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College of Applied Medical Sciences

Department of Clinical Laboratories

The Association of Serum Levels of Bacterial LPS with IL -1and IL-10 in the Disease Severity of Hepatitis Viral Infections (HBV, HCV) and Liver Cirrhosis

A thesis

Submitted to the council of the

College of Applied Medical Sciences – University of Kerbala

In Partial Fulfillment of the Requirements for the Degree of

Master in Clinical Laboratories

By

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ١١﴾

صدق الله العلي العظيم

سورة المجادلة اية ١١

Dedication

For those who helped me start this life, my mother and father (may God have mercy on him), they were my support from the softness of the roots to what I am now.

To my husband and my children, Mohammed, Batool, and Mustafa. To my loved ones, my family (my dear sisters and brothers), and friends, and to everyone who supported me.

Nawar

2023

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All my gratitude and thanks go to my sisters and friends for their efforts to help me complete my scientific career. All my gratitude and love go to my first family, especially my mother, for their constant love and prayers. To the first supporter and my life partner, all my love and gratitude.

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Supervisor's certification

I certify the thesis entitled (**The Association of Serum Levels of Bacterial LPS with IL -1and IL-10 in the Disease Severity of Hepatitis Viral Infections (HBV, HCV) and Liver Cirrhosis**.) was prepared under my supervision by (**Nawar Shamel Taher**) at the department of Clinical Laboratories\ College of Applied Medical Sciences\ University of Kerbala, in partial fulfillment of the requirements for the degree of Master in Clinical Laboratories.

Signature

Prof. Dr **. Suhad Hadi Mohammed**

Supervisor

/ / 2023

Head of Department Recommendation

In view of the available recommendation, I forward this thesis for debate by the examining committee.

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/ /2023

Committee Certification

We, the examining committee, certify that we have read the thesis entitled **" The Association of Serum Levels of Bacterial LPS with IL-1 and IL-10 in the Disease Severity of Hepatitis Viral Infections (HBV, HCV) and Liver Cirrhosis "** and have examined the student (**Nawar Shamel Taher)** in its content and that in our opinion it is accepted as a thesis for degree of Master of **Clinical Laboratories** .

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I have certified upon the discussion of the examining committee.

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Approval Certification

We certify that the thesis entitled (**The Association of Serum Levels of Bacterial LPS with IL -1and IL-10 in the Disease Severity of Hepatitis Viral Infections (HBV, HCV) and Liver Cirrhosis**.) fulfills partial requirements of the degree of Master in Clinical Laboratories.

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21 \ 9 \ 2023

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List of Abbreviations

Abbreviations	Items
ALB	Albumin
ALK	Alkaline phosphatase
ALT	Alanine transaminase
Anti-HBs	Hepatitis B surface antibody
AST	Aspartate transaminase
BMI	Body mass index
Bregs	Regulatory B cells
BT	Bacterial translocation
C5a	Complement component 5a
CD	The cluster of differentiation
CLD	Chronic liver diseases
CT	Computed tomography
CTLA-4	Cytotoxic T-lymphocyte protein 4
CTLs	Cytotoxic T lymphocytes
DAA	Direct-acting antiviral
DCs	Dendritic cells
DNA	Deoxyribonucleic acid
E.coli	*Escherichia coli*
EIA	Enzyme immunoassay
ECM	Extra cellular matrix
ELISA	Enzyme-linked immunosorbent assay
EVs	Extracellular vesicles
FOXp3	Fork head box P3

HAV	Hepatitis A virus
HBc Ag	Hepatitis B core antigen
HBeAg	Hepatitis B e antigen
HBsAg	HBV surface antigen protein
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HCV-Ab	Hepatitis C antibody
HCVcAg	HCV core antigen
HDV	Hepatitis delta virus
HE	Hepatic Encephalopathy
HEV	Hepatitis E virus
HFV	Hepatitis F virus
HGV	Hepatitis G virus
HGV	Hepatitis G virus
HRP	Horseradish peroxidase
HSCs	Hepatic Stellate cells
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
ILCs	Innate lymphoid cells
KCs	Kupffer cells
LAF	lymphocyte-activating factor
LBP	lipopolysaccharide binding protein

LPS	lipopolysaccharide
LS	Liver stiffness
LSD	Least Significant Difference
LAG3	Lymphocyte-activation gene 3
LSECs	Liver Sinusoidal Endothelial Cells
MAIT	Mucosal-associated invariant T
MCP-1	Monocyte chemoattractant protein-1
MD2	Myeloid differentiation factor 2
MDSCs	Myeloid –derived suppressor cells
METAVIR	Meta-analysis of Histological Data in Viral Hepatitis
MGN	Membranous glomerulonephritis
MLNs	Mesenteric lymph nodes
MRI	Magnetic resonance imaging
My88	Myeloid differentiation primary response88
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NF-κB	Nuclear factor kappa B
NK	Natural killer
NKT	Natural killer T cells
NPCs	Non-parenchymal cells
NS3	Nonstructural 3
OD	Optical Density
PAMPs	Pathogen-associated molecular pattern molecules
PCR	Polymerase chain reaction
PET-CT	Positron emission tomography–computed tomography

PG	Peptidoglycan
PT	Prothrombin time
RES	Reticuloendothelial system
RNA	Ribonucleic acid
SBP	Spontaneous bacterial peritonitis
sCD14	Soluble cluster diffration
SD	Standard deviation
SIBO	Small intestinal bacterial overgrowth
Sig	Significant
TGF-β	Transforming growth factor beta
TCR	T cell receptor
TLR	Toll-like receptors
TLR4	Toll-like receptor 4
TNF–α	Tumor necrosis factor
Tregs	Regulatory T cells
TIGIT	T cell immunoreceptor with Ig and ITIM domains,
TIM3	T cell immunoglobulin and mucin-domain containing-3
TRAIL-2	Tumor necrosis factor-related apoptosis-inducing ligand
TSB	Total serum bilirubin
TTV	Transfusion Transmitted Virus
WHO	World Health Organization

Summary

Viral hepatitis, which is an infection of the liver that results in its inflammation and damage, has been considered as global health problem for a very long time and is still regarded as a serious threat to human health. Hepatitis B virus (HBV) and Hepatitis C virus (HCV) can cause both acute and chronic infections.

Cirrhosis is an important cause of morbidity and mortality among patients with chronic liver disease. The major etiologies of cirrhosis are HBV and HCV infections. Globally, among individuals with cirrhosis, 42% had an HBV infection and 21% had an HCV infection.

It has been documented that as liver disease progresses, its ability to perform immune functions becomes impaired, allowing greater translocation of gut-derived microbes into systemic circulation and heightened susceptibility to systemic bacterial infections. Microbial products, such as lipopolysaccharide (LPS), is thought to be both a consequence of and promote liver disease progression.

There is limited information regarding the association between circulatory LPS and the level of some inflammatory and anti-inflammatory cytokines, the type of liver disease, and disease severity. Thus, the aim of the current study was association between bacterial LPS (as marker for bacterial translocation) and type of liver disease (HBV, HCV, and liver cirrhosis).

Across-sectional study was carried out at the College of Applied Medical Sciences/ Department of Clinical laboratories from the period 1 November 2022 to March 2023. Blood sample was collected from 89 patients with HBV, HCV, liver cirrhosis to be used in LPS, IL-1, and IL-10 measurement by ELISA technique.

Out of 45 (50.56%) of patients were infected with HBV, 21 (23.59%) infected with HCV and 23 (25.84%) with liver cirrhosis, the mean age of the patients with HBV, HCV, and Liver cirrhosis were 39.84±16.823, 42.76±15.59, and 49.87±15.9 respectively. Significant difference was noticed between mean age of HBV and Liver cirrhosis. The highest frequency of HBV, HCV, and cirrhosis was noticed within age range between 20-39 years old. More than 75% of patients were suffer from chronic diseases and a significant difference in the mean age of acute and chronic infection was observed.

No significant difference among the three studies groups were noticed according to Sex with M/F Ratio (29/16,1.8), (12/9,1.3), (10/13, 0.76) for HBV, HCV, and Liver cirrhosis respectively.

The current study revealed a significant decrease in the mean levels of LPS, IL-1, and IL-10 between HBV and the other two study groups. Also, a positive significant correlation between LPS, IL-10, and IL-1 among the three studied groups of patients.

Regarding diseases chronicity, the current study revealed that there were no significant differences in LPS, IL-1, and IL-10 levels according to disease duration. However, the mean level of LPS, IL-1, and 1L-10 were higher during the first year of the disease and the mean were higher in acute HBV than chronic HBV.

This study indicated that there were, no significant variations in the mean levels of ALT, AST, ALK, PT, and TSB among HBV with HCV and liver cirrhotic patients.

Approximately 53.5%, 33.3%, and 55.5% of HBV, HCV, and liver cirrhosis patients had an AST/ALT ratio greater than 1. Also, there was a significant positive correlation between LPS, IL-1, and IL-10 and alkaline phosphatase.

The significantly lower mean of LPS, IL-1, and IL-10 in HBV infected patients reflects the impact of HBV or its component in manipulating the inflammatory immune response during infection, which impedes pathogen clearance and results in persistent infection. A significant correlation between LPS and IL-10 and IL-1 indicated that LPS might possibly induce and amplify the inflammatory response among the studied groups.

Chapter one

Introduction

1.1 Introduction:

Hepatotropic viruses, such as those that cause hepatitis, preferentially attack hepatocytes and induce inflammation of the liver. The hepatitis B virus (HBV), or the hepatitis C virus are responsible for the majority of viral hepatitis cases around the world (HCV) in addition to hepatitis A. Frequently, HCV infection develops into a chronic, persistent infection. HBV typically spontaneously resolves in more than 90% of infected adults (Shin *et al*., 2016). Chronic HBV and HCV infections affect 248 million and 150 million persons worldwide, respectively, resulting in 780,000 and 350,000 fatalities each year (Assefa, Kiros and Delelegn, 2023) .

The liver, a crucial component of the immune system, has a high concentration of innate immune cells and is regularly exposed to nutrients and endotoxins that the gut microbiota releases into the bloodstream. The interaction between the gut and liver prevents immune activation against otherwise harmless antigens (Wang *et al*., 2021) .

A steady stream of food and commensal bacterial products, which have the potential to inflame the liver, is experienced by healthy people. The ability to tolerate these chemicals from the gut is necessary for the hepatic immune system, An ongoing Metabolic and tissue remodeling process, regular exposure to microbial metabolites, and other factors cause persistent, controlled inflammation .The liver eliminate toxic metabolic byproducts, cancerous cells, or infections with hepatotropic potential .If these potentially harmful stimuli are not eliminated and inflammation is not treated, it can lead to chronic infection, autoimmune disease, or tumor growth (Robinson *et al*., 2016) .

Chapter One Introduction

As liver disease worsens, the immune activities of liver become less effective, allowing more gut-derived germs to enter the bloodstream and systemic bacterial infection may be develop (Townsend *et al*., 2020).

Bacterial translocation (BT), also known as the transfer of bacteria or bacterial products from the intestinal lumen to mesenteric lymph nodes (MLNs) or other extra intestinal organs (Wiest *et al*., 2014). It is believed that microbial substances such peptidoglycan (PG) and lipopolysaccharide (LPS) both contribute to and facilitate the development of liver disease (Townsend *et al*., 2020).

LPS is a bacterial biomarker for bacteria translocation. In the bloodstream, LPS binds to lipopolysaccharide-binding protein (LBP), which makes it simpler for it to bind to soluble CD14, a sign for monocyte activation (sCD14) (Moon *et al*., 2019) .

Extensive studies have shown that binding of LPS to TLR4 induces the production of several cytokines such as interleukine-1 β (IL-1β), Tumor necrosis factor α (TNFα), IL-10, IL-8, Transforming growth factor -β (TGF β) from monocyte/macrophage lineage through various downstream signaling pathways (Liu *et al.*, 2018, Tucureanu *et al*., 2018) .

Understanding the relationship between bacterial translocation and the seriousness of hepatitis infection may help to understand how translocation affects chronic liver illnesses. This might result in new treatment targets for preventing infections and other cirrhosis consequences (Sehgal *et al*., 2020).

There is limited information regarding the association between circulatory LPS and the level of some inflammatory and anti-inflammatory cytokines and their association with the disease severity in patients with viral hepatitis.

The aim of the study:

To investigate the association between bacterial LPS (as a marker for bacterial translocation and the type of liver diseases (HBV, HCV, and Liver cirrhosis).

The Objectives:

1. Investigate the level of LPS in blood samples taken from HBV, HCV, and Liver cirrhotic patients

2. Study the association of LPS level with the level of some inflammatory (IL-1) and anti-inflammatory (IL-10) cytokines.

