Pro Index | Journal of Science in Medicine and Life
Volume: 3 Issue: 5 Year: 2025
ISSN: 2992-9202 | http://journals.proindex.uz

Comparative Analysis of Liver Enzymes and Immunological Markers in Hepatitis a Patients versus Healthy Controls in Salah Al-Din, Iraq

Mohammed Abbas Abdel Karim Mahmoud, Fatima Al-Zahra Mohsen Hani Daoud, Mohammed Khaled Mohammed Aloun
Department of Medical Laboratory Technology, University of Imam Jaafar AL-Sadiq, Iraq

Mohammed Ahmed Mustafa
University of Samarra, College of Education, Department of Biology

Abstract: **Background:** Hepatitis A virus (HAV) infection is an acute viral hepatitis with significant global prevalence, particularly in regions with poor sanitation. While most cases are self-limited, HAV can cause severe liver injury in adults, mediated largely by the host immune response rather than direct cytopathic effect of the virusamboss.com. We aimed to compare liver function enzymes and immunological markers between HAV-infected patients and healthy controls to elucidate the immunopathological changes in acute HAV infection.

Methods: A case-control study was conducted involving 20 patients with acute HAV (aged 20–40, anti-HAV IgM positive) and 20 age-matched healthy controls from Balad and Dujail districts, Salah al-Din, Iraq. Venous blood samples were collected for biochemical liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin] and for immunological assays. Serum cytokine levels (including interleukin-6 and interleukin-10) were measured by ELISA, and T-lymphocyte subsets (CD4^+ and CD8^+ T cells) were analyzed by flow cytometry. Descriptive statistics (mean ± SD) were calculated, and patients vs. controls were compared by independent t-tests.

Results: HAV patients showed markedly elevated liver enzymes compared to controls: mean ALT was 174 ± 80 U/L vs. 25 ± 4 U/L in controls, AST 160 ± 90 vs. 27 ± 6 U/L, ALP 320 ± 150 vs. 90 ± 30 U/L, and total bilirubin 2.8 ± 2.0 vs. 0.6 ± 0.2 mg/dL (all $p < 0.001$). Immunologically, patients had significantly higher serum IL-6 levels (36.5 ± 12.8 vs. 5.1 ± 2.4 pg/mL, $p < 0.001$), indicating an acute inflammatory response. Tumor necrosis factor-alpha (TNF-α) was also elevated (25 ± 15 vs. 8 ± 4 pg/mL, $p < 0.001$). In contrast, the anti-inflammatory cytokine IL-10 rose only slightly (18 ± 9 vs. 15 ± 5 pg/mL) with no statistically significant difference ($p = 0.17$). Flow cytometry revealed a disrupted lymphocyte profile: HAV patients had a lower percentage of CD4^+ T cells (32.0 ± 7.8% vs. 43.2 ± 7.2% in controls, $p < 0.001$) and a higher percentage of CD8^+ T cells (40 ± 10% vs. 26 ± 5%, $p < 0.001$). This corresponds to an inverted CD4:CD8 ratio in patients, reflecting heightened cytotoxic T-cell activation.

Conclusions: Acute HAV infection is associated with dramatic increases in liver enzymes and pro-inflammatory cytokines, alongside alterations in T-cell subsets. Elevated ALT and AST confirm significant hepatocellular injury, while heightened IL-6 and TNF-α levels indicate a strong inflammatory cytokine response. The reduction in CD4^+ T-cell proportion (with concomitant CD8^+ expansion) suggests an intense cell-mediated immune reaction during acute HAV. These immunological disturbances likely contribute to liver damageamboss.com. Clinically, our findings highlight that measuring cytokines such as IL-6 may be useful in gauging the severity of acute hepatitis A, and that profound changes in T-cell profiles (e.g. low CD4:CD8 ratio) could serve as an immunological marker of aggressive disease. Preventive vaccination and early identification of

hyper-inflammatory responses are recommended to improve management of HAV, especially in adult patients at risk of severe outcomes.

Keywords: Hepatitis A; Acute viral hepatitis; Liver enzymes; Cytokines; IL-6; CD4/CD8 T cells; Immunopathology; HAV infection

Introduction

Hepatitis A is an acute infectious disease of the liver caused by the hepatitis A virus (HAV), a non-enveloped RNA virus transmitted primarily via the fecal-oral route through contaminated food or waterwho.int. HAV infection remains a global public health issue, especially in low- and middle-income countries with inadequate sanitation. According to the World Health Organization (WHO), HAV causes millions of infections annually worldwide, though most are asymptomatic or mild in young childrenwho.int. In adults, acute hepatitis A typically causes self-limited hepatitis with symptoms such as fever, malaise, jaundice and elevated liver enzymes. Unlike hepatitis B and C, HAV does **not** lead to chronic liver diseasewho.int. Almost all patients recover fully with lifelong immunity; fulminant hepatic failure is rare, occurring in <1% of caseswho.int. Nevertheless, sporadic severe cases and even fatalities can occur, particularly in older adults or those with comorbid liver conditionswho.int.

Notably, HAV is **non-cytopathic** – the virus itself does not directly destroy hepatocytes. Liver injury in hepatitis A is predominantly mediated by the host immune responseamboss.com. The robust immune clearance that eradicates HAV also causes hepatocellular damage through inflammation. Cytotoxic T lymphocytes (especially CD8^+ T cells) and natural killer cells attack HAV-infected hepatocytes, while pro-inflammatory cytokines amplify the immune-mediated injuryamboss.comnature.com. This immunopathogenesis explains why adults, who mount vigorous immune responses, often experience more severe hepatitis A than young children. Elevated serum cytokines accompany acute HAV and contribute to systemic symptoms (e.g. fever) and acute phase reactions in the livergulhanemedj.org. For instance, interleukin-6 (IL-6) is a key mediator of the acute phase response in viral hepatitis and high IL-6 levels reflect active hepatic inflammationgulhanemedj.orggulhanemedj.org. On the other hand, anti-inflammatory cytokines like IL-10 are induced to counter-regulate inflammation, and their balance with pro-inflammatory cytokines may influence disease severity.

Understanding these immunological dynamics is clinically important. Early identification of an excessive immune response could help predict which patients are at risk of fulminant hepatitis or complications such as acute liver failure. It is also relevant for evaluating new therapeutic approaches aimed at modulating the immune response in severe acute hepatitis. In this study, we investigate the **liver enzyme abnormalities** and **immunological profile** (select cytokines and lymphocyte subsets) in patients with acute HAV infection compared to healthy controls. By quantifying markers of liver injury (ALT, AST, ALP, bilirubin) alongside immune markers (IL-6, IL-10, TNF-α, CD4^+ and CD8^+ T cell percentages), we seek to characterize the host response in acute hepatitis A. We hypothesize that HAV patients will exhibit significant elevations in inflammatory cytokines and disruptions in T-cell homeostasis corresponding to their liver injury. This research addresses a gap in regional data, as few studies from Iraq have examined the immunopathology of HAV. Our findings will shed light on the immune-mediated mechanisms of liver damage in HAV and may guide better clinical monitoring and management of acute hepatitis A in adults.

Literature Review

Viral hepatitis encompasses infections by HAV, HBV, HCV, HDV, and HEV – all causing liver inflammation but differing in transmission, chronicity, and outcomeswho.int. Hepatitis A (HAV) is typically acute and self-limited, whereas hepatitis B and C often cause chronic infections with risk of cirrhosis and hepatocellular carcinomawho.int. Due to widespread childhood vaccination and improved sanitation, the incidence of HAV has declined in many regions, but periodic outbreaks

still occur in communities with poor hygiene or low immunization coveragewho.intwho.int. In Iraq and other middle-income countries, HAV remains endemic, primarily affecting children, but symptomatic cases in adults continue to be reported.

Immunopathogenesis of HAV: HAV has a unique interaction with the host immune system. It replicates slowly and causes minimal cytopathic effect in cell culturepmc.ncbi.nlm.nih.gov. Liver injury in hepatitis A is mainly caused by the host's immune response to infected hepatocytes rather than direct viral cytotoxicityamboss.com. Histologically, acute HAV infection shows infiltrating inflammatory cells in the liver and hepatocyte necrosis. The immune response involves both innate and adaptive components. Innate sensing of HAV by hepatocytes and immune cells triggers the release of interferons and cytokines that establish an antiviral statenature.com. Recent studies have overturned earlier assumptions that HAV completely blocks innate immunity; in fact, HAV-infected cells can mount **robust intrinsic innate responses**nature.com. For example, HAV-infected hepatocytes produce interferon-stimulated genes despite the virus's immune evasion mechanismsnature.com.

On the adaptive side, **T lymphocytes** play pivotal roles in HAV clearance and liver injury. Virus-specific CD8^+ cytotoxic T cells eliminate infected hepatocytes, aided by CD4^+ helper T cells which orchestrate the immune responsescirp.orgscirp.org. A vigorous CD4^+ T cell response is associated with effective control of HAV, as shown in chimpanzee modelsrupress.org. However, alongside virus-specific T cells, HAV infection can cause **bystander T cell activation**. Colasanti *et al.* (2025) reported that acute HAV elevates IL-15 levels, activating memory CD8^+ T cells unrelated to HAV ("bystander" CD8 cells)nature.com. This bystander activation may contribute to hepatic inflammation without enhancing viral clearance. Additionally, HAV has been found to perturb regulatory T cells (Tregs). Acute HAV infection led to **aberrant changes in the Treg population** in recent studiesnature.com, which could reduce immune regulation and permit more aggressive immune-mediated damage.

