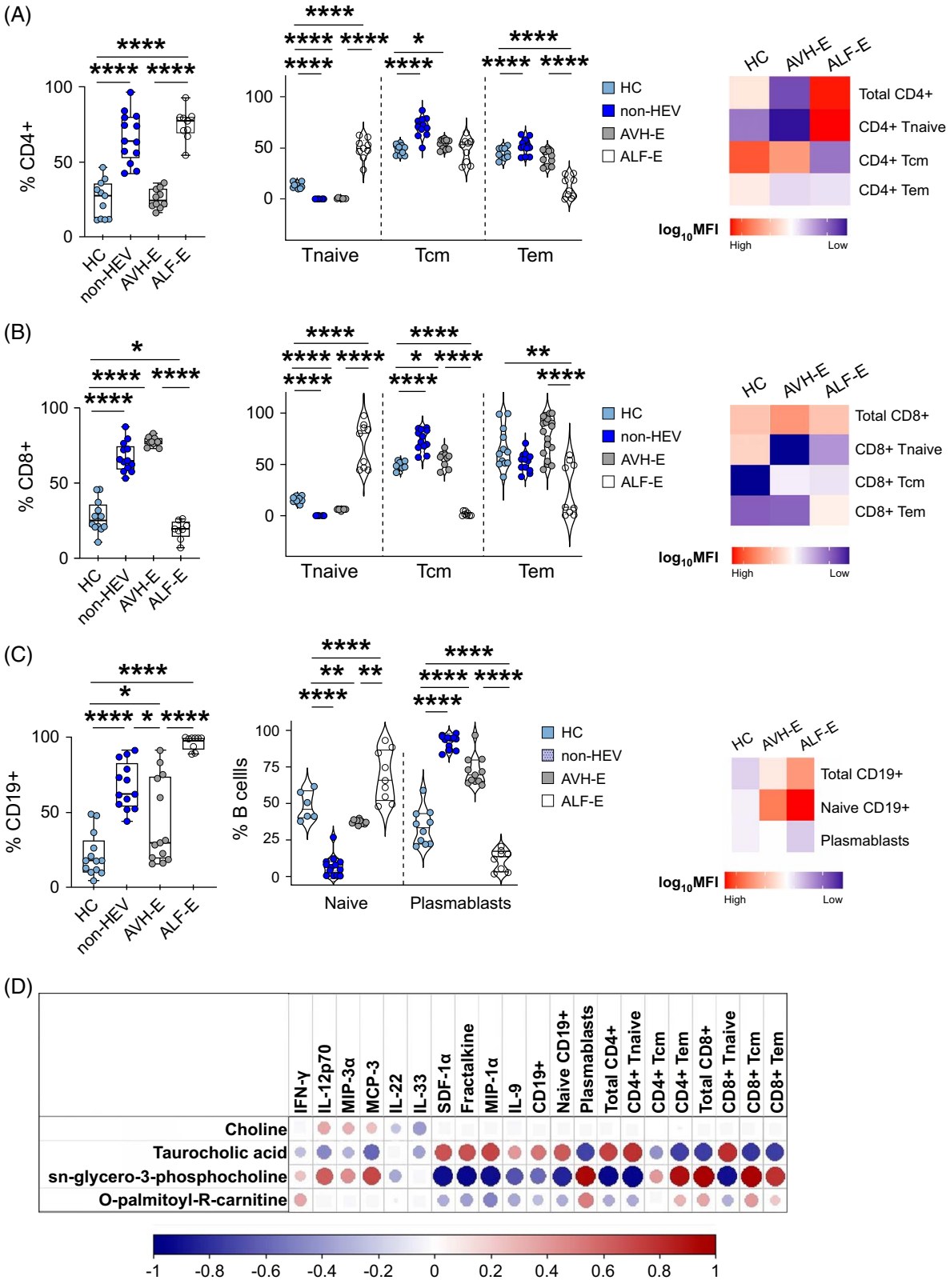


FIGURE 5 T and B lymphocytes in AVH-E and ALF-E. (A, B) Proportion of CD4+, CD8+ T cells and their subsets (Naive, T_{cm}—central memory, and T_{em}—effector memory) across HC, non-HEV, AVH-E, and ALF-E, with a heatmap (right panel), illustrating the mean fluorescence intensity (log_{10}MFI) of total and subset-specific T cells. (C) Distribution of CD19+ B cells, including naive B cells and plasmablasts, with a heatmap showing the log_{10}MFI for the total CD19+ B cells and the respective subsets. (D) Correlation matrix indicating the relationship between immune cells, metabolites, and cytokine levels, with dot color and size representing the correlation strength and direction.Statistical significance is denoted by asterisks: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$. Data are represented as mean ± SEM, and Kruskal-Wallis test was used for comparisons across multiple groups. Abbreviations: ALF-E, hepatitis E–induced acute liver failure; AVH-E, acute HEV infection; HC, healthy control.

ALF-E could lead to an inadequate T_H1 immune response, essential for effective viral clearance. IL-12p70 supports T-cell growth and function, differentiation of naive T cells into effector (T_H1) cells, and stimulates the production of IFN-γ. In addition, Wu et al[40] observed a significant T_H2 bias in patients with HEV-ALF, contributing to the aggravation of disease severity.

In our study, we observed escalating levels of taurocholic and taurochenodeoxycholic acids from acute HEV to ALF-E. Indeed, prior studies also identified increased bile acids in non-pregnant HEV-ALF, liver cirrhosis, HBV-ACLF, and DILI.[41–44] Excessive bile acids during pregnancy detrimentally affect fetal heart cells, elevating the risk of intrauterine fetal death.[41] Furthermore, elevated bile acids have been shown to suppress the cytotoxic functions of CD8+ T and B cells, impairing the ability to effectively clear viral infections.[45] These immunosuppressive effects worsen the compromised immune environment during pregnancy, heightening the risk of adverse outcomes such as maternal mortality, spontaneous preterm labor, eclampsia, DIC, and PT-PROM, as noted in our cohort.

In conclusion, our study reveals significant alterations in metabolomic and immune profiles in HEV-infected pregnant women, particularly those with ALF-E. Profound reductions in essential metabolites like choline, sn-glycero-3-phosphocholine, and *O*-palmitoyl-R-carnitine, alongside elevated levels of bile acids, such as taurocholic and taurochenodeoxycholic acids, were associated with severe obstetric complications and poor neonatal outcomes. Our findings highlight the potential for specific metabolite profiles to serve as biomarkers for predicting adverse outcomes in HEV-infected pregnancies. Furthermore, the distinct immune alterations observed in these patients suggest that HEV infection worsens immune dysfunction, leading to increased mortality.

AUTHOR CONTRIBUTIONS

Anoushka Saxena: performed all experiments, analysis, interpretation, and manuscript writing. Minal: recruitment of patients. Prabhjyoti Pahwa: healthy controls data acquisition and analysis. Jaswinder Singh Maras: helped in metabolomics data analysis. Hamda Siddiqui: patient material collection and initial processing. Jayesh Kumar Sevak: performed cytokine bead array. Y.M. Mala and Shakun Tyagi: patient stratification, clinical data analysis, and inputs in the manuscript. Shiv K. Sarin: manuscript editing and intellectual inputs. Nirupama Trehanpati: conceptual design of the study, designing of all experiments and immune work, critical revision of the article for important intellectual content, and finalized the manuscript.

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CONFLICTS OF INTEREST

The authors have no conflicts to report.

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