3. Study the association between the studied parameters with disease severity.

Chapter Two

Literature Review

Literature Review

2-1 Hepatitis

Hepatitis is defined as liver tissue inflammation. Inflammation is swelling that happens when tissues of body are injured or infected. The liver may become damaged. This swelling and damage may affect the liver's ability to function. Hepatitis can be either acute (short-term) or chronic (long-term) hepatitis. Some types of hepatitis cause only acute infections (Krishnan, 2019, JALIL *et al.*, 2020) . Long-term (chronic) inflammation can lead to scarring of tissue (fibrosis), irreversible scaring (cirrhosis),and hepatocellular carcinoma (HCC)). Other types can cause both acute and chronic infection. There are different types of hepatitis, with different causes:

1. Virus-based hepatitis is the most prevalent kind.
2. Heavy alcohol use is the cause of alcohol-related hepatitis.
3. Poisons, chemicals, medications, and dietary supplements can all cause toxic hepatitis.
4. Autoimmune hepatitis is a chronic type in which the immune system attacks the liver.
5. Genetics and environmental may have an impact (Razavi, 2020, Mehta and Reddivari, 2022)

2-2 Viral Hepatitis

Nine types of hepatotropic viruses are well characterized from A-E. Hepatitis A (HAV) sometimes called infectious hepatitis. Hepatitis B (HBV) is called serum hepatitis, Hepatic C (formerly none A or B hepatitis NABA). Hepatitis D (HDV) which is known as enteric Transmitted hepatitis. Newly discovered forms of viral hepatitis including hepatitis F (HFV), hepatitis G (HGV), Transfusion Transmitted virus (TTV) and SEN virus. They all predominantly affect and infect liver cells. Despite significant overlap in the clinical manifestation caused by them, these types of viruses differ widely in their morphology,

genomic organization, taxonomic classification and mode of replication (Adel Dawood, 2022 , Alieva and Tolipova, 2022) .

These viruses affect hundreds of millions of people globally and are thought to be a global health problem that all nations. This problem takes a multitude of different forms, with factors such as the type of hepatitis, the most common transmission pathways, and the most effective strategies for diagnosis and treatment all varying across and within countries. Thus, it is important to transform worldwide efforts to make hepatitis a public health priority in order to prevent and manage it at the national and subnational levels (Lazarus *et al*., 2013).

In May 2016, the World Health Assembly endorsed the global health sector strategy on viral hepatitis, which aims to eliminate viral hepatitis as a public health threat by 2030, to monitor and evaluate the progress of hepatitis elimination, World Health Organization (WHO) needs to collect data to assess HBV, HCV disease burden and service delivery in its 194 Member States and monitor the global, regional and country progress (World Health Organization, 2021).

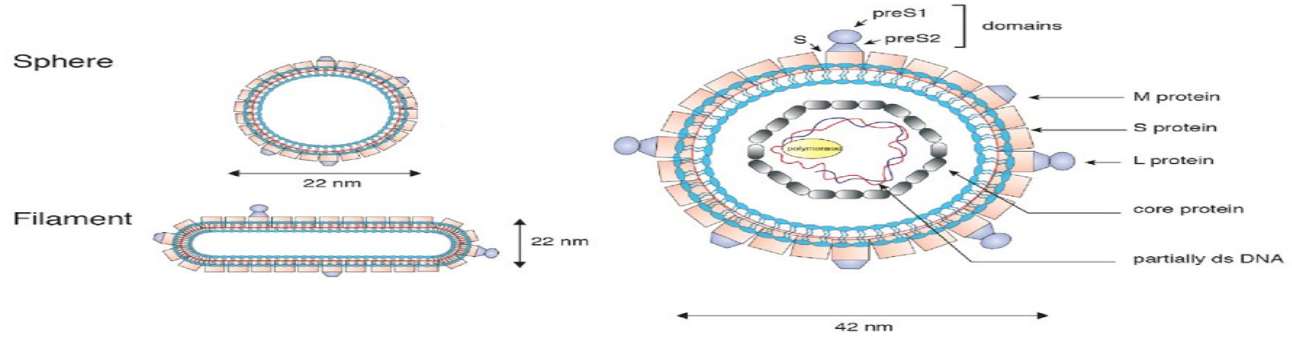
2.3 Hepatitis B Virus(HBV)

2.3.1 Structure of The Virus

HBV is a DNA virus that encodes proteins responsible for its survival and development. HBV Genome carries genes that encodes outer envelope and inner core of the virus. HBV outer core include surface proteins called as HBV surface antigen or protein HBsAg. It can be easily detected in blood of infected persons and positive test indicates infections with HBV virus (Yadav, 2022) .

HBV belongs to the Hepadnaviridae family. It infects exclusively hepatocytes of humans and some non-human primates. Its exceptional features, similar to retroviruses; it

replicates through an RNA intermediate and can integrate into the host genome. In this way, Particles of the HBV replication cycle maintain persistence of HBV infection in hepatocyte, Dane particle (42 nm), spherical (20 nm) and filamentous (22 nm) particles. All the three particles have a common HBsAg on their surface. The spherical and filamentous particles are composed of HBsAg and host-derived lipids without HBV genome, thus they are non-infectious (Tsukuda and Watashi, 2020). The infectious form, the Dane particle, has a diameter of 42 nm and contains a partially double-stranded circular DNA genome linked to a polymerase surrounded by a nucleocapsid and three envelope proteins called the large (L), middle (M), and small (S) surface proteins (Figure 2.1). The C-terminal S domain is common to all three envelope proteins. The M protein also contains an extra N-terminal preS2 domain, and the L protein contains a preS1 domain in addition to the preS2 and S domains. The envelope proteins contain domains essential for attachment to hepatocytes (Herrscher *et al*., 2020).



sub viral particle **Dane particle**

2.3.2 Disease Burden and Epidemiology (Global, Iraq)

2.3.2.1 The Global HBV Burden

Its estimated that 248 million people have HBV infection found in low- and middle-income countries like Asia, Africa, the Pacific and Latin America (Howell *et al*., 2021).

An estimated two billion people have serological evidence of past or present HBV infection and 1.5 million new infections are reported annually worldwide. In 2019, around 296 million chronic carriers of hepatitis B were documented, resulting in 820,000 deaths (Sant'Anna and Araujo, 2023).

2.3.2.2 In Iraq:

Some articles in Iraq indicated the prevalence of HBV infection were between 0.7 and 1.37% (Othman and Abbas, 2020, Hussein *et al*., 2021). The latest study in Iraq showed that the prevalence of occult HBV in the middle province is in the intermediate zone of endemicity (Salman *et al*., 2022). However, there was an underestimation of the HBV prevalence in Iraq. Most studies detected only HBsAg as a marker of prevalence and did not measure the total anti-Hepatitis B core antibodies as an additional marker. The HBV infection can become chronic and seriously threaten physical, mental, and social health. As a result, the Iraqi Ministry of Health implemented a free HBV vaccination for all newborns in Iraq (Abdulqadir *et al.*, 2023)

Its documented that Iraq is of low prevalence rate with HBsAg and that Misan province has the lowest rate (<0.5%). In Baghdad, across sectional study showed that the prevalence of HBsAg among all donors attending the National Blood Bank was 0.6. Another cross-sectional study carried in Basra province found that all blood donors has a 0.2% prevalence of HBsAg (Kadhem *et a*l., 2019).

2.3.3 Signs and Symptoms

Subclinical hepatitis, acute hepatitis, and acute liver failure are all indications of HBV infection during the acute phase. Chronic infection that has no symptoms can proceed to chronic hepatitis, cirrhosis, and HCC throughout the chronic phase of the disease. Depending on the severity of the disease, physical examination findings can range from insignificant to extraordinary.

- Acute hepatitis: Fatigue, nausea, vomiting, abdominal discomfort, low-grade fever jaundice, hepatomegaly, splenomegaly, palmar erythema, spider nevi, serum sickness-like syndrome, necrotizing vasculitis, and membranous glomerulonephritis (MGN) are some of the symptoms that may be present).
- Chronic hepatitis: Similar to acute hepatitis.
- Progressive liver disease includes hepatic decompensation, ascites, jaundice, peripheral edema, gynecomastia, atrophy of the testicles, abdominal collateral veins (caput medusa), variceal bleeding,coagulopathy, pleural effusion, hepatopulmonary, and Porto pulmonary syndrome.
- Acute liver failure ,Ascites, fever, jaundice, hepatomegaly, splenomegaly, hepatic encephalopathy, somnolence, disturbances in sleep pattern, mental confusion, coma (Inoue and Tanaka, 2016).

2.3.4 Transmission

Modes of transmission include:

1. Sharing contaminated items or using contaminated tools.

2. Sexual, either heterosexual or homosexual.

3. A needle sticks or other accident.

4. Transfusion, infusion or inoculation of blood or blood products from an infected person or plasma pool before use, so this risk is now extremely low.

5. Contact of infective fluid with a mucosal surface (e.g., a splash of blood to the mouth or eye).

6. Contact of lacerated, scratched, or otherwise broken skin with blood or contaminated environmental surfaces (for example, countertops, blood smear slides or specimen tubes in laboratories).

7. Biting by an infected person or scratching with saliva-contaminated nails leading to percutaneous introduction of virus (Owusu-Dommey and Ladd-Wilson, 2021).

8. Vertical transmission from mother to fetus range from70 – 90 % for HBeAg - positive mothers to 4-10% form HBeAg - negative mothers (Veronese *et al.*, 2021).

2.3.5 Risk Factor

It was discovered that age, parity, abortion, nationality, tattoos, and marriage style, dental extraction and Vaccinators were all substantially connected with the prevalence of HBV infection (Mehmood *et al*., 2020, Khalid *et al*., 2022).

2.3.6 Laboratory Diagnosis

For effective treatment of HBV infection, a precise diagnosis is essential. HBV infection can occur in multiple stages, every one of which can be identified

by the presence of specific indicators like viral DNA or human antibodies to viral antigen. Therefore, detection techniques are separated into serological assays with varied sensitivity and specificity and molecular assays for HBV DNA detection using various types of PCR (Al-Sadeq *et al*., 2019).

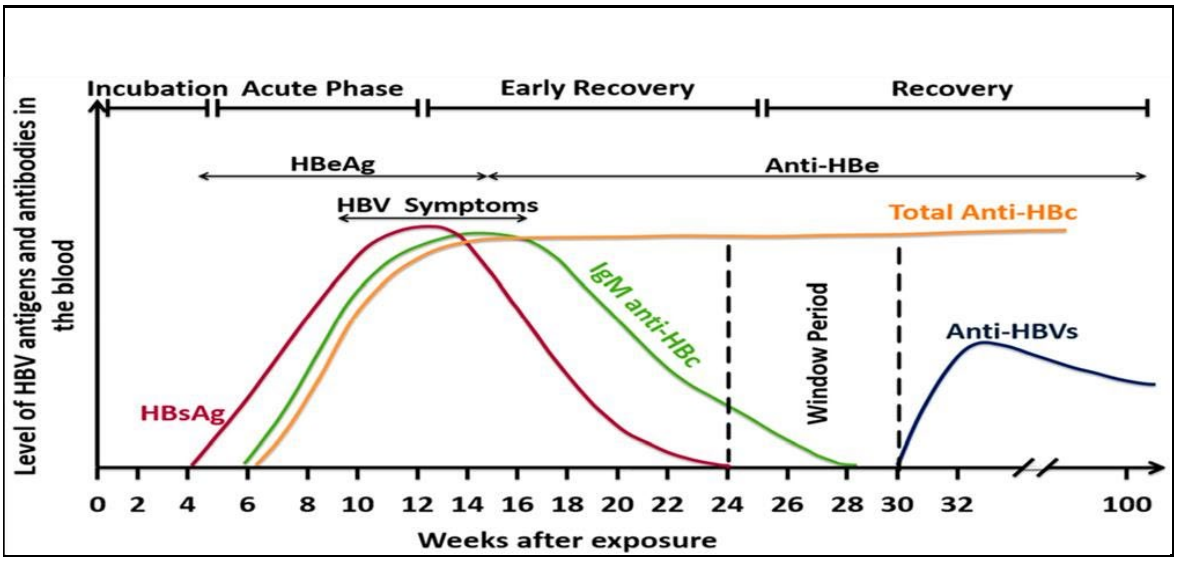


Figure (2-2) displays the pathogenic activity experienced during HBV infection. Very early in the acute stage of the infection, HBsAg can be identified. Within 23–24 weeks after infection, HBsAg levels in serum start to decline until they are undetectable. The Hepatitis B e antigen (HbeAg), which denotes the potential for infection, comes next. Hepatitis B core antigen (HBc) IgM is the initial HBV antibody made, and it can last for up to 28 months after infection. Therefore, the presence of IgM indicates an acute HBV infection. IgG becomes detectable and lasts longer than IgM during the phase of a chronic infection, though. Anti-HBs appears few weeks after HBsAg has been eliminated. During the healing process, HBsAg

and anti-HBs may both test negative. In acute infection, this is referred to as the window phase. Later, anti-HBs will be created, and immunity will be built by the immune system as a result of a real infection (Al-Sadeq *et al*., 2019).