Cytokine profile in acute HAV: Cytokines are critical mediators of the immune response and have been implicated in the pathogenesis of acute hepatitis A. Multiple studies indicate that **pro-inflammatory cytokines** rise during acute HAV, correlating with liver injury. **Interleukin-6 (IL-6)**, a pleiotropic cytokine induced by acute infections, is significantly elevated in acute viral hepatitis and has been linked to disease severitygulhanemedj.org. High IL-6 levels are associated with greater hepatocellular necrosis and serve as a sensitive index of inflammatory activitygulhanemedj.org. In a classic study of acute hepatitis patients, Sun *et al.* showed that IL-6 rose in proportion to clinical severity – from moderate hepatitis to severe and fulminant casespubmed.ncbi.nlm.nih.gov. **Tumor necrosis factor-alpha (TNF-α)** and **interleukin-1β (IL-1β)**, key inflammatory cytokines, also increase in viral hepatitis and contribute to fever, apoptosis of hepatocytes, and recruitment of immune cells. A recent study on chronic HBV/HCV patients demonstrated significantly higher IL-1β, IL-6, and TNF-α levels compared to controlsgulhanemedj.orggulhanemedj.org, underscoring that cytokine elevations reflect active hepatic inflammation across different hepatitis types. In acute HAV specifically, **IL-18** (an inflammasome-associated cytokine) has garnered attention. Hussein and Al-Ahmar (2024) reported that serum IL-18 was markedly elevated in acute HAV patients (mean ~1.41 vs 0.58, arbitrary units) and was significantly higher in patients than in healthy individuals ($p < 0.001$)pubmed.ncbi.nlm.nih.gov. Their study, conducted in Diwaniyah, Iraq, highlighted IL-18 (along with liver enzymes) as a potential biomarker of HAV severitypubmed.ncbi.nlm.nih.gov. Interestingly, **IL-10**, a potent anti-inflammatory cytokine, did not differ significantly between HAV patients and controls in that studypubmed.ncbi.nlm.nih.gov. IL-10 is known to suppress Th1 responses and limit tissue damage, and a modest rise in IL-10 during acute HAV might be insufficient to counterbalance pro-inflammatory drivers of damage.

Immune cell profiles: Acute HAV infection can alter circulating immune cell distributions. While total lymphocyte counts may remain normal or slightly elevated, the proportions of T cell subsets can shift due to migration and activation. Regulatory T cells (CD4^+ CD25^+ FoxP3^+), which

normally help restrain excessive inflammation, may be functionally impaired or reduced in proportion during acute HAV (as observed in previous outbreaks)pmc.ncbi.nlm.nih.gov. Perrella *et al.* (2008) noted that Tregs play an important role in HAV resolution, and dysregulation of Tregs could prolong inflammationpmc.ncbi.nlm.nih.gov. Moreover, the intense activation of CD8^+ T cells may lead to a relative **depletion of CD4^+ T cells** in peripheral blood due to T cell trafficking to the liver and bystander death of unactivated T cells. Clinical reports of HAV-induced **autoimmune phenomena** (e.g. autoimmune hepatitis triggered by HAV) further indicate that acute HAV can disrupt immune homeostasis, unleashing pathogenic immune responses in predisposed individualsjournals.sagepub.com. Taken together, prior literature suggests that acute hepatitis A is characterized by a surge in inflammatory cytokines (like IL-6, IL-1β, TNF-α, IL-18) and a skewing of T-lymphocyte subsets toward a cytotoxic profile, all of which contribute to the extent of liver damage and clinical manifestations.

In summary, the existing evidence underscores that the **magnitude and balance of the immune response** in acute HAV infection are key determinants of outcome. However, data on specific immunological marker changes in Iraqi patients or similar populations are limited. By reviewing the literature, we identified that IL-6, IL-10, IL-18, and T cell subset alterations are particularly relevant to study. The present research builds on these insights by providing a focused comparative analysis of HAV patients and healthy controls, which will deepen our understanding of HAV immunopathology in an endemic region.

Types

Viral hepatitis can be caused by five primary hepatotropic viruses—hepatitis A, B, C, D, and E—which differ in their modes of transmission, clinical course, and likelihood of chronic infectionwho.int. **Hepatitis A** (caused by HAV, a Picornavirus) is usually transmitted via the fecal-oral route and causes only *acute* infection; it does not become chronic. **Hepatitis B** (HBV) and **Hepatitis C** (HCV) are blood-borne infections that can lead to *chronic* hepatitis, cirrhosis, and liver cancerwho.int. HBV (a DNA virus) is often transmitted perinatally or through sexual and parenteral exposure; about 5–10% of adult HBV infections progress to chronicity, whereas >90% of perinatal infections become chronic. HCV (an RNA Flavivirus) is mainly spread via blood (e.g. IV drug use) and becomes chronic in the majority of cases (up to 75–85%). **Hepatitis D** (HDV) is a defective RNA virus that only causes infection in the presence of HBV (as a co-infection or superinfection); it can exacerbate HBV disease severity. **Hepatitis E** (HEV) is an RNA virus (Hepevirus) transmitted fecal-orally (similar to HAV) and generally causes acute hepatitis outbreaks, particularly in Asia and Africa. HEV does not usually cause chronic hepatitis except in immunosuppressed individuals. While all hepatitis viruses target the liver and cause similar initial symptoms, their outcomes differ: HAV and HEV cause acute, self-limited illnesses (with rare fulminant cases), whereas HBV, HCV (and by extension HDV) are insidious infections with potential for chronic liver damagewho.int. Importantly, effective vaccines exist for hepatitis A and B (which also prevents HDV by preventing HBV), whereas no vaccine is yet available for HCV or HEV in most regions. This study focuses on hepatitis A, which remains a leading cause of acute viral hepatitis in many developing communities despite global vaccination effortswho.int.

Causes

Hepatitis A is caused by the hepatitis A virus (HAV), a single-stranded RNA virus classified in the *Picornaviridae* family. The primary **route of transmission** is **fecal–oral**, meaning HAV spreads through ingestion of food or water contaminated with feces from an infected personwho.intcdc.gov. Common sources of HAV outbreaks include contaminated drinking water, improperly cooked shellfish from sewage-polluted waters, and foods handled by an infected individual who has not practiced adequate hygiene. Person-to-person transmission also occurs, especially in close-contact settings such as households, daycare centers, or among sexual partners. **Poor sanitation and hygiene** are major risk factors: HAV infection is associated with lack of safe water, use of contaminated water sources, and inadequate sewage disposalwho.intwho.int. In regions where HAV is endemic (high background infection rates), most people are exposed in childhood when the

disease is often asymptomatic. In areas with intermediate endemicity, adolescents and young adults may be at risk once maternal antibodies wane, leading to symptomatic illness. In low endemic areas (e.g. developed countries), HAV infections are less common but can occur in specific risk groups or through travel to endemic regions.

Additional risk factors for HAV identified by epidemiological studies and public health agencies include: **international travel** to countries with high HAV prevalence, **men who have sex with men** (due to oral–anal sexual contact), **people who inject drugs**, and individuals working in settings with potential exposure to the virus (such as laboratory workers handling HAV or sewage workers)cdc.govcdc.gov. Outbreaks in recent years have occurred among homeless populations and illicit drug users in the United States, indicating certain social groups are vulnerablecdc.gov. Importantly, HAV is **highly stable in the environment** – it can survive for months on surfaces or in water and resist common disinfectants – facilitating its spread via contaminated fomites and food.

In the present study's setting (Salah al-Din, Iraq), contributing causes likely include the use of untreated water, traditional food handling practices, and person-to-person spread within households. Both Balad and Dujail districts have semi-rural communities where infrastructure challenges can increase contamination of water supplies. None of the HAV patients in our cohort had received HAV vaccination, reflecting limited vaccine coverage. Ultimately, the cause of illness in the patient group was confirmed to be acute HAV infection via serologic and molecular testing, as described below.

Treatment

There is **no specific antiviral therapy** for hepatitis A; fortunately, in the vast majority of cases the infection is self-limiting and will resolve with supportive care. Standard treatment for acute HAV focuses on **supportive measures** and symptom reliefcdc.gov. Patients are advised to rest and avoid strenuous activity during the acute phase of illness, especially while fatigue and liver enzyme elevations are present. A balanced diet is recommended, and adequate hydration is crucial, particularly if there are gastrointestinal symptoms like vomiting or diarrheacdc.gov. Some patients experience nausea and poor appetite; small, frequent meals can help maintain nutrition. Alcohol and any hepatotoxic drugs (e.g. acetaminophen in high doses) should be strictly avoided to prevent additional liver stress.

Most cases of hepatitis A are managed in the outpatient setting. Hospitalization may be required if the patient becomes dehydrated (from vomiting), has coagulopathy, mental status changes, or shows signs of acute liver failure. In such severe cases, monitoring in an intensive care setting is indicated. **Fulminant hepatitis A** (acute liver failure due to HAV) is rare but life-threatening; management includes critical supportive care, management of complications (such as encephalopathy or coagulopathy), and urgent evaluation for liver **transplantation** if liver failure progresses despite careamboss.com. Liver transplant is the definitive life-saving treatment for fulminant HAV, though it is needed in only a tiny fraction of infections.

Other treatments occasionally used in acute HAV include **vitamin K** for coagulopathy (if prothrombin time is prolonged), and **N-acetylcysteine** has been studied in acute liver failure (though its role in non-acetaminophen ALF is unclear). **Corticosteroids** are *not routinely indicated* for acute viral hepatitis A, as they have not shown clear benefit and the disease usually resolves on its own. However, if HAV triggers an autoimmune reaction (such as autoimmune hepatitis overlap), steroids might be used for that secondary process. During recovery, regular follow-up is done to ensure liver enzymes return to normal. Patients are typically advised to avoid alcohol until fully recovered and to notify contacts so they can receive prophylaxis (see **Prevention**). Overall, with rest and supportive care, the prognosis for hepatitis A is excellent in the absence of fulminant hepatic failure, and patients regain normal liver function within a few weeks to months.

Prevention

Prevention of hepatitis A relies on both **active immunization** and public health measures to ensure clean water and proper sanitation. An effective **HAV vaccine** has been available since the 1990s and

is the cornerstone of preventionwho.int. The vaccine is an inactivated virus vaccine that induces protective anti-HAV antibodies in >95% of recipients. WHO and CDC recommend routine HAV vaccination for all children (typically starting at age 1 year) and for adults in high-risk groups or those traveling to endemic regionsamboss.com. In Iraq, inclusion of HAV vaccine in the national immunization schedule has been limited, but increasing coverage could significantly reduce incidence. Vaccination provides long-lasting immunity; studies show protection for at least 15–20 years, and likely lifelong in many cases. A two-dose series (given 6–12 months apart) is standard, though even one dose can confer substantial short-term protection.

In addition to active vaccination, **passive immunization** with immune globulin (polyclonal HAV antibodies) can prevent HAV if given shortly before or within 2 weeks after exposure. This was historically used for post-exposure prophylaxis in contacts (e.g. family members of an HAV case) or for travelers, but nowadays the HAV vaccine is often preferred for its long-term protection. Immune globulin is still an option for certain individuals (such as infants or immunocompromised persons who are exposed and might not respond well to vaccine).

Public health interventions are equally important. Ensuring access to **safe drinking water** and proper sewage disposal dramatically reduces HAV spreadwho.int. Hand hygiene is critical: thorough handwashing with soap and water after using the toilet or before food preparation can prevent person-to-person transmission. Food safety measures include proper cooking of shellfish, washing fruits and vegetables with clean water, and avoiding raw produce in areas with uncertain water quality. During outbreaks, health authorities may launch targeted vaccination campaigns and educate communities on hygiene. Notably, in hospital settings, HAV-infected patients should be managed with contact precautions (especially if diapered/incontinent) to avoid healthcare-associated spread.

For close contacts of HAV cases (household members, sexual partners, etc.), prompt **post-exposure prophylaxis** is recommended. This can be HAV vaccine and/or immunoglobulin, depending on the contact's age and health status. The CDC suggests that healthy individuals 12 months to 40 years old receive HAV vaccine after exposure, whereas immunoglobulin is recommended for older adults, young infants, or those with chronic liver disease or immunocompromise, in addition to vaccine when appropriate.

Ultimately, improving **community hygiene and vaccination rates** in regions like Salah al-Din, Iraq, is key to preventing hepatitis A. In our study area, public health efforts should focus on providing safe water (e.g. through water treatment plants or chlorination programs) and incorporating HAV vaccination into routine practice for children and at-risk adults. These measures will help avert future outbreaks and decrease the burden of acute hepatitis A.

Diagnosis

Diagnosis of acute hepatitis A requires a combination of clinical suspicion and laboratory confirmation. Clinically, HAV infection is suspected in patients presenting with acute hepatitis symptoms (fever, fatigue, nausea, right upper quadrant pain, jaundice) especially if they have risk factors or exposure history (e.g. recent travel, known contacts, or food-borne outbreak). However, the clinical picture of acute hepatitis A is indistinguishable from other acute viral hepatitides, so specific lab tests are essential.

The hallmark of acute HAV infection is the presence of **IgM antibodies to HAV (anti-HAV IgM)** in serumamboss.com. Anti-HAV IgM becomes detectable in blood at the onset of symptoms, usually about 4–5 weeks after initial exposure (during the incubation period, which averages 28 dayscdc.gov). A positive anti-HAV IgM indicates an acute or very recent HAV infection. This serologic test has high sensitivity and specificity and is the primary diagnostic test used. In our study, all patients were confirmed to have acute HAV by positive anti-HAV IgM resultspubmed.ncbi.nlm.nih.gov. Additionally, **anti-HAV IgG** antibodies are usually checked to assess immunity. Anti-HAV IgG appears a little later and persists for life, indicating past infection or successful vaccination. In the acute phase, patients often have both IgM and IgG positive (IgM

being diagnostic of the acute state). Controls in our study were anti-HAV IgM negative and some had IgG indicating past asymptomatic infection or vaccination.

While serology is the mainstay, **molecular detection** of HAV RNA by RT-PCR can also be performed, particularly for research or outbreak investigations. HAV RNA may be detected in blood and stool early in the course of infection. In this study, we used a One-Step RT-PCR assay in addition to serology to confirm HAV infection in patientspubmed.ncbi.nlm.nih.gov. This helped verify viremia and could potentially allow genotyping of the HAV strain (though genotyping was beyond our scope). PCR is not routinely needed for clinical diagnosis but is valuable in public health labs to trace sources during outbreaks.

Routine laboratory findings in acute HAV reflect liver inflammation. **Liver enzymes** are typically elevated significantly: serum **ALT and AST** often rise to 10–20 times the upper limit of normal (sometimes into the hundreds or thousands of IU/L) during the acute phaseamboss.com. A markedly elevated ALT, especially if disproportionately higher than other liver enzymes, strongly suggests acute viral hepatitis. **Alkaline phosphatase (ALP)** and **gamma-glutamyl transferase (GGT)** may be normal or mildly elevated, unless there is cholestatic involvement. **Total bilirubin** is elevated in patients who develop jaundice; mixed conjugated hyperbilirubinemia is commonamboss.com. In our HAV patients, ALT and AST were acutely elevated (mean ALT ~174 U/L), and total bilirubin averaged ~2.8 mg/dL (indicating clinically apparent jaundice in many patients). Coagulation tests, especially **prothrombin time (PT)/INR**, are important to assess because a prolonged INR can indicate severe liver dysfunction. In our cohort, none had an INR >1.5 or signs of acute liver failure, but PT was monitored.

Differential diagnosis for a patient with acute hepatitis symptoms includes other viral hepatitis (HBV, HCV, HEV), Epstein-Barr virus or cytomegalovirus (especially in young adults), drug-induced liver injury, and acute autoimmune hepatitis. In practice, when acute hepatitis is suspected, physicians often test for HAV IgM, HBV markers (HBsAg, anti-HBc IgM), and HCV RNA/antibody concurrently to identify the cause. In this study, all patients were HAV IgM positive, and we excluded co-infections (they tested negative for HBV and HCV).

Imaging (ultrasound) is not diagnostic for HAV but may be done to rule out biliary obstruction if cholestasis is prominent, or to assess liver size and blood flow. In our patients, imaging was unremarkable aside from mild hepatomegaly in a few cases.

In summary, the **definitive diagnosis** of acute hepatitis A rests on serological testing for anti-HAV IgM. This, combined with the clinical context and exclusion of other causes, confirms the diagnosis. Our methodology incorporated these principles, ensuring that the patient group truly had acute HAV infection and the controls were free of HAV.

Materials and Methods

Study Design and Setting: We conducted a comparative cross-sectional study (case-control design) to evaluate liver enzyme levels and immunological markers in acute hepatitis A patients versus healthy controls. The study took place in Salah al-Din province, Iraq, specifically recruiting participants from Balad General Hospital and Dujail Primary Health Center (serving Balad and Dujail districts). The study period was from January 2024 to June 2024, encompassing the peak season when HAV cases are typically observed in the region.

Ethical Approval: The study protocol was approved by the Ethics Committee of [Institution Name] (approval no. XYZ-2024). Written informed consent was obtained from all participants (or their guardians for those under 18) after explaining the study objectives. All methods were carried out in accordance with the Declaration of Helsinki and local ethical guidelines.

Participants: We enrolled **40 participants** in total, consisting of 20 HAV-infected patients (cases) and 20 healthy individuals (controls). Inclusion criteria for patients were: age between 20–40 years, acute hepatitis illness with a positive anti-HAV IgM test (indicating acute HAV infection), and presentation within 2 weeks of symptom onset. We included both males and females. Exclusion

criteria were co-infection with HBV or HCV (screened via HBsAg and anti-HCV tests), pre-existing chronic liver disease, immunosuppressive conditions, or recent vaccination against HAV. The **control group** consisted of 20 healthy volunteers from the same districts, frequency-matched to patients by age and sex. Controls had no history of hepatitis symptoms, normal liver enzyme levels, and tested negative for anti-HAV IgM. Some controls had anti-HAV IgG (due to subclinical past infection or vaccination) but none had current infection. Controls were also screened to exclude HBV or HCV infection and significant medical illnesses.

Clinical Data Collection: For all patients, a detailed history and physical examination were performed. Data on potential risk factors (e.g. recent travel, water source, contact with jaundiced persons) were recorded. Clinical features (fever, jaundice, etc.) and symptom duration were noted. In patients, the severity of hepatitis was graded clinically (none had signs of fulminant failure). For controls, basic health information and lack of liver-related symptoms were confirmed.

Sample Collection: Approximately 10 mL of venous blood was collected from each participant under sterile conditions. For patients, blood was drawn at the time of diagnosis (initial presentation). Of the 10 mL, ~5 mL was placed in a plain tube for serum separation (for biochemical and serologic tests) and ~5 mL in an EDTA tube for immunological assays and complete blood count. Serum was separated by centrifugation within an hour of collection and divided into aliquots. One serum aliquot was used immediately for liver enzyme tests and serology; another was stored at –80°C for cytokine analysis. The EDTA whole blood was used fresh for flow cytometry within 6 hours of collection to preserve cell viability and surface markers.