2.3.7 Detection of HBV by PCR

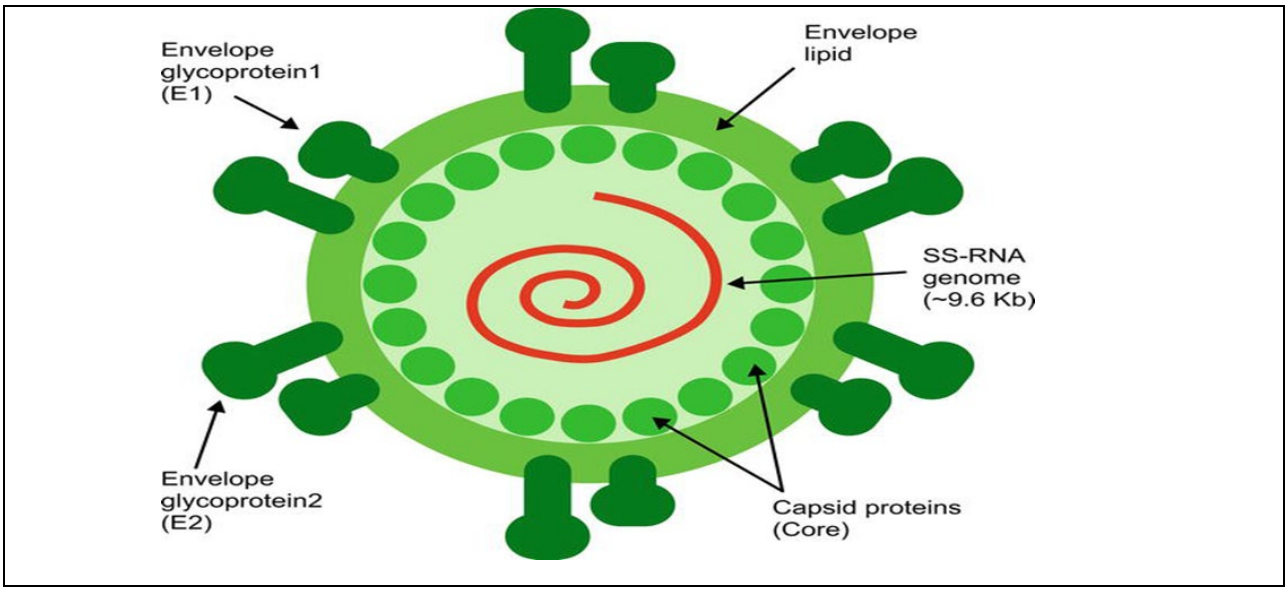
Nucleic acid detection is increasingly crucial for early and precise diagnosis, individualized treatment, and preventive screening in many medical domains (Nikolaou *et al.*, 2022). Quantitative measurement of HBV DNA and HBsAg can be used to separate patients during the course of HBV infection and to monitor therapy (Liu & Yao, 2015).

2.4 Hepatitis C Virus (HCV)

2.4.1 Structure of The Virus

HCV is a positive Sense, single-stranded RNA virus that belongs to the Flaviviridae family and is a member of the genus Hepacivirus. It has about 9600 nucleotides (Roger *et al*., 2021) .

The HCV particle is made up of a lipid bilayer that hosts the heterodimerized viral envelope proteins (E1 and E2) and a nucleocapsid that houses the single-stranded RNA genome associated with to the viral core protein. Three proteins that are crucial to the HCV life cycle include the NS3/4A protease, NS5A protein, and RNA-dependent RNA polymerase NS5B protein (Manns *et al.*, 2017).



2.4.2 The Burden of Disease and Epidemiology (Global, Iraq)

2.4.2-1The Global HCV Burden

WHO estimates that 58 million people worldwide have HCV infection. More than 1.5 million people newly contract HCV every year, and more than 290000 deaths was documented (Mbaga *et al*., 2022,Yang *et al*., 2023).

2.4.2.2 In Iraq:

The HCV prevalence in Iraq has been reported with a range between 0.32% to 7.1% (Bakir *et al*., 2023). The prevalence rate of HCV infection among Iraqi citizens is 3.2 percent, which is comparable to the rates found in the majority of Asian and non-Asian populations that have been studied.

The incidence of Infection was higher in males and inversely correlated with patient age (Khudhair *et al*., 2020). HCV was also widely observed in Alnajaf city Alkufa district, an ALmanathira district, with Almishkhab and Alabbasiya having the lowest distribution (Abusaiba, 2020).

2.4.3 Signs and Symptoms

Most patients who contract HCV for the first time do not exhibit any symptoms. If symptoms do show up, they are often mild and can include jaundice, which is a yellowing of the eyes and skin, as well as nausea and stomach ache. Acute HCV infection is the first stage. Although some patients with acute HCV fully recover, the majority are develop chronic HCV infection (Jin, 2020)

2.4.4 Transmission

The main causes of HCV infection were blood transfusions and renal dialysis (Khudhair *et al*., 2020). Homosexual behaviors among men in high-income nations and drug injection are among important routes of HCV transmission (Midgard *et al.*, 2016). During pregnancy and childbirth, HCV can be passed from mother to child (Utero, 2017). Furthermore, medical procedures like colonoscopy, and endoscopy are also implicated in HCV transmission (Ebrahimzadeh *et al*., 2016).

2.4.5 Risk Factors

Include: Surgical Procedures, People that inject drugs, Dental Surgery, Blood Transfusions, Tattoos/Piercing, Receiving Dialysis, Lifetime partners for sexual activity (Hagan *et al*., 2019).

2.4 .6 Laboratory Diagnosis

The current method of detecting HCV infection relies on a third-generation enzyme immunoassay for the detection of anti-HCV antibodies (EIA). Third generation enzyme immunoassays use a multiantigen format that comprises antigens from the core, NS3, NS4, and NS5 regions to identify anti-HCV antibodies. These tests have greater than 99% sensitivity and specificity (Fourati *et a*l., 2018). The identification of HCV-RNA by PCR after a positive serology is the gold standard confirmatory test for HCV infection (Yuhan Wang *et a*l., 2021). Figure 2-4 showed the onset of many infection markers during an HCV infection, including HCV RNA, HCV-c Ag, anti-HCV

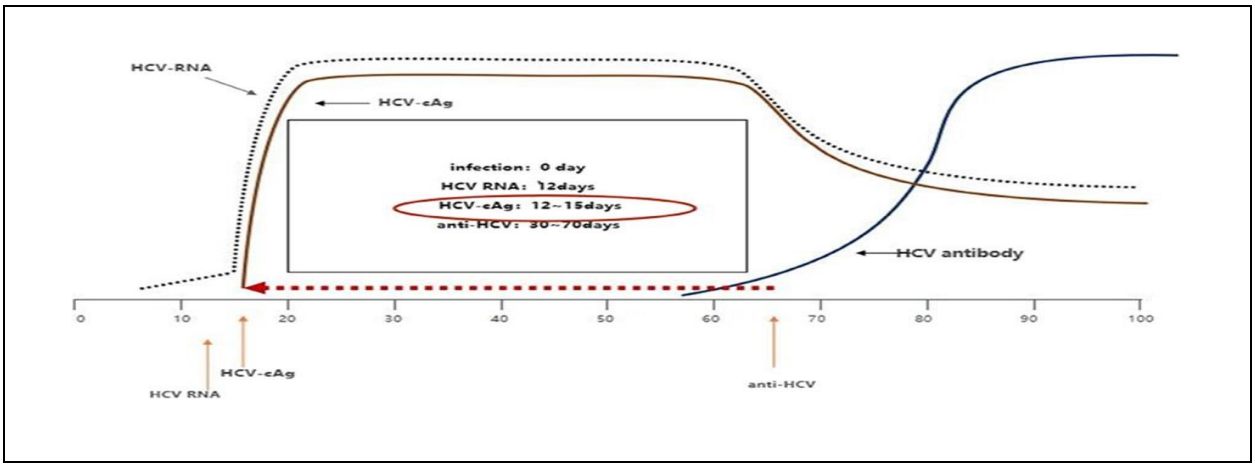


Figure 2-4 Emergence of HCV infection laboratory markers (Wang *et al.*, 2021).

-Infection

HBV-HCV co-infection is posing serious threat and has fatal consequences to human population around the world (Haq *et al*., 2022). It is reported that the incidence of coinfection ranges from 1% to 15%, which may be under estimation

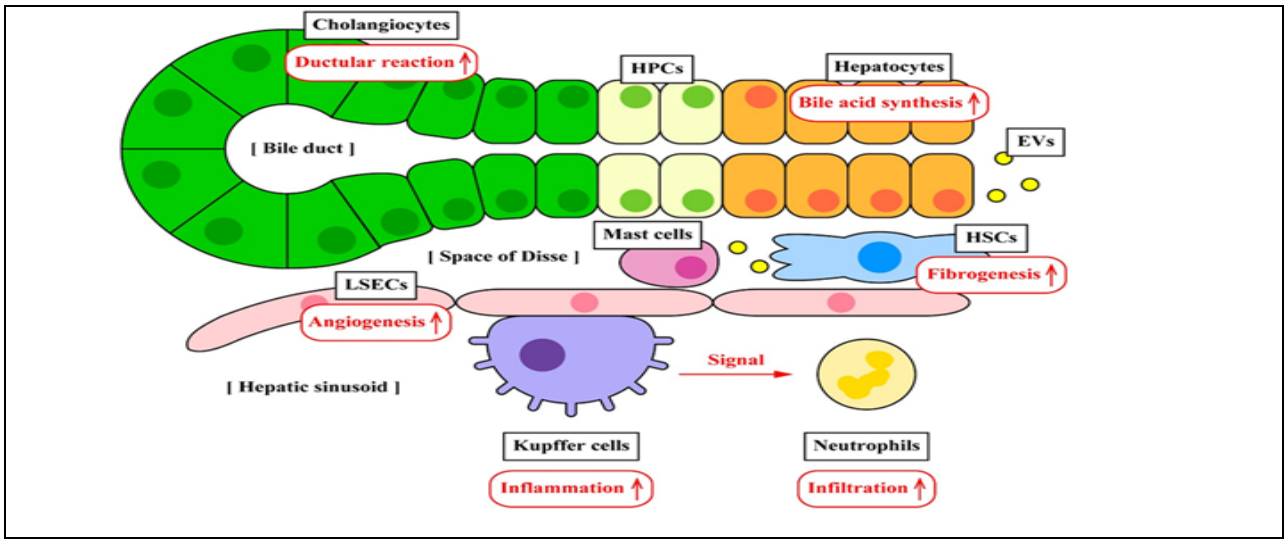
due to the possibility of unrecognized occult HBV infection. Coinfection of HBV/HCV would result in higher rates of cirrhosis and increased severity of liver disease compared to mono-infection of HBV or HCV. Some reports showed that the risk of advanced liver disease increased (2 – 3) fold in the co-infected patients (Zhang *et al*., 2021).

2.6 Liver As Immunological Organ

The liver is the largest solid organ in the body and has many unique immunological properties, including induction of immune tolerance, strong innate immunity poor adaptive immune response versus over reactive autoimmunity and hematopoiesis in the fetal liver. Thus, the liver (as an immunological organ) (Gao, 2016). Its responsible of the production of acute phase proteins, complement components, cytokines and chemokines, and contains large, diverse populations of resident immune cells (Robinson *et al*., 2016).

A Healthy liver is composed of, as shown in Figure 2-5, (i) parenchymal cells, the hepatocytes (70% of liver cells; participate in liver immune response), (ii) three liver-specific non-parenchymal cells (NPCs): The Liver Sinusoidal Endothelial Cells (LSECs) (20%; compose the sinusoidal wall), the Hepatic Stellate cells (HSCs) (5%-8% when quiescent; liver fibroblasts) and Kupffer cells (KCs) (4%; resident macrophages) and (iii) liver-unspecific NPCs, found in the circulation (monocytes) and/or infiltrating the liver: Dendritic cells (DCs) and lymphocytes. Concerning the latter, some subsets display innate functions, as the Natural Killer T (NKT) cells and Mucosal-associated invariant T (MAIT) cells which are equipped with antigen recognition capacities. MAIT cells can be activated by cytokines during virus infections. Moreover, innate lymphoid cells (ILCs), despite lacking antigen-specific receptors, can mediate immune responses and regulate tissue homeostasis and

inflammation. Natural Killer cells and type 1 innate lymphoid cells are the most abundant ILCs in the liver (Delphin *et al*., 2021).



2.7 Immunopathogenesis of Viral Hepatitis

There is general agreement that viral genome variation, viral titers, and suppression of viral components against the host immune system are related to persistent infection and liver damage. An important component for virus clearance failure and liver inflammation is the malfunctioning of innate immune cells (NK cells, monocyte/macrophages, NKT cells, etc.) and adaptive immune cells (antigen-presenting cells, T cell**)** (Wu *et al*., 2020) **.**

- **In HBV**

During acute or self-limiting HBV infection, HBV-specific CD8+ effector T cells can control HBV infection effectively by producing pro-inflammatory cytokines, such as Interferon γ (IFN-γ) and TNF-α, and direct killing of HBV-infected hepatocytes through perforin and granzyme. HBV-specific CD8+ T cells become exhausted in the event of persistent HBV infection. Exhausted CD8+ T cells demonstrate decreased proliferation and are prone to apoptosis (figure 2-6).

Although the effector function of CD8+ T cell decreases when these cells are exhausted, a partially activated status is maintained, which causes persistent hepatocyte injury, recurrent DNA damage, genomic instability, mutation accumulation, and eventually, neoplastic transformation (Cho & Cheong, 2021).

During chronic HBV infection, there are complicated interactions among immune cells. Activation of CD8+ T cells is enhanced by CD4+ T and monocytes; and then activated CD8+ T cells recruit and activate macrophages. Activation of NKT cells can be promoted by Kupffer cells, and further activate NK cells and HSCs. On the other hand, suppressive Tregulatory (Tregs), Kupffer cells and B regulatory (Bregs) inhibit the activation of CD4+ T, CD8+ T, and NK cells. Furthermore, Tregs, Kupfer cells, and Myloid –derived suppressor cells (MDSCs) contribute to the formation of exhausted CD8+ T and NK cells. A variety of cytokines including IFN-γ, TNF-α, IL-6, MCP-1, IL-4, IL-13, IL-12, IL-17, IL-10, and TGF-β are involved in the cross-talk between immune cells. Additionally, complement protein such as C5a positive regulate the activation of HSCs (Chen & Tian, 2019).

- **In HCV**

During acute HCV, the immune system is typically unable to get rid of the virus, allowing it to replicate for a long period in hepatocytes and other cells. It is well known that the viral antigens have a low immunogenicity and do not cause noticeable immune reactions. Patients frequently exhibit a detectable humoral and cellular immune response to the virus's structural and non-structural proteins (Khayrulla *et al*., 2021).