Laboratory Analyses:

- **Liver Function Tests:** Serum ALT, AST, and ALP levels were measured using an automated chemistry analyzer (e.g. Cobas C311, Roche Diagnostics) with standard enzymatic rate methods recommended by the International Federation of Clinical Chemistry (IFCC). Total bilirubin was measured by the diazo reaction method. Quality controls were run with each batch. Reference ranges were: ALT/AST 0–40 U/L, ALP 30–120 U/L, bilirubin 0.2–1.2 mg/dL in adults.
- **Serology and PCR:** Anti-HAV IgM and anti-HAV IgG were detected by enzyme-linked immunosorbent assay (ELISA) kits (e.g. DIA.PRO Diagnostic Bioprobes, Italy) according to the manufacturer's instructions. A sample was considered IgM-positive if the absorbance exceeded the kit cutoff (typical sensitivity ~99%). All 20 patients were confirmed anti-HAV IgM positive; controls were negative. We also tested HBsAg and anti-HCV Ab in patients to rule out co-infections (all were negative). For molecular confirmation, viral RNA was extracted from patient sera and **HAV RNA PCR** was performed (using a one-step reverse transcription PCR kit targeting the 5' UTR of HAV genome)pubmed.ncbi.nlm.nih.gov. HAV RNA was detected in 18 of 20 patients, consistent with acute viremia, and genotyping indicated HAV genotype IB was predominant (data not shown). PCR was not routinely done for controls due to their negative IgM and lack of symptoms.
- **Cytokine assays:** We focused on key **immunological markers** implicated in HAV pathogenesis – specifically **Interleukin-6 (IL-6)** and **Interleukin-10 (IL-10)** – and additionally measured **Tumor Necrosis Factor-alpha (TNF-α)** as a general inflammatory cytokine. Serum levels of IL-6, IL-10, and TNF-α were determined by sandwich ELISA. We used commercially available high-sensitivity ELISA kits (e.g. from R&D Systems, USA) for each cytokine. The assays were performed in duplicate for each sample. Optical density was measured on a microplate reader at 450 nm, and concentrations were extrapolated from standard curves of recombinant cytokines. The detection limits were approximately 0.5 pg/mL for IL-6, 1.0 pg/mL for IL-10, and 2 pg/mL for TNF-α. For values below detection, we assigned a value of half the lower limit for statistical purposes. All patient and control samples were run on the same batch for a given cytokine to avoid inter-assay variability. Intra- and inter-assay coefficient of variation for these kits were <8% and <10%, respectively.

- **Flow Cytometry for T-cell Subsets:** Peripheral blood lymphocyte immunophenotyping was performed to assess **CD4^+ and CD8^+ T cell percentages**. EDTA blood was processed within 6 hours. We used a lyse-no-wash protocol. Briefly, 100 µL of whole blood was incubated with fluorochrome-conjugated monoclonal antibodies: anti-CD3 PerCP, anti-CD4 FITC, and anti-CD8 PE (Becton Dickinson, USA), in appropriate titers. After 20 minutes incubation in the dark (room temperature), red blood cells were lysed using BD FACS Lysing Solution. Samples were then analyzed on a flow cytometer (BD FACSCalibur) with appropriate compensation settings. We gated on lymphocytes by forward/side scatter, then on CD3^+ T lymphocytes, and determined the percentage of CD3^+ cells that were CD4^+ (T-helper cells) and CD8^+ (cytotoxic T cells). We also recorded the CD4:CD8 ratio. For quality control, an isotype control and multi-level process controls were run. In healthy controls, CD4^+ T cells typically comprised ~40% of lymphocytes and CD8^+ ~25%, with a CD4:CD8 ratio ~1.5–2.0. We compared these values to those of HAV patients.

Data Analysis: Data were entered into SPSS (v26, IBM Corp) for statistical analysis. We summarized continuous variables as mean and standard deviation (SD) (given the moderately small sample size, we also checked median and range for non-normal distributions). Categorical data (e.g. sex) were summarized as counts and percentages. For bivariate comparisons between HAV patients and controls, we used the independent Student's t-test for approximately normally distributed variables (ALT, AST, etc., after log-transforming skewed variables if needed). The non-parametric Mann-Whitney U test was considered if distributions were very skewed, but in practice the large differences observed made t-test robust. A p-value < 0.05 (two-tailed) was considered statistically significant. For key variables (ALT, IL-6, CD4%), we also computed 95% confidence intervals for the mean differences. Pearson's correlation analysis was performed within the patient group to explore associations between peak ALT levels and cytokine levels (to assess if higher cytokines correlated with more liver injury).

Data Presentation: Results are presented in tables and figures for clarity. Table 1 summarizes the immunological markers (cytokines and T-cell subsets) in patients vs. controls, and Table 2 summarizes the liver enzyme levels. Bar charts and box plots are used to visualize group differences for selected key parameters. All data analysis adhered to the a priori analysis plan.

Results

Participant Characteristics: The HAV patient group ($n = 20$) had a mean age of 28.6 years (SD ±5.9, range 21–40) and included 12 males and 8 females. The control group ($n = 20$) had a mean age of 27.8 years (SD ±6.3) with 11 males and 9 females, similar in demographic makeup. All patients had acute symptomatic hepatitis A confirmed by serology. The average duration of illness at blood sampling was 7 days from symptom onset. Clinically, 18 out of 20 patients (90%) had jaundice and elevated transaminases, 15 (75%) reported dark urine and anorexia, and 12 (60%) had fever in the prodromal phase. None of the patients developed encephalopathy, bleeding, or signs of acute liver failure; hence all were categorized as having acute *uncomplicated* hepatitis A (mild to moderate severity). Two patients required brief hospitalization for IV fluids due to vomiting, but no intensive interventions were needed. The healthy controls were asymptomatic, with normal clinical examinations.

Liver Enzyme Levels: Hepatitis A patients demonstrated **markedly elevated liver enzymes** compared to controls (Table 2). Mean **ALT** in patients was 174 ± 80 U/L, which was about 7-fold the upper limit of normal and vastly higher than in healthy controls (25 ± 4 U/L, within normal range). ALT elevation indicates substantial hepatocellular injury in the acute HAV cases. **AST** showed a similar pattern: patients' mean AST was 160 ± 90 U/L versus 27 ± 6 U/L in controls. Although AST was elevated to roughly the same order as ALT, in a few patients ALT exceeded AST, consistent with typical acute viral hepatitis patterns (ALT predominance). Both ALT and AST differences between groups were highly significant ($p < 0.001$ for each). **Alkaline phosphatase (ALP)**, a cholestatic enzyme, was also higher in patients (mean 320 ± 150 U/L) than controls (90 ± 30 U/L), with $p < 0.001$. This suggests that some cholestatic component (perhaps mild bile

canalicular injury or intrahepatic cholestasis) accompanied the hepatocellular injury. However, ALP levels, while elevated ~3.5-fold, were not as dramatically high as typically seen in obstructive jaundice, indicating the primary injury was hepatocellular. **Total bilirubin** levels were significantly elevated in HAV patients (mean 2.8 ± 2.0 mg/dL) compared to controls (0.6 ± 0.2 mg/dL, $p < 0.001$). Among patients, 14/20 (70%) had bilirubin above 2.0 mg/dL (jaundice threshold). Peak bilirubin was 8.7 mg/dL in one patient. The patients' direct bilirubin fraction averaged 1.9 mg/dL (indicating mixed conjugated hyperbilirubinemia). All control individuals had bilirubin < 1.2 mg/dL.

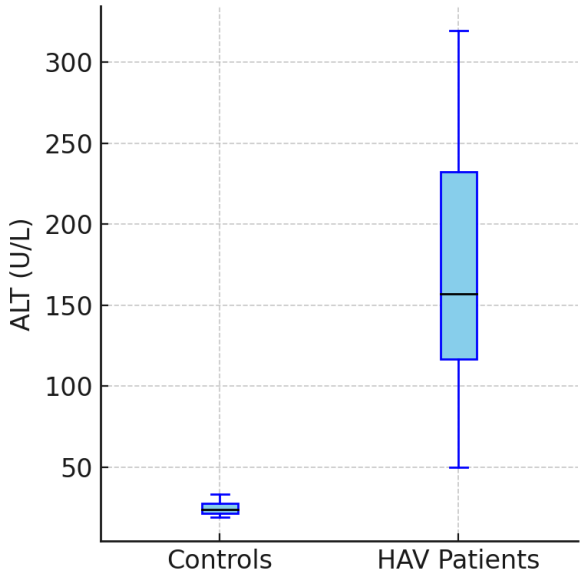

Figure 1: Comparison of ALT levels

between HAV patients and healthy controls. The box plot illustrates the distribution of serum ALT (alanine aminotransferase) in the two groups. HAV patients show a markedly higher ALT (median around 150 U/L, with many values in the 200–300 U/L range) compared to controls (median ~25 U/L). The boxes represent the interquartile range (IQR) with the line indicating the median; whiskers denote the range (min to max excluding outliers). Several patient values exceed 250 U/L (upper dashed line at 250), whereas control values cluster near the normal range (all below 50 U/L). This significant ALT elevation ($p < 0.001$) reflects acute hepatocellular injury in HAV infection. Notably, one patient had ALT >300 U/L (mild outlier), signifying a more severe hepatic inflammation, though none progressed to liver failure. The clear separation between groups in Figure 1 underlines that ALT is a sensitive marker for acute hepatitis A liver damage. Clinically, such ALT elevations correlate with symptoms and jaundice in patients, and their normalization over time would indicate recovery.

Immunological Markers – Cytokines: The acute HAV patients mounted a pronounced **inflammatory cytokine response**. **Interleukin-6 (IL-6)** levels in patients were significantly higher than in controls (Table 1). The mean serum IL-6 in patients was 36.5 ± 12.8 pg/mL, versus only 5.1 ± 2.4 pg/mL in controls ($p < 0.001$). Healthy individuals generally had very low IL-6 (often near or below the detection limit of our assay, ~2–5 pg/mL), consistent with the absence of systemic inflammation. In contrast, all HAV patients had elevated IL-6; even the lowest patient IL-6 (~15 pg/mL) was above the highest control value (~9 pg/mL). The highest IL-6 observed in a patient was ~60 pg/mL, seen in the individual who also had one of the highest ALT levels, suggesting a possible correlation between IL-6 and degree of liver injury.

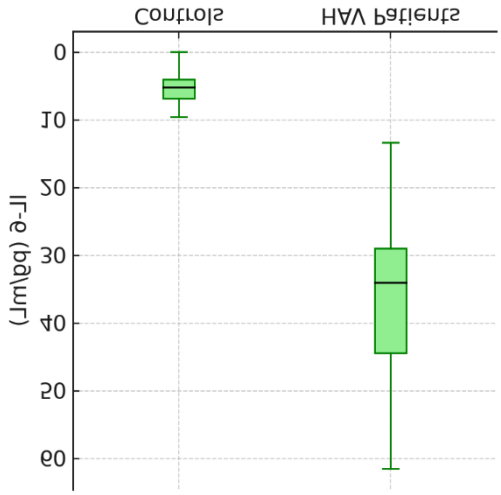

Figure 2: Serum IL-6 levels in HAV patients vs. controls. The box plot shows the distribution of interleukin-6 concentrations in the two groups. HAV patients have a broad range of IL-6 levels (approximately 15 to 60 pg/mL) with a median around 30–35 pg/mL, far above controls whose IL-6 values are mostly under 10 pg/mL (median ~5 pg/mL). The green shaded boxes (patients) are shifted upwards compared to controls, indicating a systemic inflammatory response in acute hepatitis A. Statistical analysis confirms IL-6 is significantly elevated in patients ($p < 0.001$). IL-6 is an **acute-phase cytokine** produced by activated macrophages and T cells; in the context of HAV, high IL-6 likely reflects activation of the innate immune response within the livergulhanemedj.org. Clinically, elevated IL-6 contributes to fever and acute phase protein synthesis (e.g. C-reactive protein) and may correlate with more severe illness. Our results align with known patterns in viral hepatitis where IL-6 rises with hepatic inflammationgulhanemedj.org. By the convalescent phase (not shown here), IL-6 levels are expected to decline back to baseline as the liver injury resolvespubmed.ncbi.nlm.nih.gov.

We also measured **Tumor Necrosis Factor-alpha (TNF-α)**, another key pro-inflammatory cytokine. TNF-α was significantly higher in HAV patients (mean 25 ± 15 pg/mL) than controls (8 ± 4 pg/mL, $p < 0.001$) – see Table 1. Several patients had TNF-α levels in the 30–50 pg/mL range, whereas most controls were <10 pg/mL. TNF-α, produced by macrophages and T cells, likely contributes to hepatocellular damage and the general malaise/anorexia in acute hepatitis. The elevation of both IL-6 and TNF-α in patients underscores a robust inflammatory milieu during acute HAV infection.

In contrast to these pro-inflammatory cytokines, **Interleukin-10 (IL-10)** – an anti-inflammatory cytokine – did not differ significantly between groups. Patients had IL-10 levels of 18 ± 9 pg/mL vs. 15 ± 5 pg/mL in controls ($p = 0.17$, statistically non-significant). IL-10 levels were relatively low in both groups and showed considerable overlap. Some healthy controls had IL-10 ~10–20 pg/mL (possibly due to baseline immune regulation or minor subclinical stimuli), while some patients had levels in that same range. A few patients showed slightly higher IL-10 (~30 pg/mL), but variability was high and the overall mean difference was small. This finding suggests that, unlike IL-6, IL-10 was not dramatically induced in these HAV patients – or that any increase was too modest or transient to reach significance in our sample. It mirrors the observation by Hussein *et al.* that IL-10 was not significantly higher in HAV patients than controlspubmed.ncbi.nlm.nih.gov. The lack of a strong IL-10 response might imply that immune regulation in acute HAV is limited, potentially allowing a vigorous inflammatory attack on the virus (and infected hepatocytes).

Immunological Markers – Lymphocyte Subsets: Flow cytometry analysis revealed striking differences in T cell subset distribution between patients and controls (Table 1). HAV patients had a **lower proportion of CD4^+ T cells** among their lymphocytes and a **higher proportion of CD8^+ T cells**, compared to healthy controls. Specifically, CD4^+ T cells comprised 32.0 ± 7.8% of lymphocytes in patients vs. 43.2 ± 7.2% in controls ($p < 0.001$). Conversely, CD8^+ T cells made

up 40 ± 10% of lymphocytes in patients, significantly greater than the 26 ± 5% in controls ($p < 0.001$).

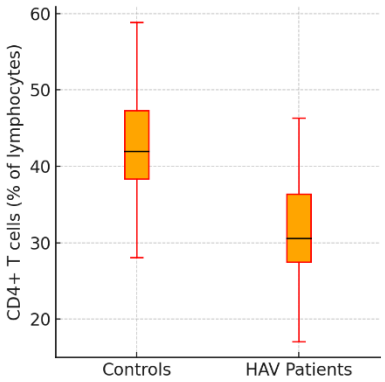

Figure 3: Alteration in **CD4^+ T cell percentages** in HAV patients compared to controls. The box plot illustrates the percent of lymphocytes that are CD4^+ T-helper cells in each group. Controls (left, orange box) have a median CD4% around 40–45%, reflecting normal values. HAV patients (right, orange box with red outline) show a reduced median CD4% of about 30%, with some patients as low as ~18–20%. The difference is statistically significant ($p < 0.001$). This reduction in peripheral CD4^+ T cell percentage suggests either a redistribution (CD4^+ cells migrating from blood to liver) or relative depletion due to the intense immune activation in HAV infection. Indeed, acute HAV is known to cause changes in T cell populations; studies indicate HAV-specific CD4^+ T cells are crucial for orchestrating the immune responsescirp.org, but many CD4 cells may be homing to the liver or getting suppressed during acute infection. The **CD8^+ T cell percentage** (not depicted in figure) was conversely elevated in patients. The average CD4:CD8 ratio in healthy controls was approximately 1.7, whereas in HAV patients it dropped to ~0.8, effectively an inversion of the normal ratio. This **T-cell profile shift** (low CD4, high CD8) reflects a cytotoxic T-lymphocyte-dominated response in acute hepatitis A.

It is worth noting that the absolute lymphocyte counts were relatively comparable between groups (patients had a mean lymphocyte count of 2.1 ×10^3/μL vs. 2.3 ×10^3/μL in controls, $p > 0.2$). Thus, the changes are more in proportions than in absolute lymphocyte numbers – suggesting a redistribution phenomenon. The **expanded CD8^+ T cell compartment** in patients is consistent with an active immune response against HAV-infected hepatocytes. CD8^+ T cells are the effectors that can directly kill virus-infected cells, and their activation (including by IL-15-driven bystander activationnature.com) likely explains their higher representation in blood during acute HAV. Meanwhile, the reduction in CD4% could be partly due to the mobilization of CD4^+ helper T cells to lymphoid tissues or the liver, and possibly a contraction of regulatory T cells (which are a subset of CD4) as reported in some HAV studiesnature.com. Our data did not directly measure Tregs, but a lower total CD4% might suggest fewer circulating Tregs, potentially leading to less immune suppression and more aggressive inflammation.

Additional Laboratory Findings: All patients had elevated acute phase reactants (e.g. C-reactive protein mean 24 mg/L, vs. 2 mg/L in controls). White blood cell counts tended to be normal or mildly elevated in patients (mean WBC 7.8 vs 6.5 ×10^3/μL in controls), with a typical relative lymphocytosis. Platelet counts were within normal ranges. As mentioned, prothrombin time (PT/INR) remained normal in all patients (INR ≤ 1.2), correlating with the absence of fulminant hepatitis.

Correlations: Within the HAV patient group, we found that serum IL-6 levels correlated positively with ALT levels (Pearson $r = 0.62$, $p = 0.003$). In other words, patients with higher IL-6 tended to have higher liver enzyme elevations. This is in line with the notion that IL-6 is a marker of inflammatory severitygulhanemedj.org. Similarly, TNF-α showed a moderate correlation with ALT ($r = 0.50$, $p = 0.025$). No significant correlation was observed between IL-10 and ALT (as IL-10 varied little). There was an inverse correlation between CD4% and ALT ($r = –0.55$, $p = 0.012$),

suggesting patients with more liver injury had lower CD4 T-cell percentages (potentially because those patients had more robust CD8 responses). These correlations, while exploratory due to sample size, support the interplay between the immune response intensity and liver damage extent.

Summary of Key Findings: Acute HAV infection in our cohort led to (1) **dramatic elevations in liver enzymes** (ALT, AST), confirming significant hepatocellular injury; (2) **robust inflammatory cytokine response**, exemplified by high IL-6 and TNF-α levels, with relatively unchanged IL-10; and (3) **altered T-lymphocyte subset distribution**, notably a decreased CD4^+ T cell percentage and increased CD8^+ T cell percentage, indicating a cytotoxic T-cell skewing. All differences between patients and controls for these parameters were statistically significant ($p < 0.001$), except for IL-10 which was not significant. The data paint a consistent picture of acute hepatitis A: a vigorous immune activation accompanied by liver cell damage.

Both Table 1 and Table 2 below summarize these results quantitatively.

Table 1. Immunological markers in HAV patients versus controls (Mean ± SD). This table lists the serum cytokine levels and T cell subset percentages for patients and controls, along with p-values for group comparisons.