Cellular response during the acute phase of HCV infection is critical for rapid viral clearance. Within six months of the onset of symptoms, in the majority of individuals who ultimately spontaneously eliminate the virus, cellular CD4 and CD8T-cell responses can be detectable to structural and non-structural HCV antigens. It is believed that this response control virus replication and multispecific T-cell response during the early phase of acute HCV infection are associated with disease resolution and spontaneous clearance (Kaźmierczak *et al*., 2016). The first major mechanism of chronic infection is the loss of T cell function due to chronic T cell activation. Essentially, T cell function is inhibited and their cytotoxic capacity is lost (Saraceni&Birk,2021).

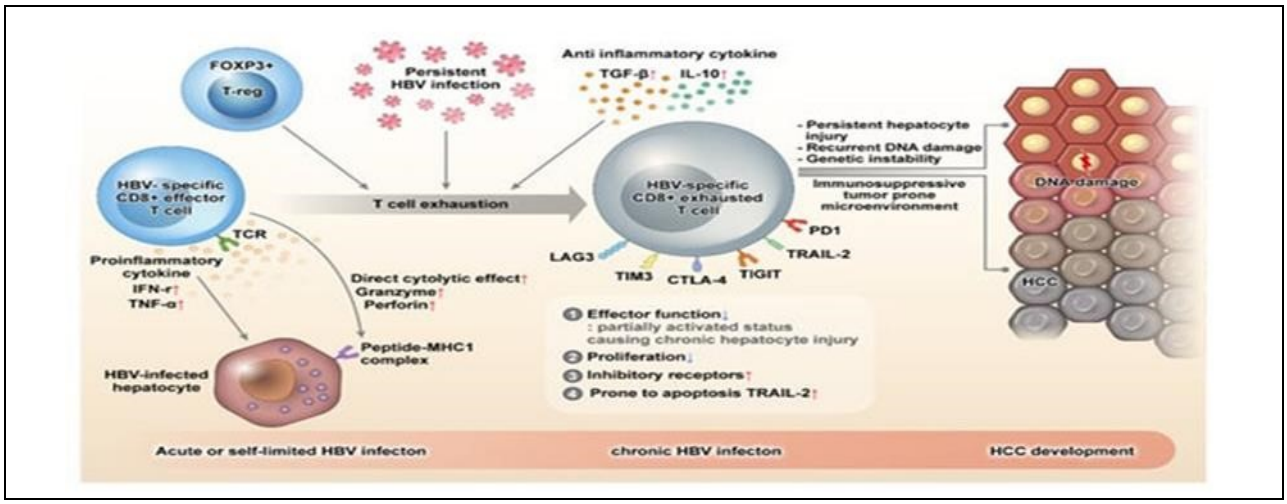


Figure 2-6 Immunopathogenesis of HBV(Cho & Cheong, 2021). HBV: Hepatitis B virus, TIGIT: T cell immunoreceptor with Ig and ITIM domains, PD-1: programmed cell death protein-1, TNF: tumor necrosis factor, CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), LAG-3 (Lymphocyte-activation gene 3), TIM3 (T cell immunoglobulin and mucin-domain containing-3) , TRAIL-2 (Tumor necrosis factor-related apoptosis-inducing ligand), TGFβ (Transforming growth factor-beta) , IFN-γ (Interferon gamma) , TCR (T cell receptor)

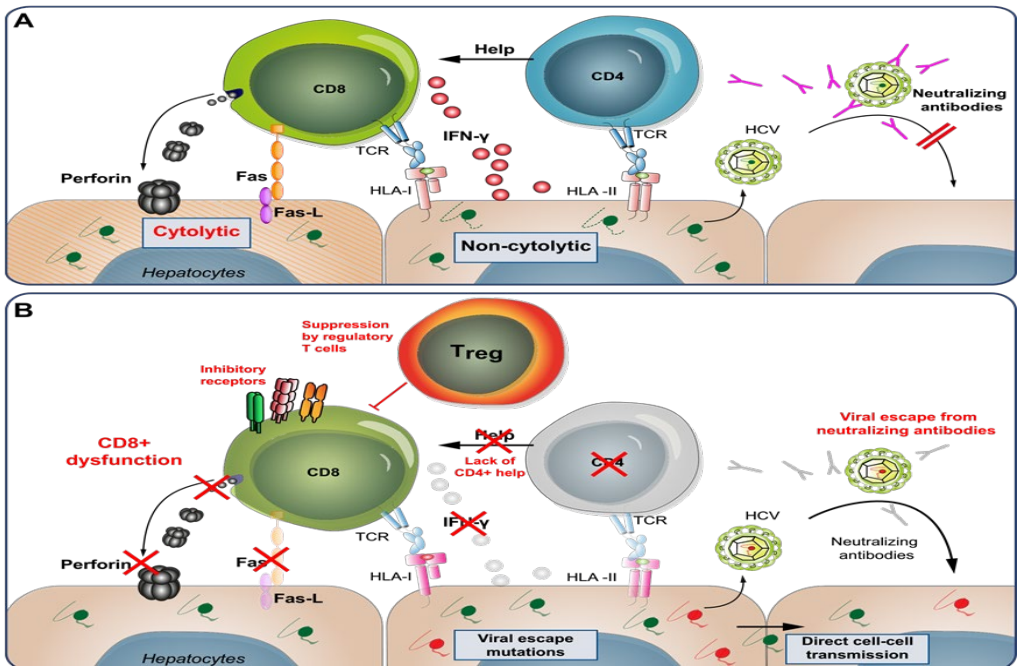


Figure 2-7 shows, (A) CD8+ T cells can inhibit viral replication by cytolytic (perforin) and non-cytolytic (IFN-γ) effector mechanisms. Help by CD4+ T cells is required. Neutralizing antibodies may block the virus and thus infection of further hepatocytes. (B) Several different mechanisms contribute to the failure of adaptive immune responses in HCV infection, such as viral escape, T cell exhaustion, as indicated by the expression of inhibitory receptors, lack of CD4+ T cell help or the action of regulatory T cells (Heim & Thimme, 2014).

2.8 Liver Fibrosis

Liver fibrosis is the outcome of chronic liver damage caused by a number of conditions, such as alcohol use, non-alcoholic steatohepatitis (NASH), viral hepatitis (hepatitis B and C), autoimmune hepatitis, non-alcoholic fatty liver disease (NAFLD), and cholesteric liver diseases. All of these factors have the same impact of generating a chronic inflammation that results in an abnormal wound healing response (Aydin & Akcali, 2018) .

2.9 Liver Cirrhosis

Liver cirrhosis represents the final stage of liver fibrosis (Pinter *et al*., 2016) and is the common outcome of chronic liver injury (Grüngreiff *et al*., 2016). It is develop after a long period of inflammation that results in replacement of the healthy liver parenchyma with fibrotic tissue and regenerative nodules, leading to portal hypertension (Ginès *et al*., 2021). Alcoholic liver disease and chronic infections due to HBV and/or HCV constitute the main causes of liver cirrhosis worldwide (Jung & Yim, 2017).

More than 160 million people suffered from cirrhosis in 2017 around the world, and more than 0.8 million patients with cirrhosis died every year, Although the antiviral therapy has been continuously improved, the burden of cirrhosis caused by hepatitis is still quite large (Ye *et al*., 2022).

HCV and alcoholism are so common among Iraqi patients with liver cirrhosis, ascites and encephalopathy are the most common presentation at medical wards from all causes of liver cirrhosis (Al-Khazraji *et al*., 2021) , causes of liver cirrhosis is demonstrated in figure (2-8).

Fatty liver diseases Alcohol-related liver disorders NO-alcoholic fatty liver diseases	Viral Hepatitis B Hepatitis C Hepatitis D	Autoimmune Hepatitis primary biliary cirrhosis cholangitis IgG4 cholangiopathy
Rare cause Medications Porphyrin	Cardiovascular diseases Budd-Chiari syndrome Right heart failure Osler diseases	Storage diseases Hemochromatosis Wilson diseases
	Chronic biliary diseases Recurrent bacterial cholangitis Bile duct stenosis	

Figure 2- 8 Causes of liver cirrhosis (Wiegand and Berg, 2013)

2.10 Liver Damage

2.10.1 Development and Clinical Diagnosis

In general, once chronic liver injury of any etiology has occurred, damaged hepatocytes, activated sinusoidal cells, platelets, and recruited inflammatory cells release various profibrogenic cytokines, including transforming growth factor-β,

and reactive oxygen species, which activate hepatic stellate cells (Figure 2-9). This process is responsible for deposition of the majority of excess extracellular matrix (Iida-Ueno *et al.,* 2017).

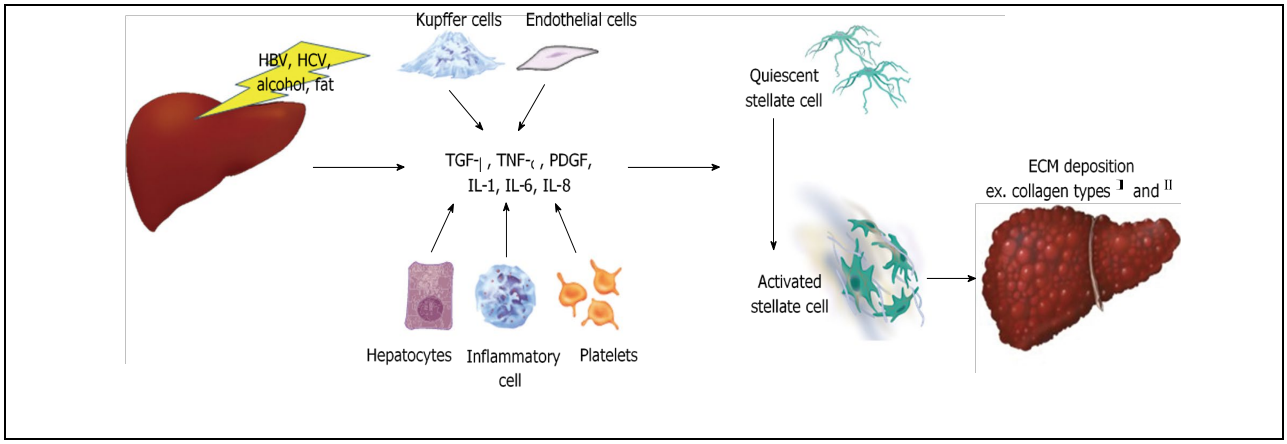


Figure 2-9 The mechanisms of activation of hepatic stellate cells during chronic liver injury(Iida-Ueno *et al.*, 2017). ECM: Extracellular matrix; IL: Interleukin; PDGF: Platelet-derived growth factor; TGF-β: Transforming growth factor-β; TNF-α: Tumor necrosis factor-α.

Further development of liver fibrosis may lead to structural disorders of the liver, nodular regeneration of hepatocytes and the formation of cirrhosis. Hepatic fibrosis is histologically reversible if treated aggressively during this period, but when fibrosis progresses to the stage of cirrhosis, reversal is very difficult, resulting in a poor prognosis (Bao *et al*., 2021) . Both the Meta-analysis of Histological Data in Viral Hepatitis (METAVIR) and Brunt systems—arguably the most commonly used systems—stage liver fibrosis on a scale of 0–4 through visual assessment of the amount and distribution of fibrous tissue on histopathology slide. While semi quantitative scoring systems differ in the number of categories, they distinguish the following stages: 0, absence of liver fibrosis; 1, mild fibrosis with portal fibrosis, defined as a stellate enlargement of portal tracts; 2, significant fibrosis with portal

fibrosis and a few septa between portal tracts or hepatic veins; 3, severe fibrosis with septal fibrosis; and 4, liver cirrhosis with diffuse fibrosis delineating regenerative nodules (Petitclerc *et al*., 2017).

Liver biopsy is the gold standard for fibrosis assessment because it allows detailed evaluation and localization and captures a larger amount of fibrosis. However, its well-known drawbacks have made this procedure unappealing to doctors and patients (technical considerations, invasiveness, and potential severe complications). Considering this, efforts have been made in the last years for developing non-invasive strategies for assessing liver fibrosis. The several broad categories include serological markers (direct and indirect), imaging studies consisting of computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography–computed tomography (PET–CT), and methods assessing physical properties of the liver tissue (liver stiffness, attenuation, and viscosity) (Popa *et al.*, 2023 ,Chowdhury & Mehta, 2023) .

Elastography techniques are used to evaluate the stage of fibrosis by quantifying the shear wave velocity or tissue displacement generated by an ultrasonic or physical impulse, which represents liver stiffness (LS) (Alnimer & Noureddin, 2023). Fibroscan has high accuracy, simplicity, and reproducibility to assess hepatic steatosis and fibrosis (Yip *et al*., 2023) .

2.10.2 Markers of Liver Damage

The increased production of liver enzymes (AST, ALT, ALP and TSB, ALb) were due to ongoing destruction of hepatocytes as the disease progresses, thus liver enzymes are still more important in diagnosis of HBV infection (Al-Madany & Sarhat, 2018, Hammood *et al*., 2022, Mohammed, 2022).

Unfortunately (85%) HCV people are free of any symptoms, usually they are discovered because the liver enzymes in their blood are above normal limits , and their doctors do more blood tests to find the cause , or they are discovered during their donation for blood in blood bank and this is also true for HBV as (15%) of the patients can easily passed unnoticed as some of them have no outward signs or symptoms , and others do experience just "flu-like" symptoms (Abdulrazzaq *et al.*, 2022).

2.10.2.1 Serum Alanine AminoTransferase (ALT), Aspartate Amino Transferase (AST)

(ALT) and (AST) are the common liver enzymes of liver function tests and well-known markers of liver damage. Among the liver enzymes, ALT is the most specific marker of liver function and is used as an indirect marker of liver inflammation or injury, but AST is the less specific marker because it also exists in other tissues (Chen *et al*., 2016 a, Abulude *et al*., 2017, Al-rubaey *et al*., 2019).

2.10.2.2 Alkaline Phosphatase (ALK)

Is an enzyme that is primarily found in the hepatobiliary tract, bone, placenta, and to a smaller extent in intestinal tissue. ALK is involved in multiple dephosphorylating reactions. The normal range for ALK is between 30-120 IU/L. ALK is generally higher in children and adolescents due to the increased osteoblastic activity associated with the bone growth (Kalas *et al*., 2021).

2.10.2.3 Albumin(Alb)

Is the most abundant circulating protein found in plasma. It represents half of the total protein content (3.5 g/dL to 5 g/dL) of plasma in healthy human patients. Albumin is synthesized by liver hepatocytes and rapidly excreted into the

bloodstream at the rate of about 10 gm to 15 gm per day. Very little albumin is stored in the liver, and most of it gets rapidly excreted into the bloodstream. In humans, serum albumin functions as a significant modulator of plasma oncotic pressure and transporter of endogenous and exogenous (i.e. drugs) ligands. In clinical medicine, serum albumin can be measured via standard serum laboratory testing, and this measure has been advocated as a marker for an individual patient's nutritional status. Albumin is also a colloid fluid administered to patients in need of fluid resuscitation, especially in the setting of trauma (i.e. hypovolemic shock) or in the setting of large-volume paracentesis. As a laboratory value, serum albumin can also aid clinicians regarding insight into patients' liver function or the ability to biosynthesize proteins and factors vital to total body homeostasis (Moman *et al.,* 2017).

2.10.2.4 Total Serum Bilirubin(TSB)

Metabolism of hemoglobin or myoglobin produces bilirubin in the spleen. Bilirubin then circulates in the body, bound to albumin. The liver dissociates this complex and converts unconjugated bilirubin to conjugated bilirubin. Jaundice is clinically visible when total bilirubin is greater than 2 mg/dl. As in chronic liver disease, there is the destruction of liver parenchyma, and it does not conjugate bilirubin, which deposits in various tissues of the body (Sharma and Nagalli, 2021).

2.10.2.5 Prothrombin Time(PT)

The liver produces clotting factors, so the patients with chronic liver diseases have coagulopathies and manifest or contribute to easy bruising and bleeding per gastrointestinal tract is reduced production of clotting factors leading to raised PT (Sharma and Nagalli, 2021) .

2.10.2.6 AST to ALT Ratio

In previous years the ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT) in patients of chronic liver disease of various origins has gained much attention. This variable is readily available, easy to interpret, and inexpensive and the clinical utility of the AST/ALT ratio in the diagnostic workup of patients with chronic liver diseases (CLD) is quite promising. revealed that the AST/ALT ratio is correlated with both histologic stage and clinical evaluation in patients with related CLD and progressive liver functional impairment is reflected by an increase in the AST/ALT ratio. Though liver biopsy is considered the gold standard for assessing patients of chronic hepatitis for cirrhosis but has its own drawbacks and inconvenience. So by using the AST/ALT ratio we can negate the necessity of doing the cumbersome procedure (Karim *et al.*, 2015) .

2.11 Bacterial translocation (BT)

2.11.1 General Overview

BT is defined as the migration of live microorganisms or bacterial endotoxins (e.g. bacterial [LPS], peptidoglycan, lipopeptide) from the intestinal lumen to the mesenteric lymph nodes and extra intestinal sites. Its important mechanism in the development of infection in liver cirrhosis (Alexopoulou *et al*., 2017).

The concept of BT can be traced back to the 1960s. In 1966 , Wolochow first proposed the term “bacterial translocation”, which was defined as the phenomenon by which resident bacteria of the intestinal tract enter the lamina propria through the mucosal epithelium, subsequently entering the mesenteric lymph nodes and even

distant organs. In 1979, Berg *et al.* extended the definition of BT to include all phenomena by which microorganisms or their products pass through the intestinal mucosal barrier. Since then, researchers have continued to expand and enrich this concept. It is now believed that the normal flora and their endotoxins or peptidoglycans and metabolites that originally colonized the intestine penetrate large numbers of tissues and organs outside the intestine through the intestinal mucosal barrier (Wang, Yan-hua.2021).

Significant increases in potential microbial translocation, especially along the oral–gut axis, have been identified in many immune-related and inflammatory diseases, such as inflammatory bowel disease, colorectal cancer, rheumatoid arthritis, and liver cirrhosis (Jin *et al*., 2022) .

There are a wide variety of micro-organisms in the human gut, which are harmless and have an important role in human nutrition and health, promoting nutrient supply, preventing pathogen colonization, and shaping and maintaining normal mucosa immunity. Under certain circumstances, these normally harmless bacteria pose an imminent threat when intestinal bacteria translocate to extra-intestinal organs (Chen *et al.*, 2016 b) .

The most common bacteria involved in BT are derived from the family of Enterobacteriaceae (Escherichia coli [E. coli], Klebsiella spp, etc.), Enterococci and Streptococci spp., while species of anaerobic microorganisms are rarely responsible for BT (Alexopoulou *et al*., 2017). The gut microbiome and the translocation of gut-derived bacterial products into the circulation may contribute to a proinflammatory state in the liver that promotes liver disease. Although it is currently believed that the liver does not contain a microbiome of its own, it receives approximately 70%

of its blood supply from the portal vein, which carries blood from the colon. Factors such as a high-fat diet, smoking, alcohol abuse, and intestinal disease can upset the balance between beneficial and potentially pathogenic bacterial species, creating a state of intestinal dysbiosis characterized by altered microbiota composition and decreased bacterial diversity. For example, increased abundance of Enterobacteriaceae, Veillonellaceae, and Streptococcaceae and decreased abundance of Lachnospiracea have been reported in association with cirrhosis (Yang *et al*., 2019).

2.11.2 Effects on Liver Disease

Changes to the intestinal microbiota, intestinal permeability and local immune responses lead to increased BT. In combination with reduced systemic immunity in the liver (reduced reticuloendothelial system (RES) and macrophage activity), these changes may lead to complications of liver disease including increased infections such as spontaneous bacterial peritonitis, acute decompensation of cirrhosis, and increased mortality (Skinner *et al*., 2020).

Several authors have reported that chronic inflammation and liver fibrosis in chronic HCV infection, are associated with microbial translocation, from the gut lumen to the systemic circulation. Indeed, LPS, which is a component of the outer layer of Gram-negative bacteria, is able to stimulate liver resident KCs and HSCs, thereby contributing to the development of liver fibrosis (Lattanzi *et al*., 2018).

Gut microbial alteration is a cause of systemic immune activation in chronic HBV infection. Translocation of gut microbial products, bacterial peptidoglycan, and metabolic by-products has been suggested to exacerbate the clinical course of

patients with chronic HBV infection, and intestinal dysfunction in liver cirrhosis is well known. A better understanding of the pathophysiological correlation between gut microbiota alteration and its impact on the hepatic immune response is crucial for the development of new therapy to treat chronic hepatitis B virus infection (Kassa *et al.*, 2021).

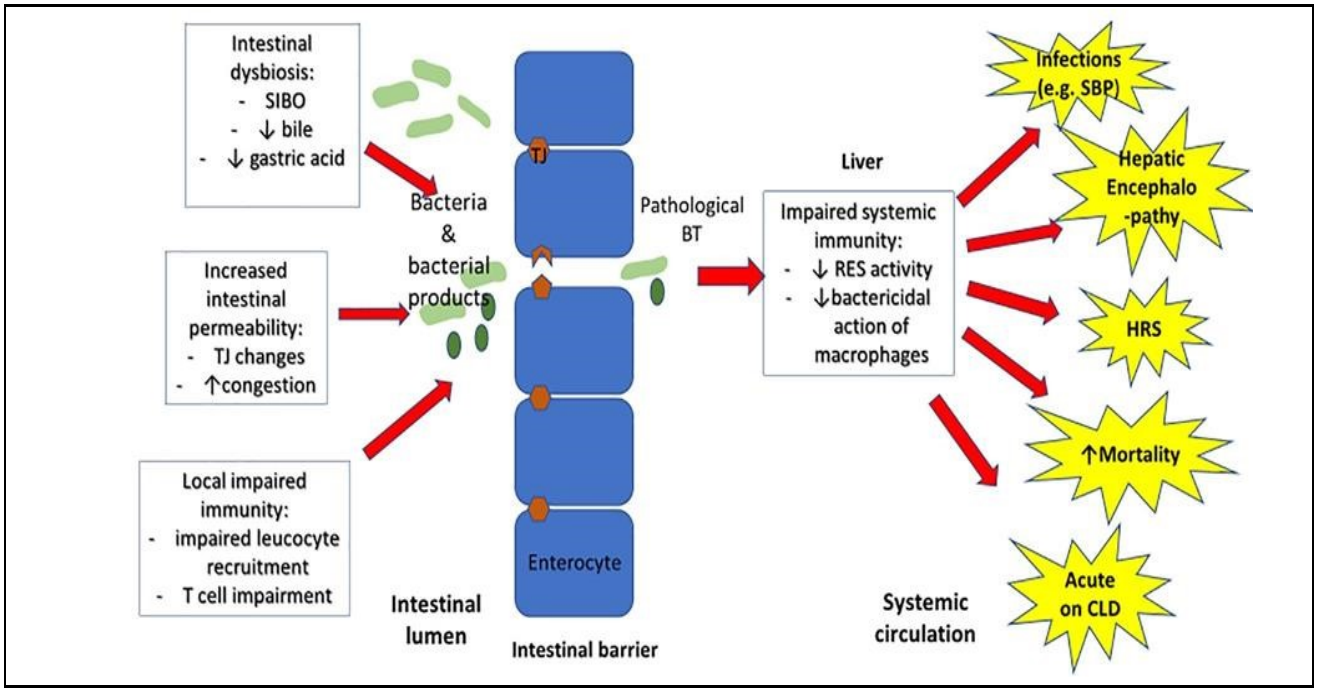


Figure 2-10 Bacterial translocation mechanisms and effects in liver disease (Skinner *et al.*, 2020) BT, bacterial translocation; CLD, chronic liver disease; HE, hepatic encephalopathy; HRS, hepatorenal syndrome; RES, reticuloendothelial system; SBP, spontaneous bacterial peritonitis; SIBO, small intestinal bacterial overgrowth.

2.11. 3 BT Pathogenesis in Cirrhosis

Pathological factors leading to BT in cirrhotic patients are small intestinal bacterial overgrowth (SIBO) , increased intestinal permeability and alterations of the local host immune system. cirrhotic patients are exposed to a higher risk of dysbiosis

because of a variety of pathological interactions between the liver and the gastrointestinal tract. The alteration in intestinal motility, the higher gastric pH and the reduced bile acid concentration in the colon seen in patients with cirrhosis, may lead to a failure in the control of bacterial intestinal growth (Giannelli *et al.*, 2014).

In cirrhosis, there is change in the bacteroides/fermicutes ratio and more prevalence of pathogenic microbes like the Enterobacteriaceae and Streptococcaceae, as well as less prevalence of beneficial microbes like the Lachnospiraceae, and it may affect the prognosis. The change in the microbiota in cirrhosis disturbs the intestinal immune homeostasis, favors BT and impairs the host defense against them, and contributes to the development of hepatic encephalopathy (HE), Spontaneous bacterial peritonitis (SBP) and variceal bleeding. These disturbances in gut microbiota lead to various bacterial complications in cirrhosis (Noor & Manoria, 2017) .

At the decompensated stage, due to portal hypertension and the leaky gut, persistent BT further activates the immune system. In response to the continuous influx of Pathogen-associated molecular pattern molecules (PAMPs), the levels of pro-inflammatory cytokines and leukocyte activation antigens significantly increase. Numerous cytokines and activation antigens are involved in this initial "pro-inflammatory" phenotype, such as TNF-α, IL1β, IL-6, INF-γ, IL-17, IL-18. Concomitantly, the levels of anti-inflammatory cytokines (e.g., IL-10, TGF-β) are decreased. In the more advanced stages of cirrhosis, the immune system is exhausted and unable to mount functional innate and adaptive immune responses, resembling an endotoxin tolerance scenario. At this point, an "immunodeficient" phenotype is

observed, characterized by increased levels of anti-inflammatory cytokines and leukocyte inhibitory antigen and deteriorated immune cell function (Dirchwolf & Ruf, 2015).