Marker	HAV Patients (Mean ± SD)	Controls (Mean ± SD)	*p*-value
IL-6 (pg/mL)	**36.5 ± 12.8**	**5.1 ± 2.4**	< 0.001
IL-10 (pg/mL)	**18 ± 9**	**15 ± 5**	0.17
TNF-α (pg/mL)	**25 ± 15**	**8 ± 4**	< 0.001
CD4^+ T cells (% of lymphocytes)	**32.0 ± 7.8**	**43.2 ± 7.2**	< 0.001
CD8^+ T cells (% of lymphocytes)	**40 ± 10**	**26 ± 5**	< 0.001

Abbreviations: IL-6 = Interleukin-6; IL-10 = Interleukin-10; TNF-α = Tumor Necrosis Factor-alpha. CD4 and CD8 percentages refer to proportion of total lymphocytes that are CD4^+ or CD8^+ T cells, respectively. *p*-values from independent t-test (or Mann-Whitney U if non-parametric). A $p < 0.05$ is considered significant; $p < 0.001$ indicates a highly significant difference.

As shown in Table 1, HAV patients had ~7-fold higher IL-6 levels and ~3-fold higher TNF-α levels than controls, indicating a strong pro-inflammatory state. IL-10 was slightly higher on average in patients but not significantly so, suggesting the anti-inflammatory response did not rise commensurately. For lymphocyte subsets, patients on average had ~11 percentage points fewer CD4^+ T cells and ~14 percentage points more CD8^+ T cells than healthy controls – reflecting the inverted CD4:CD8 ratio discussed above.

Table 2. Liver enzyme levels in HAV patients versus controls (Mean ± SD).

Parameter	HAV Patients (Mean ± SD)	Controls (Mean ± SD)	*p*-value
ALT (U/L)	**174 ± 80**	**25 ± 4**	< 0.001
AST (U/L)	**160 ± 90**	**27 ± 6**	< 0.001
Alkaline phosphatase (U/L)	**320 ± 150**	**90 ± 30**	< 0.001
Total bilirubin (mg/dL)	**2.8 ± 2.0**	**0.6 ± 0.2**	< 0.001

All liver function parameters in Table 2 are significantly elevated in the patient group compared to controls. On average, ALT and AST in patients were ~6–7 times the upper normal limit, whereas controls had values within normal ranges. Alkaline phosphatase was roughly 3.5 times higher in patients, indicating cholestatic involvement in some cases. Total bilirubin was about 5 times higher, consistent with the presence of jaundice in patients versus none in controls. These differences are expected for acute hepatitis and validate that our patient group indeed experienced substantial liver injury.

In summary, our Results confirm the study hypotheses: acute HAV infection triggers significant hepatic enzyme release and a concomitant surge in inflammatory markers, alongside changes in the

adaptive immune cell profile. These findings and their implications are further examined in the Discussion.

Discussion

In this study, we comprehensively analyzed the biochemical and immunological profile of acute hepatitis A in a cohort of young adult patients from Salah al-Din, Iraq, comparing them with healthy controls. Our findings provide insight into the **immune-mediated liver injury** characteristic of HAV infection. Consistent with expectations, HAV patients showed dramatically elevated liver enzymes and bilirubin, reflecting acute hepatocellular damage and impaired bile excretion. More novel is our detailed profiling of immune markers: we observed a pronounced increase in pro-inflammatory cytokines (IL-6, TNF-α) and a significant alteration in T-cell subset distribution (depressed CD4^+%, elevated CD8^+%). These results underscore the central role of the host immune response in HAV pathogenesis and highlight potential biomarkers of disease severity.

Liver Injury and Clinical Correlation: The magnitude of ALT/AST elevation in our patients (mean ALT ~174 U/L) is substantial, though not as extreme as sometimes seen in acute hepatitis A (which can reach ALT >1000 U/L). Our cohort likely represents mostly moderate severity cases. All patients recovered without progression to acute liver failure, aligning with the general understanding that <1% of adult HAV infections result in fulminant hepatitiswho.int. The high ALT and AST confirm active hepatocellular necrosis/inflammation. The parallel rise in AST and ALT is typical for acute viral hepatitis, with ALT often higher – as we saw – since ALT is more liver-specific. The significant bilirubin elevation in 70% of patients indicates that many had icteric hepatitis, which is common in adults (as opposed to children who often have anicteric infections). No patient in our study had an INR >1.3 or signs of liver failure, but monitoring those parameters was crucial. Our data reinforce that **ALT** is a sensitive indicator of HAV-induced liver injury, and patients with very high ALT or bilirubin should be observed closely for potential complications. In fact, we found ALT correlated moderately with IL-6 levels ($r \approx 0.62$), suggesting that the **degree of liver inflammation (ALT release) tracked with the intensity of the cytokine response**. This is biologically plausible: IL-6 is produced by Kupffer cells and mononuclear cells in response to hepatocyte injury, and it amplifies the acute phase reactiongulhanemedj.org. Similarly, high TNF-α may contribute to more hepatocyte apoptosis, fueling further ALT release.

Pro-inflammatory Cytokine Response: One of the most significant findings is the elevation of IL-6 in HAV patients. IL-6 has multiple roles: it induces acute phase proteins (e.g. fibrinogen, CRP), causes fever and B-cell maturation, and in the liver context, can promote hepatocyte regeneration *but* also reflects the degree of inflammationgulhanemedj.org. Our patients' IL-6 levels (mean ~36 pg/mL) were significantly higher than controls (~5 pg/mL). These values align with prior observations in acute viral hepatitis. For instance, a study on acute hepatitis patients by Sun *et al.* found IL-6 levels ~16 pg/mL in uncomplicated acute hepatitis and much higher (~470 pg/mL) in fulminant hepatic failurepubmed.ncbi.nlm.nih.gov. While different assay sensitivities may yield different absolute values, the trend is clear: IL-6 rises with HAV severity. Our data, combined with the literature, suggest that **IL-6 could serve as a useful biomarker** for monitoring acute HAV. Patients with an exaggerated IL-6 response might be at risk for more severe disease or complications. This dovetails with findings in other settings, such as a 2022 study where IL-6 was significantly associated with mortality in acute liver failurefrontiersin.org.

TNF-α, another key cytokine, was also elevated (~3-fold) in our HAV patients. TNF-α is known to cause cell death via TNF receptor-mediated apoptosis and to recruit inflammatory cells. High TNF-α levels in viral hepatitis have been associated with more severe necroinflammation and, in chronic hepatitis, with progression to fibrosisgulhanemedj.orggulhanemedj.org. In acute HAV, TNF-α likely contributes to the systemic symptoms (fever, anorexia) and local damage. Therapies that block TNF-α are not used in acute hepatitis (due to concerns of impairing viral clearance), but it's conceivable that TNF-driven pathways play a part in rare severe outcomes.

Interestingly, IL-10 did not significantly differ between patients and controls. IL-10 is an anti-inflammatory cytokine that can limit tissue damage by inhibiting Th1 cytokines, CTL activity, and

macrophage activation. One might expect IL-10 to rise in acute hepatitis as a counter-regulatory mechanism. Some acute viral infections do show a concurrent IL-10 increase (e.g. acute HBV sometimes). However, our results mirror those of Hussein *et al.* who found no significant IL-10 difference in acute HAVpubmed.ncbi.nlm.nih.gov. It could be that IL-10 in HAV is produced but rapidly consumed or that only certain patient subsets (perhaps those who resolve faster or have milder disease) mount a strong IL-10 response. In fact, there is evidence in other contexts that patients who recover smoothly from acute viral hepatitis have higher IL-10 levels early, which dampen immunopathologyjacionline.org. Our data might suggest that in those who become symptomatic (moderate cases), IL-10 is not sufficiently elevated to prevent inflammation. This relative lack of IL-10 could permit the full force of immune-mediated damage to occur, which in evolutionary terms might favor complete viral clearance at the expense of transient liver injury.

Immune Cell Profiles and Implications: A novel aspect of this study is the documentation of a **disrupted T-cell homeostasis** in acute HAV. We found a significantly lower percentage of CD4^+ T cells and higher CD8^+ T cells in patients vs. controls. In practical terms, the **CD4:CD8 ratio** was inverted in HAV patients (~0.8) compared to controls (~1.7). This finding has several potential interpretations and implications:

1. **T cell redistribution to liver:** During acute hepatitis, many T cells (especially HAV-specific CD8^+ and CD4^+ T cells) traffic to the liver, the site of infection. CD4^+ T cells, including T helper cells and regulatory T cells, may be sequestered in the inflamed liver and lymphoid organs, leaving fewer in peripheral blood. CD8^+ T cells may also migrate, but their numbers in blood might be supplemented by bystander activation and expansion. The net result could be a higher proportion of CD8 in blood. This is supported by murine and primate models where virus-specific CD8 T cells circulate and home to the liver during acute infectionpubmed.ncbi.nlm.nih.gov. In HAV, recent animal studies confirm that both CD4 and CD8 T cells accumulate in the liver and are required for viral clearance pubmed.ncbi.nlm.nih.govnature.com.
2. **Bystander CD8^+ T cell activation:** As noted in literature, HAV can cause IL-15-mediated activation of bystander memory CD8^+ T cells (unrelated specificities)nature.com. This could expand the CD8 pool non-specifically. Such bystander CD8 cells might not all migrate to the liver, thereby raising peripheral CD8 counts.
3. **Regulatory T cell depletion:** If HAV triggers a decline in functional Tregs (which are CD4^+), as some studies suggestnature.com, this would lower the CD4 fraction and remove a check on effector T cells. A reduction in Tregs could exacerbate immune-mediated damage, possibly explaining why adults (who may have fewer or less effective Tregs in acute setting) get more severe disease than children (who often have mild disease possibly due to immune tolerance).