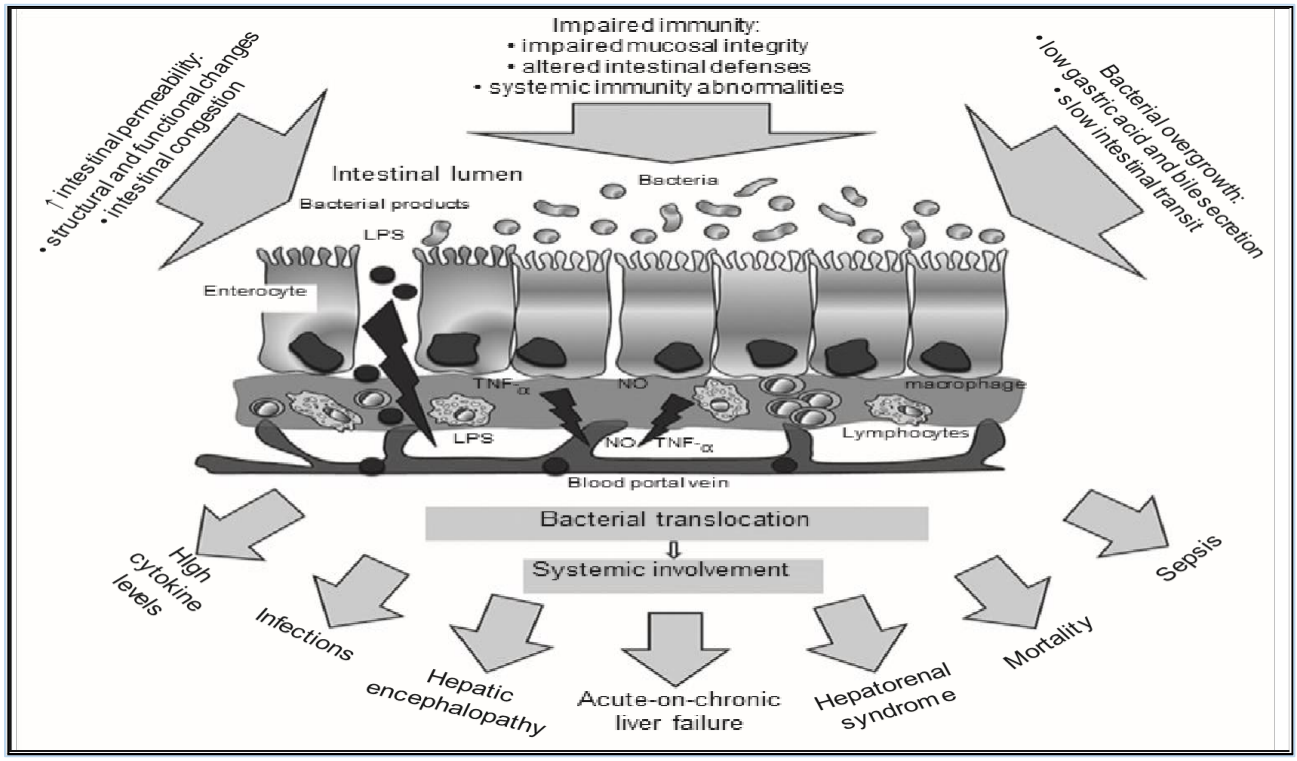


Figure 2-11 Bacterial translocation mechanisms in liver cirrhosis (Alexopoulou *et al.*, 2017)

2-11-4 Lipopolysaccharide(LPS):

LPS is the major component of the outer membrane of all Gram-negative bacteria and called endotoxin that elicits septic shock in animal. LPS is composed of two major important components, hydrophilic lipid A and hydrophilic polysaccharide (O-region). Both are important for endotoxin biological activity (Sampath, 2018).

LPS recognized by the body's innate immunity and considered as a potent stimulator of host immune response and thus a promoter of proinflammatory cytokine secretion. However, this response appears to be dependent on the microorganism (i.e., commensal or pathogenic) from which LPS originates and, consequently, on the LPS structure. The liver receives a larger influx of endotoxin in comparison with other tissues due to the portal vein. For instance, LPS concentration in the portal system has been reported to be 10 times greater versus concentrations in peripheral blood (Mohr *et al*., 2022).

The major consequence of a disruption in the intestinal barrier is the increased Para cellular transport of LPS into systemic circulation. In blood, LPS is carried by

LBP or lipoproteins and interacts with surface receptors eg, Toll like receptor (TLR4) on immune cells initiating an inflammatory response. TLR4 by itself cannot bind LPS but requires CD14 as a cofactor, which facilitates the transfer of LPS to TLR4 and Myeloid differentiation factor 2 (MD2) that modulates LPS recognition. LPS binding protein shuttles LPS to CD14. The association of these auxiliary molecules triggers the signal resulting in the homodimerization of TLR4 and the consequent intracellular signaling via Myeloid differentiation primary response 88 (MyD88). This cascade then leads to the activation of Nuclear factor kappa B (NF-κB) that results in increased transcription of proinflammatory cytokines such as TNFα, IL-1β, and IL-6. Infiltration of activated macrophages or direct activation of resident macrophages in peripheral tissues by circulating LPS results in tissue inflammation (Ghosh *et al*., 2020). LPS is the best-studied microbial marker for assessing bacterial translocation and the resulting host responses (Moon *et a*l., 2019).

2.12 Inflammatory and Anti-inflammatory Cytokines

2.12.1 Interlukine1 (IL-1)

IL-1 is a proinflammatory mediator in both acute and chronic inflammation and among the most powerful inducers of innate immunity. IL-1 induces both its own production as well as the synthesis and expression of multiple secondary inflammatory mediators, including IL-6 (Ridker, 2016). The main cells that produce IL-1 in the body are monocytes and macrophages, as well as cells that share a common origin with macrophages. IL-1 is also produced by fibroblasts, lymphocytes, NK cells, keratinocytes, endothelial cells, and neutrophils (Rakhmatullaeva *et al.*, 2021).IL-1 is a master regulator of inflammation via controlling a variety of innate immune processes. IL-1 has a wide range of biological functions, which include acting as a leukocytic pyrogen, a mediator of fever and a leukocytic endogenous mediator, and an inducer of several components of the acute-phase response and lymphocyte-activating factor (LAF) (Kaneko *et al*., 2019).

2.12.2 Interlukine10 (IL-10):

IL-10, which is encoded by the *IL10* gene, is a major immune-regulatory cytokine produced primarily by monocytes, macrophages, dendritic cells, and lymphocytes with profound anti-inflammatory functions (Sharifinejad *et al.*, 2022). IL-10 is a key component of cytokine system that regulates and suppresses the expression of proinflammatory cytokines during the recovery phases of infections and consequently reduces the damage caused by inflammatory cytokines. IL-10 binds IL-10R, a dimeric receptor composed of a high affinity IL-10 R1 chain predominantly expressed on leukocytes and unique to IL-10 recognition, and an

universally expressed IL-10 R2 chain involved in the recognition of other cytokines from the IL-10 family (IL-22, IL-26, IL-28A, IL-28B, and IL-29) (Rojas *et al.,* 2017)

In vitro studies showed that immune responses to HBcAg are weak because of IL-10 which is a potent immunosuppressive cytokine and involved in immune tolerance towards HBV (Trehanpati & Vyas, 2017). The production of IL-10 is significantly enhanced by increased HBV titers, and IL-10 may suppress IFN- γ production in NK cells in patients (Ma *et al.*, 2020 , Witanto *et a*l., 2022) .

During acute viral infections, proinflammatory signals are produced by DCs after recognition of pathogen patterns. In parallel, NK cells recognizing pathogen patterns and/or stimulated by proinflammatory signals further enhance inflammation. In this proinflammatory context, DC can promote antiviral T cell responses that clear the infection. Activation of DC, T cells, and NK cells also results in the production of the immunoregulatory cytokine IL-10 to balance inflammation. In this context, IL-10 expression controls immunopathology and leads to the resolution of the inflammation and T cell responses once the pathogen is cleared. During persistent infections, the virus uses the production of IL-10 by DCs to exhaust antiviral T cells. High IL-10 levels produced by DCs suppress their antigen presenting capacity and lead to inefficient T cell activation. Chronic antigen presence further exhausts T cells and induces IL-10 production. T cells therefore become “tolerant” to viral antigens and infection persists. To establish chronicity and latent infections, the virus produces viral IL-10 homologs that favor anti-inflammatory responses (Rojas *et al*., 2017).

Chapter Three

Materials and Methods

Materials and Methods:

3.1 Subjects:

Across-sectional study was carried out at College of Applied Medical Sciences, Department of Clinical Laboratories, University of Kerbala. Out of 89 patients with HBV, HCV infections, and with liver cirrhosis , after they diagnosed by physician whom they visit Karbala health department, AL Hussain Medical City, Karbala Health Deportment / women obstetrics and gynecology hospital, Karbala Health Department/ Karbala Center for Diseases and Surgery of Digestive System and Liver, were enrolled in this study, as shown in Figure 3.1. Blood samples was collected from the period of from 1 November 2022 to March 2023.

1.Inclusion Criteria include: patients either with HBV and HCV infections with no specific age range. Both sex were included.

2. Exclusion criteria included active interferon treatment, evidence of other etiologies of chronic liver disease, bacterial infection, antibiotic treatment.

3.2 Sample collection

Five milliliters of venous blood were drawn from all participants by using a disposable syringe. Drawn blood was put into gel tubes and centrifuged at 4000 xg for 20 minutes to get serum used to analyze LPS, IL-1, IL-10 automatically using ELISA technique. Biomarkers were collected from patient’s reports.

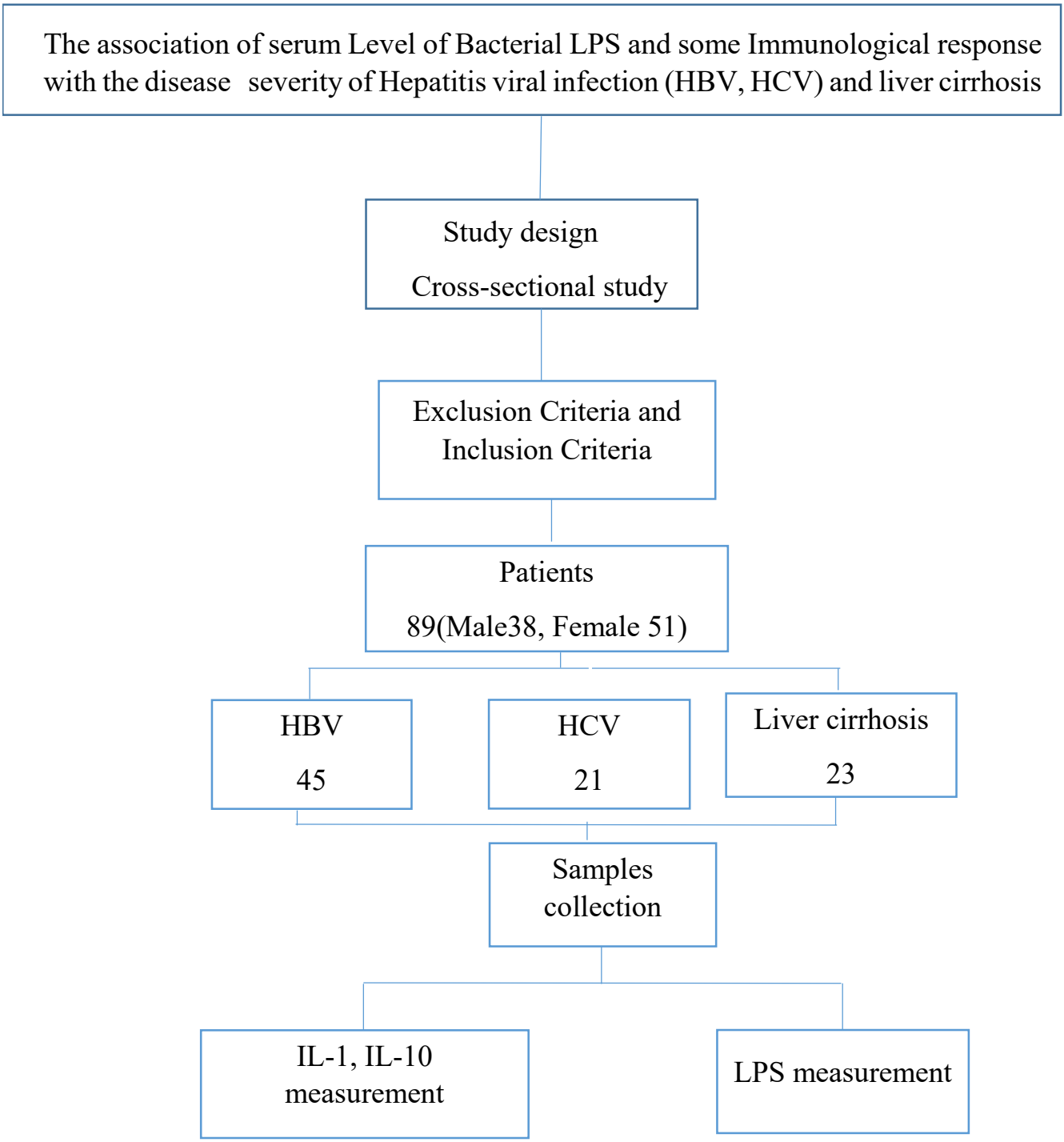


Figure 3-1 study design

3.3 Materials:

3.3.1 Devices and Equipment

Table (3.1) demonstrates the tools and equipment used in this study.

Table 3.1 Tools and Equipment

NO	Equipment	company	Manufacture
1.	Deep freeze	Concord	German
2.	Micro centrifuge	Guohua	German
3.	Eppendorf tube	Arth Al.Rafidin	German
4.	Micropipette	Arth Al.Rafidin	Iraq
5.	Elisys Uno Fully Automated ELISA Analyzer	Human Gesellschaft Für Biochemica Und Diagnostica Mbh	German
6	Incubator	Human Gesellschaft Für Biochemica Und Diagnostica Mbh	German
7	Human Reader	Human Gesellschaft Für Biochemica Und Diagnostica Mbh	German
8	Vortex stirrer	Bioscan	German
9	Combi wash	Human Gesellschaft Für Biochemica Und Diagnostica Mbh	German
10	Thermo shaker	Bioscan	German

3.3.2 Kits and Chemicals Materials

Chemicals and kits used in the current study are listed in table (3-2)

Table 3.2: Chemicals and Kits

NO	Kits	company	Manufacturer
1	LPS	Biont	Shanghai YL biont
2	IL-10	Biont	Shanghai YL biont
3	1L-1	Biont	Shanghai YLbiont

3.4 Methods

3.4.1 Determination of Human(LPS) Using ELISA Kit

Human Lipopolysaccharides was measured according to procedure mentioned by manufacture.