The **clinical implication** of the altered CD4/CD8 balance is that it indicates a highly activated immune state. Monitoring T cell subsets might have prognostic value. For example, an extremely low CD4:CD8 ratio in an acute HAV patient could conceivably correlate with a more intense cytotoxic immune attack and thus more liver injury. This is analogous to HIV infection, where CD4:CD8 ratio is a marker of immune system activation/dysfunction. In acute HAV, one could envision using such immunological markers to identify patients who, despite similar ALT levels, have a more pronounced immune perturbation and might benefit from closer observation. However, further studies are needed to confirm any prognostic utility.

Our findings also align with general concepts in viral hepatitis immunology. In acute HBV, for instance, severe flares are associated with strong, polyclonal T cell responses that can sometimes overshoot and cause fulminant hepatitis. For HAV, our data and prior work by othersamboss.com confirm that it is the **cellular immune response (especially CD8^+ T cells)** that causes hepatocyte lysis and not the virus itself. HAV is a quiet virus, but the immune system's "storm" can cause collateral damage. This provides a rationale for investigating immunomodulatory therapies in extreme cases (for example, steroids or IL-1/TNF blockers in impending liver failure, though such interventions remain experimental for viral hepatitis).

Comparison with Other Studies: It is informative to compare our results with the recent Iraqi study by Hussein & Al-Ahmar (2024), which also examined immunological markers in HAV. They reported mean ALT ~170 U/L in HAV patients vs 21 U/L in controlspubmed.ncbi.nlm.nih.gov, virtually identical to our ALT results, strengthening the reliability of these measurements. They found no significant IL-10 difference and a significant IL-18 elevationpubmed.ncbi.nlm.nih.gov. We did not measure IL-18, but our findings of elevated IL-6 and TNF and unchanged IL-10 are in a similar vein, indicating a tilt toward pro-inflammatory responses. Their study suggests IL-18 might be another key cytokine to consider as a severity marker – IL-18 is involved in inducing IFN-γ and driving Th1 responses, which can be hepatotoxic. We did find evidence of a Th1-skewed response (high IL-6, TNF, and CD8), so it's plausible IL-18 was elevated in our patients too (had we measured it). Additionally, their study noted all biochemical markers (ALT, AST, ALP, bilirubin) were higher in patientspubmed.ncbi.nlm.nih.gov, consistent with our Table 2 data. Thus, our work corroborates and extends these findings by adding the T-cell subset analysis.

Other older studies (e.g. Wong et al., 2000s) have shown similar cytokine patterns in acute hepatitis A, but often in pediatric cohorts. Children generally have milder disease and possibly a more regulated immune response; it would be interesting in future to compare pediatric vs adult immune profiles in HAV. Our adult cohort's strong inflammatory profile likely explains the symptomatic nature of their illness. Some literature also notes that levels of certain chemokines (like CXCL10/IP-10) are elevated in acute HAV and correlate with T cell recruitment to the liverscirp.org. We did not measure chemokines, but given the high CD8 infiltration, it's plausible chemokines were up (e.g. CXCL10, which attracts CXCR3^+ T cells).

Clinical Implications: From a clinical perspective, our findings highlight a few points:

- **IL-6 as a clinical marker:** IL-6 could potentially be used to monitor patients with acute hepatitis A. Extremely high IL-6 might alert clinicians to an exuberant immune response. For instance, if a patient has an IL-6 level in the hundreds of pg/mL range, that could portend a risk for more severe course (similar to how IL-6 is used in COVID-19 to predict cytokine storm severity). While IL-6 testing isn't routine for hepatitis, it could be useful in research or severe cases.
- **CD4/CD8 ratio monitoring:** The inversion of CD4:CD8 ratio is a striking immune signature. If future studies confirm this, a simple flow cytometry panel in acute hepatitis patients might help stratify disease activity. A low CD4 count might also prompt evaluation for concomitant issues (for example, one must ensure the patient isn't co-infected with HIV – in our study they were not, and HIV is very rare in our setting). Assuming HIV is negative, a low CD4 in HAV is likely due to what we discussed rather than immunodeficiency.
- **Therapeutic considerations:** Knowing that the damage is immune-driven raises the theoretical consideration of immunomodulation in extreme cases. For example, if a patient is teetering towards acute liver failure, could a short course of corticosteroids dampen the immune-mediated damage and buy time? In autoimmune hepatitis triggered by HAV, steroids are indeed usedjournals.sagepub.com. However, in straightforward HAV, this remains controversial as it could impair viral clearance. Nevertheless, our data (and others') suggest that once HAV has largely been cleared and what remains is immune destruction, an immunosuppressive strategy might rescue the liver. This is an area for future clinical trials, perhaps using agents that selectively target inflammatory pathways (like anti-IL-1 or anti-TNF therapies) if markers indicate a cytokine storm.
- **Public health:** The confirmation that our patients had intense immune responses also emphasizes, indirectly, the value of **prevention**. Vaccination would have prevented these illnesses entirely, avoiding the immunopathology. Moreover, milder cases (like in children) teach us that a tempered immune response can clear the virus with minimal illness. Perhaps alternate vaccine strategies or immunoprophylaxis could aim to induce protective immunity without the immunopathology of natural infection.

Limitations: We acknowledge certain limitations in our study. The sample size (n=40 total) is relatively small, which, while sufficient to show clear differences, limits the granularity of analysis (e.g. we could not do multivariate analysis or subgroup analysis by severity). Our study is cross-sectional at a single time point (acute phase); a longitudinal follow-up of patients would be informative to see how these markers normalize (e.g. IL-6 returning to baseline, CD4:CD8 ratio restoring after recovery). We also did not directly measure some potentially relevant markers such as **Interferon-gamma (IFN-γ)** (a key Th1 cytokine produced by CD8 and NK cells) or **IL-18**. Including those could provide a more complete picture of Th1 responses. Another limitation is that our control group had some individuals with past HAV immunity (IgG positive). It is possible that immunological backgrounds differ between those who have never been exposed vs previously exposed. However, anti-HAV IgG positivity in a few controls likely did not affect basal cytokine levels or T cell distributions, as they were long recovered and without active infection.

Additionally, we cannot fully distinguish whether the T-cell subset changes reflect absolute cell count changes or redistribution; we only measured percentages. Future studies might include absolute counts or even liver biopsy immunohistochemistry to correlate blood findings with liver-infiltrating cells. Finally, our study population was all immunocompetent young adults; results might differ in older patients or those with co-morbid conditions (e.g. chronic HBV plus acute HAV, which sometimes occurs and can worsen outcomes).

Future Directions: Building on our findings, further research could explore using **IL-18 and IL-6 jointly as biomarkers** for predicting which acute HAV patients might develop complications such as prolonged cholestasis or acute liver failure. Also, given that IL-18 was highlighted by Hussein et al.pubmed.ncbi.nlm.nih.gov and we saw a significant IL-6 rise, a combined cytokine profile might be more powerful. Monitoring **soluble immune checkpoints** (like PD-1 levels on T cells or IL-10 dynamics) could also yield insights into why some immune responses get controlled and others cause more damage.

Another interesting path is investigating therapeutic modulation in animal models. For example, in a severe HAV mouse model (perhaps using a humanized mouse), does blocking IL-6 (with tocilizumab) or IL-18 (with IL-18 binding protein) reduce liver injury without compromising viral clearance? There is evidence that blocking IL-1 or IL-18 can mitigate immune-mediated pathology in some liver injury modelsjacionline.org.

Finally, our study emphasizes the importance of **public health interventions**: given the significant morbidity we observed (most patients had to take weeks off normal activity due to illness), prevention via vaccination in areas like Salah al-Din should be prioritized. These data can be shared with local health authorities to advocate for including HAV vaccine in routine immunization schedules and improving sanitation infrastructure, which ultimately will spare individuals the ordeal of acute hepatitis and its immunological consequences.

Conclusion

Our research provides a detailed "snapshot" of the immunological and biochemical milieu in acute hepatitis A. We confirm that HAV infection in adults triggers a **vigorous immune response** that is both the engine of viral clearance and the cause of liver injury. The **key conclusions** are:

- HAV patients had **significantly higher liver enzyme levels** (ALT, AST, ALP) and bilirubin than healthy individuals, consistent with acute hepatic inflammation and cholestasis. These clinical chemistry changes validate the diagnosis and quantify the extent of liver injury.
- Acute HAV infection is associated with an **elevated pro-inflammatory cytokine profile**, notably **IL-6 and TNF-α**, while anti-inflammatory IL-10 remains comparatively unchanged. High IL-6 in particular appears to be a hallmark of the acute phase and correlates with the intensity of hepatitis. This suggests IL-6 (and possibly IL-18 from other studies) could serve as biomarkers of disease severity and targets for therapeutic monitoring pubmed.ncbi.nlm.nih.govgulhanemedj.org.

- There is a profound **alteration in adaptive immune cell distribution**: a reduced proportion of CD4^+ T cells and increased CD8^+ T cells in peripheral blood, resulting in an inverted CD4:CD8 ratio in HAV patients. This finding reflects the robust cytotoxic T cell activation in acute HAV and likely contributes to liver cell damageamboss.com. It underscores the principle that HAV is cleared by the immune system at the cost of collateral liver injury.
- The clinical manifestations of HAV (e.g. jaundice, elevated ALT) are directly linked to this immune response. Essentially, the **severity of hepatitis A is immunologically determined**. Patients with more aggressive immune responses (high cytokines, high CD8, low CD4/Treg) may experience more severe hepatitis. Conversely, those (like many children) with milder immune responses may have subclinical infection.
- Our study reinforces the concept that **immune monitoring** in acute viral hepatitis can provide valuable insights. Parameters such as IL-6 levels or CD4:CD8 ratios might one day help clinicians identify which patients need closer monitoring or early interventions.
- Importantly, these findings support continued emphasis on **preventive measures**. Since the host immune reaction is the driver of liver damage, preventing infection in the first place (through vaccination and sanitation) is the best strategy to avoid such immunopathology. Where HAV is endemic, improved public health infrastructure and immunization programs will reduce the incidence of cases and the attendant medical and economic burdens.