3.4.1.1 Principle

This kit uses ELISA, an enzyme-linked immunosorbent test, to measure human LPS using a biotin-double antibody sandwich method. Wells coated with LPS monoclonal antibody bind with LPS in sample. After that, biotin-labeled anti-LPS antibodies added to generate an immunological complex by combining with Streptavidin-Human Radish Peroxidase HRP (typically used in the immunodetection of biotinylated proteins. This conjugate is suitable for use in ELISA) . Enzymes that are not bound will be removed after washing and incubation. Supplied A and B are added. The impact of the acid will then cause the fluid to turn from blue to yellow. The colors of the solution and the amount of human LPS are positively correlated. The optical density (OD) is determined spectrophotometrically at a wavelength of 450 nm. Human LPS levels and the OD value have a linear relationship.

3.4.1.2 Kit components

Table 3.3 shows the details of the kits

Table 3.3 LPS ELISA kit components

No	Items
1	Coated ELISA plate
2	Washing concentrate(30x)
3	Chromogen A
4	Chromogen B
5	Streptavidin-HRP
6	Stop solution
7	Standard dilution
8	Standard solution(4800EU/L)
9	Anti LPS antibodies labeled with biotin
10	Seal plate membrane

3.4.1.3 Procedure

1.The standard solution should be diluted as follows: This kit had a standard of original concentration, which could be diluted in small tubes, details are shown in tables 3-4 .

Table 3-4 Standard solution dilution

2400 EU/L	Standard No.5	120 µl original standard+120 µl standard diluents
1200 EU/L	Standard No.4	120 µl standard NO 5+120 µl standard diluents
600 EU/L	Standard No.3	120 µl standard NO4+120 µl standard diluent
300 EU/L	Standard No.2	120 µl standardNO3+120 µl standard diluent
150 EU/L	standard No.1	120µl standard NO2+120 µl standard diluent

2. Sample injection:

1) Only the stop solution, chromogen solutions A, and B were put to the blank well.

2) Fifty µl of standard solution and 50 µl of streptavidin-HRP were added.

3) Forty µl of sample, 10µ l of LPS antibodies, and 50µ l of streptavidin-HRP were all present in the test sample well. The plate was covered with seal membrane and genital shaking was done to mix them up. The plate was incubated at 37 °C for 60 minutes.

3. Preparation of the washing solution: The washing concentration (30X) solution was diluted with distilled water for later use.

4. Washing: The seal membrane was removed and the liquid was drained. Each well was filled with washing solution, which was then drained after standing for three seconds. This step was repeated five times.

5. To develop the color, 50 µl of chromogen solution A and 50µ l of chromogen solution B were added to each well, respectively, and the wells were gently shaken to combine the two solutions. The plate was incubated for 10 minutes at 37°C without light in order to develop the color.

6. Stop of the reaction: 50µ l of stop solution was add to each well.

7. Assay: The absorbance (OD) was measured at 450 nm within 10 minutes of adding the stop solution.

8. Using the standards' concentrations and related OD values, the linear regression equation for the standard curve was created. Using specialist software and the samples' OD values, the concentration of the linked samples was then calculated.

3.4.2 Measurement of Human Interleukin (IL-1) Using ELISA Kit

IL-1measured according to procedure mentioned by manufacturer.

3.4.2.1 Principle

This kit uses a sandwich ELISA technique. Each well is coated with IL-1 mono clonal antibody. After the addition of sample, biotin labeled anti IL-1 antibody, streptavidin-HRP, immunological complex will be formed. substrate will be add after washing the unbound enzyme. The optical density is determined spectrophotometrically at wave length 450 nm. The amount of human IL-1present in the sample can be calculated by comparing the OD of the samples with standard curve .

3.4.3.2 Kit components

Table 3.7, shows the details of the kit

Table 3.5 IL-1 ELISA kit components

No	Items
1	Coated ELISA plate
2	Washing concentrate(30x)
3	Chromogen A

4	Chromogen B
5	Streptavidin-HRP
6	Stop solution
7	Standard dilution
8	Standard solution(320ng/L)
9	AntiIL-1 antibodies labeled with biotin
10	Seal plate membrane

3.4.2.3 Procedure

1. Standard solution dilution: This kit had a standard of original concentration, which could be diluted in small tubes, details are shown in in table (3-8).

Table 3-6 Dilution of standard solution

160 ng/L	Standard No.5	120 µl original standard+120 µl standard diluents
80 ng/L	Standard No.4	120 µl standard No 5+120 µl standard diluents
40 ng/L	Standard No.3	120 µl standard No4+120 µl standard diluent
20 ng/L	Standard No.2	120 µl standardNo3+120 µl standard diluent
10 ng/L	Standard No.1	120 µl standard No2+120 µl standard diluent

2.Sampling injection

1) Blank well: chromogen solution A, B, and stop solution was added.

2) Standard solution well: 50µl standard and 50µl streptavidin- HRP was added.

3) Sample well: 40 µl of sample, 10µ l of IL-1 antibodies, and 50 µl of streptavidin-HRP was added. Then, the plate was covered with seal membrane. The plate was shacked gently to mix and the plate was incubated at 37°C for 60 minutes.

3. Washing solution preparation: washing solution with concentration 30x was diluted with distilled water for later use

4. Washing the wells: The seal membrane was removed, the liquid was drained and each well was filled with washing solution. The washing liquid was left for 3 seconds and then drained. This step was repeat five times.

5.Color development: 50 µl of chromogen solution A was add first to each well and then 50 µl of chromogen B was added. shaking was done to mix up. The plate was incubated for 10 minutes at 37°C.

6. Stop of the reaction: 50 µl of stop solution was added to each well

7. Reading the assay: OD was measured for each well under 450 nm wave length After 10 minutes from adding the stop solution

8. According to standard concentration and corresponding OD values, the linear regression equation of the standard curve was calculated, then according to OD values of samples, the concentration of each sample was calculated.

3.4.3 Determination of Human Interleukin (IL-10) using ELISA Kit

IL-10 was measured according to the procedure mentioned by manufacture.

3.4.3.1 Principle

Similar to Principle to IL-1

3.4.3.2 Kit components

Table 3.5, shows the details of the kit

Table 3.7 1L-10 ELISA kit components

NO	Items
1	Coated ELISA plate
2	Washing concentrate(30x)
3	Chromogen A
4	Chromogen B
5	Streptavidin-HRP
6	Stop solution
7	Standard dilution
8	Standard solution(640ng/ml)
9	AntiIL-10 antibodies labeled with biotin
10	Seal plate membrane

3.4.3.3 Procedure

Similar to IL-1 Procedure

3.5 Statistical Analysis

SPSS, version 22 software (IBM Corp., NY, and USA), was used to analyze data. Descriptive statistics including frequencies and cross tabulation were analyzed. Bivariate correlations between the studied variables were examined to find both

significant positive and negative Correlations. To compare mean levels, Both the Least Significant Difference (LSD) test and the Analysis of Variance (ANOVA) test were used. The chi-square test was used to examine the category variables. The statistical significance was set at P value ≤ 0.05.

3.6 Ethical considerations

This study was approved by ethical Committee at College of Applied Medical Science/ University of Kerbala. All subjects involved in this work were informed and agreement was obtained verbally from each one before sample collection.

Chapter Four

Results and Discussion

Results and Discussion

4.1 Demographic data

In total, 89 patients' blood samples were obtained during from 1 November 2022 to March 2023 in Karbala Governorate, Iraq. Out of 45 (50.56%) of patients were infected with HBV, 21 (23.59%) were infected with HCV and 23 (25.84%) with liver cirrhosis, as shown in table (4-1). The information of each patients was documented according to questionnaire as shown in Appendix.

4.1.1 Distribution of HBV, HCV, and Liver cirrhosis patients according to Age

The current study found that the mean age ± SD of the patients with HBV, HCV, and liver cirrhosis were 39.84 ± 16.823, 42.76 ± 15.59, and 49.87 ± 15.9, respectively, as shown in table (4-1). Comparable results were reported in previous studies in which the mean age of HBV infected patients were documented as 42.1 ± 11.80 (Choi *et al.*, 2022) and 35.7 ± 11 years (Osasona *et al.*, 2023). For HCV infection the mean age was reported as 44.3 ±12.7 (Johannesson *et al.*, 2022) and 42.5 ± 10 (Hashmi *et al.*, 2023)**.** For liver cirrhosis the mean age were reported as 49.94 (Duah *et a*l., 2022), and 48.60 48.60 ± 11.70 (Rajpurohit *et al.*, 2023) **.**

Additionally, this study found that there were statistically significant difference in the mean ages of patients with HBV infection and liver cirrhosis. A higher mean age was found in patients with liver cirrhosis, as shown in table (4-1). This result agrees with previous studies (Yang *et al.*, 2020, Suriguga *et al.*, 2022).

Higher mean age found in liver cirrhotic patients might possibly due to life style and accumulated exposure to environmental factors. Together with drinking alcohol and being exposed to harmful substances, these factors all contribute to the progression of persistent liver inflammation which may results in cirrhosis (Radonjić *et al.*, 2022)**.**

The frequency of patients by age was displayed in table (4-2), There were significant different among the three studied groups according to age categories. Higher frequency of with HBV, HCV, was found below of 40 years, whereas high frequency of the patient with liver cirrhosis was found with age above 40 years old. Additionally, the current study revealed that more than 75 % of patients were suffer from chronic disease and that significant differences existed in the mean age of patients between acute and chronic infection, as shown in table (4-3).

Age is considered as an important risk factor for hepatitis virus infection. It was documented that infection during the early age of life increase the risk of chronic infection and the development of liver cancer (de Mattos *et al*., 2021). Older age have universally been described as independent risk factors for the development of HCC in patients with a chronic HBV, HCV infection (Alqahtani & Colombo, 2020)

Chronic HBV infection occurs in approximately 90% of newborns infected prenatally, 30 % of children aged under 5 years, and < 5 % of immunocompetent adults (McMahon *et al*., 1985 , Hyams,1995) . Immunosuppressed individuals are also more susceptible to developing chronic HBV infection after the acute infection" (Hadler & Judson, 1991, Bodsworth *et a*l., 1991, Kang *et al*., 2022) .

Ryerson *et al* , reported that " During 2018 the highest rate of acute HCV infection was in patients aged 20- 29 years (3.1 per100.000), followed by patients aged (30 -39) years (2.6), (40- 49) years (1.3) ,(50 - 59) years 0.9 and ≥ 60 (0.4) , and the lowest rate (0-1) was in patients aged < 20 years "(Ryerson *et al.*, 2020).

Regarding liver cirrhosis, comparable result was documented by (Sajja *et a*l., 2014) who found that the average age of cirrhosis patients was 52 ± 11 .

Table 4.1 Mean age of patients

Virus types	Mean age	No (%)	SD	Multiple Comparisons LSD			
				(I) virus types	(J) virus types	Mean Difference (I-J)	Sig.
HBV	39.84	45 (50.56)	16.823	HBV	HCV	-2.917	.500
					Liver cirrhosis	-10.025*	.019
HCV	42.76	21 (23.5)	15.595	HCV	HBV	2.917	.500
					Liver cirrhosis	-7.108	.152
Liver cirrhosis	49.87	23 (25.8)	15.907	Liver cirrhosis	HBV	10.025*	.019
					HCV	7.108	.152
ANOVA test *P*- value	0.061						

*. The mean difference is significant at the 0.05 level, SD: Standard Deviation, LSD: Least Significant Difference

Table 4.2 Frequencies of Patients According to Age Categories

		Virus Types			Total
		HBV	HCV	Liver cirrhosis	
Age Categories	≤40 years	27(60)	11(52.38)	6(26.08)	44(49.43)
	>40 years	18(40)	10(47.61)	17(73.91)	45(50.56)
Total		45(100)	21(100)	23(100)	89(100)
P-value		0.029			

Table (4.3) Differences in Mean Age According to Disease Chronicity

Virus types	Diagnosis	Mean age	No%	SD	*P value*
HBV	Acute	34.64	11(25.5)	16.194	0.269
	Chronic	41.16	32(74.4)	16.797	
	Total	39.49	43(50)	16.702	
HCV	Acute	37.75	4(21.1)	14.569	0.618
	Chronic	42.33	15(78.9)	16.347	
	Total	41.37	19(22.3)	15.713	
Liver cirrhosis	Chronic	49.87	23(100)	15.970	
Total	Acute	35.47	15(17.64)	15.32	0.049*
	Chronic	44.27	70(82.35)	16.65	
	Total	42.72	85(100)	16.686	

**P* value Significant at 0.05 level, SD Standard deviation

4.1.2. Distribution According to Sex

The Sex distribution of patients enrolled within this study were shown in table (4-4A), there were no significant differences. However, higher frequency of infection among female was found in case of HBV and HCV infection while lower frequency was found in case of cirrhosis. Male/ female ratio (29/16,1.8), (12/9,1.3), (10/13, 0.76), for HBV, HCV, and liver cirrhosis, respectively. Higher ratio was recorded in previous studies (Riaz *et al*., 2022, Agbozo *et al*., 2022)

Sex is considered as risk factor in different types of diseases. Actually, there are many differences between female and male in health and disease status. older age was seen in female than in male but usually female are not healthier. Sex differences in immune response has been documented. The immunological response to viral infections was found to be stronger in females. The course of liver disease appears to differ across the sexes, and numerous clinical trials have demonstrated that postmenopausal women

and men with chronic hepatitis caused by HBV and HCV progresses more quickly to cirrhosis (Licata *et al.*, 2023) **.**

Table 4.4 A Distribution of Patients with HBV, HCV, and liver Cirrhosis According to Sex

Sex	Type of Disease No (%)			
	HBV	HCV	Liver cirrhosis	Total
Male	16 (35.55)	9(42.8)	13 (56.52)	38 (42.69)
Female	29 (64.44)	12 (57.1)	10 (43.47)	51 (57.30)
Total	45 (100)	21 (100)	23 (100)	89 (100)
P- Value	0.255			

Additionally, this study showed that there was significant difference between HBV infected male and female regarding disease chronicity. Higher percentage of females had chronic HBV infection, as shown in table 4-4B.