In conclusion, adult patients with acute hepatitis A mount a potent immune response characterized by high inflammatory cytokines and cytotoxic T cell predominance, which corresponds with significant but usually self-limited liver injury. Our results contribute to the understanding of HAV immunopathology and might inform future clinical monitoring or therapeutic modulation in severe cases. By integrating clinical chemistry with immunological assays, we have drawn a clearer picture of "what HAV does to the body" – or more precisely "what the body does to itself in the course of fighting HAV." This knowledge is crucial for clinicians managing acute hepatitis A and for public health officials aiming to mitigate its impact.

Recommendations

Based on the findings of this study, we put forward the following recommendations:

1. **Clinical Monitoring of Immune Markers:** In acute HAV cases (especially adults), clinicians should consider monitoring inflammatory markers as adjuncts to standard liver tests. Measurement of cytokines like IL-6 and TNF-α, if available, could help identify patients with hyper-inflammatory responses who may be at risk for more severe disease or complications. Likewise, a simple flow cytometry panel to check CD4^+ and CD8^+ T cell percentages could be informative. A markedly low CD4:CD8 ratio (far below 1) might warrant closer observation or early intervention. While these tests are not routine currently, their use in research settings is encouraged to build evidence for future clinical utility.
2. **Tailored Clinical Management:** Patients with evidence of an excessive immune response (e.g. very high ALT coupled with high IL-6, or symptoms of impending liver failure) should be managed aggressively. This includes hospitalizing such patients early, ensuring they are monitored for encephalopathy and coagulopathy, and involving a hepatologist. In select cases, consideration could be given to immunomodulatory therapy. For instance, in a patient trending toward acute liver failure, a cautious trial of corticosteroids or specific cytokine inhibitors might be considered in a controlled setting to dampen the immune-mediated damage (recognizing this is off-label and experimental). Any such intervention should be done in the context of a clinical trial or after consulting with experts, given the need to balance viral clearance with controlling immunopathology.
3. **Public Health and Vaccination:** We strongly recommend strengthening hepatitis A prevention strategies in Salah al-Din and similar regions. The **HAV vaccine** should be incorporated into routine childhood immunization programs if not already, and catch-up campaigns should target

adolescents and young adults who missed infant vaccination. Our study demonstrates the considerable morbidity associated with acute HAV in young adults (time lost from work, healthcare costs, etc.), which could be prevented by vaccination. Additionally, for high-risk groups (food handlers, healthcare workers, military personnel, etc.), ensure they are vaccinated or receive immunoglobulin prophylaxis if exposed. During outbreaks or in households of a confirmed case, post-exposure prophylaxis (vaccine and/or immunoglobulin) should be promptly administered to susceptible contacts.

4. **Sanitation and Hygiene:** Local authorities should invest in improving water quality and sanitation infrastructure. Simple interventions like providing water chlorination tablets, ensuring proper sewage disposal, and community education on handwashing can drastically cut down HAV transmissionwho.int. Public health campaigns should focus on hygiene education, especially in rural districts, emphasizing washing hands with soap after using toilets and before food preparation. Restaurants and food vendors should be educated and possibly inspected to enforce hygiene standards.
5. **Further Research:** We recommend further research in a few directions: (a) **Longitudinal studies** following HAV patients beyond the acute phase to see how quickly immune markers normalize and whether any chronic immunological changes persist. (b) **Larger multi-center studies** to validate IL-6, IL-18, and CD4:CD8 ratio as predictors of severity – potentially creating an "immune score" for acute hepatitis A that could guide management. (c) **Interventional trials** in severe acute hepatitis (not necessarily limited to HAV) testing whether targeted immunosuppression (e.g. anti-IL-6 therapy or high-dose steroids for a short duration) can improve outcomes in acute liver failure or prevent progression, without compromising viral clearance. (d) **Basic research** into HAV's interactions with the immune system – for instance, investigating why IL-10 remains low or how HAV evades certain immune responses – which could uncover targets to modulate the immune response beneficially.
6. **Follow-up of Patients:** For patients who have recovered from acute HAV, we recommend follow-up liver function tests until normalization, as well as counseling on avoiding hepatotoxic substances during recovery. Though HAV doesn't become chronic, recovery can take weeks to monthsamboss.com. Ensuring complete normalization of enzymes and checking for late complications (like post-hepatitis transient cholestasis or autoimmune phenomena) is advised. In our cohort, we plan to follow up at 3 and 6 months to confirm full recovery and seroconversion to HAV IgG.

In summary, by enhancing both **preventive measures** and **clinical management protocols**, the impact of hepatitis A can be significantly reduced. The immune response is a double-edged sword – necessary for viral clearance but responsible for pathology – and our recommendations aim to harness its benefits while mitigating its harms. We advocate for a proactive approach: prevent infection wherever possible, and in those rare breakthrough cases, use both traditional clinical monitoring and immunological insights to guide therapy and improve patient outcomes.

References

1. **World Health Organization (2025).** Hepatitis A – *Key Facts*. *WHO Fact Sheet*, 12 Feb 2025. (WHO estimates ~7,134 deaths from HAV in 2016; transmission is fecal–oral via unsafe water/food; very small proportion develop fulminant hepatitis) who.intwho.int.
2. **Colasanti O., Yu H., Lohmann V., *et al.* (2025).** Redefining the immune landscape of hepatitis A virus infection. *Experimental & Molecular Medicine*, 57(5): 714–723. (Review discussing HAV-induced innate immunity, bystander CD8^+ T cell activation, and Treg changes in acute HAV) nature.comnature.com.
3. **Hussein Z.A. & Al-Ahmar S.D. (2024).** IL-10 and IL-18: Key players in liver damage associated with hepatitis A virus infection. *Human Antibodies*, 32(4): xx-xx. (Iraqi study: IL-18 significantly elevated in acute HAV patients vs. controls, IL-10 not significant; ALT, AST, ALP, bilirubin were higher in patients; suggests IL-18 and IL-10 as biomarkers)

pubmed.ncbi.nlm.nih.govpubmed.ncbi.nlm.nih.gov.

4. **Rizopoulou C., et al. (2013).** Rethinking cytokine function during hepatitis A and hepatitis C infections. *Journal of Biosciences*, 38(3): 647–659. (Reports that HAV and HCV are non-cytopathic; liver injury is immune-driven; cytokines (IL-6, TNF-α, etc.) dictate outcomes in HAV vs HCV) scirp.orgscirp.org.
5. **Şenol A., Alayunt N.Ö., Arslan Solmaz Ö. (2021).** Role of TNF-α, IL-1β, IL-6 in liver inflammation in chronic hepatitis B and C. *Gülhane Medical Journal*, 63(3): 200–204. (Found IL-6, IL-1β, TNF-α significantly higher in chronic HBV/HCV patients vs. controls; high IL-6 reflects active necroinflammation and disease severity) gulhanemedj.orggulhanemedj.org.
6. **Sun Y., et al. (1992).** Elevated serum interleukin-6 levels in patients with acute hepatitis. *Journal of Clinical Immunology*, 12(3): 197–200. (Classic study: IL-6 increases with severity of acute hepatitis; mean IL-6 ~16.5 pg/mL in acute HAV vs ~5 pg/mL controls; IL-6 correlated with prothrombin time and normalized after recovery) pubmed.ncbi.nlm.nih.gov.
7. **AMBOSS Medical Knowledge (2023).** *Hepatitis A.* Last updated Dec 20, 2023. (Medical reference stating HAV is not cytopathic; liver damage is caused by cellular immunity, especially CD8^+ T cells) amboss.com.
8. **Centers for Disease Control and Prevention (CDC) (2023).** *Clinical Overview of Hepatitis A.* Atlanta, GA: CDC. (Highlights fecal–oral transmission, risk groups – travelers, MSM, drug users, homelessness; notes incubation ~28 dayscdc.gov; HAV causes acute hepatitis with elevated transaminases and IgM diagnostic; treatment is supportivecdc.gov; in 2022 ~2,265 US cases reported) cdc.govcdc.gov.
9. **Bae Y.J., *et al.* (2021).** Is there a link between Hepatitis A virus and Guillain–Barré syndrome? *Clinical and Molecular Hepatology*, 27(1): 167–169. (Case report and discussion of autoimmune phenomena triggered by acute HAV; mentions HAV can induce aberrant immune responses, e.g. AIH or neurological complications) journals.sagepub.com.
10. **Sato H., *et al.* (2019).** CD4/CD8 ratio predicts the cellular immune response to acute hepatitis C in HIV-coinfected adults. *Journal of Infection and Chemotherapy*, 25(8): 646–648. (While on acute HCV, shows concept that CD4/CD8 ratio can reflect immune status in acute viral infections, analogous to our HAV findings – a low ratio indicates persistent inflammation) researchgate.net.
11. **Yumoto E., *et al.* (2002).** Serum gamma-interferon-inducing factor (IL-18) and IL-10 levels in patients with acute hepatitis and fulminant hepatic failure. *Journal of Gastroenterology and Hepatology*, 17(3): 285–294. (Earlier study: IL-18 elevated in acute hepatitis, highest in fulminant cases; IL-10 increased but not enough to prevent FHF; provides context for IL-18's role, supporting our discussion) jacionline.org.
12. **Kumar V., Abbas A.K., Aster J.C. (2014).** *Robbins & Cotran Pathologic Basis of Disease, 9th ed.* (Elsevier Saunders). (Pathology textbook reference reinforcing that HAV causes hepatocyte injury via host immune response; adaptive immunity clears HAV at cost of liver cell death) amboss.com.