Understanding the clinical variations between sex is crucial for enhancing the prognosis of HBV-infected individuals. According to some studies conducted, there are sex differences in the demographic, physical, and psychosocial characteristics of HBV-infected patients, including lifestyle elements (like alcohol consumption and cigarette smoking) and social behaviors that are connected to the emergence of HBV-related liver diseases. For instance, prior research discovered that the higher prevalence of smoking and alcohol consumption in men may be partially attributable to the sex discrepancy in liver disorders. There may be significant sex disparities in clinical signs and symptoms, and of HBV-associated liver disorders because males

and females have different hormones related to sex, immune systems, vulnerability to infections, and HBV exposure (Liu *et al*., 2022a).

Course and results of numerous acute and chronic liver illnesses varies between males and females according to sex. X chromosome contains the majority of the genes controlling this response, which explains why females have a stronger viral response (Sayaf *et al*., 2022).

There have been a few theories put up to try and explain the genesis of the sex disparities in HCV prevalence. Some documents has established the significance of sex in placing women at higher risk of exposure to HCV infection, while others support the idea that HCV infection appears to be more common and spreads more quickly in males than in females. On the other hand, some documents discovered that HCV infection rates do not differ significantly by sex and do not vary even slightly. Furthermore, studies have shown that women experience direct-acting antiviral (DAA) side effects more frequently than males do and are less likely to develop liver cirrhosis and HCC (Abdel-Gawad *et al*., 2023) .

Table 4-4 B. HBV and HCV sex differences according to disease chronicity.

Virus Types		Sex No (%)		Total No (%)	*P-value*
		Male	Female		
HBV	Acute	8 (50)	3 (11.11)	11 (25.58)	0.01
	Chronic	8 (50)	24 (88.8)	32 (74.41)	
	Total	16 (100)	27 (100)	43 (100)	
HCV	Acute	1 (12.5)	3 (27.27)	4 (21.05)	0.6
	Chronic	7 (87.5)	8 (72.72)	15 (78.94)	
	Total	8 (100)	11 (100)	19 (100)	

4.1.3 Risk factors associated with HBV, HCV and Liver cirrhosis

The current study revealed that the most common types of possible routs of infection with HBV, HCV, liver cirrhosis infection include tooth extract 22 (52.4%), 9(45%), 13(56.5%), followed by surgical3 Procedure 21(48.8%), 9 (42.9%), 10

(43.52), respectively, as shown in figure (4.1). High frequency of tooth extract and surgical Procedure were reported in previous studies (Serraino *et a*l., 2020 , Samo *et a*l., 2021, Khalid *et al*., 2022).

This is because auto-lock injections are not available, unsafe surgical techniques are used during small and large surgical procedures, unsafe dental extraction and processing equipment, unsafe root canal therapy equipment, and unsafe gum treatment equipment (Samo *et al.,* 2021). In addition to education of people about illnesses and their transmission mechanisms, it is important to thoroughly sterilize surgical and dental devices in order to prevent the spread of infections (Riaz *et al.,* 2022).

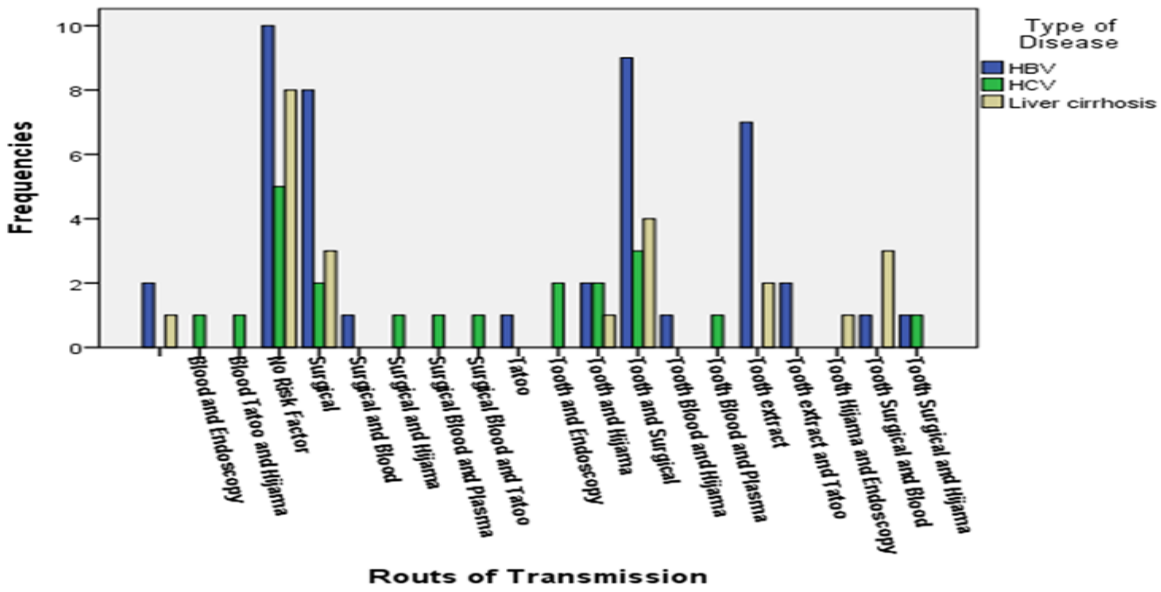


Figure (4.1): Possible Routs of transmissions associated with HBV, HCV, and Liver cirrhosis

According to BMI, there were no significant differences between type of viral infection, cirrhosis with the BMI of patients, as shown in table (4.5), no significant difference were found in previous studies between BMI and HBV infection (Liu *et al.*, 2022b), HCV infection (Ismail *et al.,* 2021), and liver cirrhosis (Kanwal *et al*., 2022).

The present study showed that more than a third of the patients were in normal weight whereas two third of them were in over weight and obese groups, as shown in table (4.5).

Obesity is frequently present in patients with chronic HBV or HCV and it is considered an additional HCC risk factor in these patients suggesting a possible synergistic effect of metabolic factors and viral hepatitis (Saitta *et al*., 2019).

Also, it has been documented that obesity can accelerate the progression of liver cirrhosis (Nishikawa *et al*., 2021).

Regarding family history, the current study revealed that there were no significant difference between the types of diseases and family history, most of patients had no family history, as shown in table (4.5). However, more than 23% of HBV, 10% of HCV, 4.3% of liver cirrhotic patients had family history. The results of the current study were similar to those of previous research (Hong *et al*., 2022). but differ from those of other previous studies (Zang *et a*l., 2020, Rahimzadeh *et al.,* 2022).

Concerning history of smoking, no significant differences in type of disease and history of smoking was found and most of patients were not smokers, as shown in Table (4.5), Similar finding was reported previously (Huang *et al.,* 2021). Inversely significant difference with smoking were documented in previous research (Hsiao *et al.,* 2022, Kumar *et al.,* 2023). The differences in the results seems to be due that the majority of the participants in this study were female and the majority of them in the Iraqi society were not smokers.

The present study revealed that there were no statistically significant differences among the three studies groups regrading academic achievement, marital status, living condition, as shown in table (4.5).

The results of this study are in agreement with those of earlier studies (Fortini *et al*., 2019 , Pouri *et al*., 2020 , Vaz *et al*., 2020, Farshadpour *et al.*, 2021) but in consistent with other previous studies (Israr *et al*., 2021, Zhang *et al*., 2022).

In the current study, the number of cases were more common among the lower educational levels, and this may be because for health awareness among educated people was in agreement other previous studies (Owiti *et al*., 2015 , Kim *et al*., 2019)

4.5 Risk Factors Associated with HBV, HCV, Liver Cirrhosis Infection

Type of Disease		HBV (%)	HCV (%)	Cirrhosis ((%)	Total (%)	*p-value*
BMI	Normal weight	15 (35.7)	7 (36.8)	8 (34.8)	30 (35.7)	.949 (N.S)
	Overweight	15 (35.7)	8 (42.1)	10 (43.5)	33 (39.3)	
	Obesity	12 (28.6)	4 (21.1)	5 (21.7)	21 (25.0)	
	Total	42 (100)	19 (100)	23 (100)	84 (100)	
Family history	N0	32 (76.2)	18 (90)	22 (95.7)	72 (84.7)	.086 (N.S)
	Yes	10 (23.8)	2(10.0)	1 (4.3)	13 (15.3)	
	Total	42 (100)	20 (100)	23 (100)	85 (100)	
Smoking	Smoking	5 (11.11)	5(23.8)	7 (30.4)	17 (19.9)	.112 (N.S)
	Non smoking	40 (88.9)	16 (76.2)	16 (69.6)	72 (80.9)	
	Total	45 (100)	21 (100)	23 (100)	89 (100)	
Academic achievement	IIIiteracy	16 (35.6)	4 (19.0)	9 (39.1)	29(32.6)	.834 (N.S)
	Primary	21 (48.9)	12 (57.2)	10 (43.4)	44 (49.4)	
	Secondary	1 (2.2)	1 (4.8)	2 (8.7)	4 (4.5)	
	College or Institute	6 (13.3)	4 (19.1)	2(8.7)	12 (13.4)	
	Total	45(100)	21 (100)	23 (100)	89 (100)	
Marital status	Married	35(77.8)	15 (71.4)	25 (108)	75 (84.2)	.465 (N.S)
	single	10(22.2)	6 (28.5)	2 (8.69)	18 (20.22)	

	Total	45 (100)	21 (00)	23 (100)	89 (100)	
Living condition	Very good	1 (2.2)	1 (4.8)	1 (4.3)	3 (3.4)	.273 (N.S)
	Good	20 (44.4)	6 (28.6)	6 (26.1)	32 (36.0)	
	Medium	21 (46.7)	8 (38.1)	12 (52.2)	41 (46.1)	
	Poor	3 (6.7)	6 (28.6)	4 (17.4)	13 (14.6)	
	Total	45 (100)	21 (100)	23 (100)	89 (100)	

*Chi-Square Test

4.2. Association of HBV, HCV, and Liver cirrhosis with Bacterial components (LPS) and Immune response.

4.2.1 Differences in the mean level of LPS, IL-1, and IL-10 among the three studied groups.

The current study revealed the presence of significantly lower mean of LPS, IL-1, and IL-10 between HBV and HCV infection and between HBV and liver cirrhotic patients, as show in table (4.6). This results were consistent with those of earlier studies (Sadik *et al*., 2015, Faure-Dupuy *et al.,* 2019), Whereas no significant differences were found between HCV and liver cirrhotic patient which was in consistent with other previous study was found higher mean level of LPS(Fan *et al.,* 2020).

The presence of significantly lower mean of LPS, IL-1and IL-10 among HBV infected patients might possibly due to immune response manipulation by the virus. It has been documented that during chronic HBV infection causes the virus and its component (HBeAg), to manipulate a number of mechanisms that prevent IL-1ß from expressing and having a biological effect. Additionally, it prevents the NLRP3 inflammasome (nucleotide-binding and oligomerization domain-like receptor family pyrin domain-containing 3) is activated by LPS. the generation of IL-1ß, and the activation of NK- κB ,all of which may encourage the development of chronic infection (Yu *et al*., 2017, Papadakos *et al*., 2022) .

Interestingly has been demonstrated that persistent HCV infection causes a considerable elevation in LPS, which is a marker of liver failure. Dysbiosis of the gut microbiota in chronic hepatitis B infection affects the etiology of the disease and frequently results in liver failure. Indication of the spread of bacteria and inflammation during the development of the illness (Sehgal *et al*., 2020).

Additionally, dysbiosis of gut microbiota and intestinal innate immune response are linked to cirrhosis (Hasa *et a*l., 2022). Pathogen-associated molecular patterns (PAMPs), such as LPS, are the main cause of systemic inflammation in cirrhosis and are the result of bacterial translocation (Soffientini *et al*., 2021)

Table (4.6): Differences in LPS, IL-1, and IL-10 mean among HBV, HCV and Liver cirrhotic patients.

	Type of disease	No	Mean	SD	Min	Max	Post Hoc	Kruskal-Wallis test *P-value*
LPS (U/I)	HBV	37	105.89	110.02	2	476	b	.001*
	HCV	20	232.29	131.03	18	514	a	
	Liver cirrhosis	22	299.64	133.49	9	619	a	
IL-10 (ng/ml)	HBV	38	17.89	15.63	2.289	67.60	b	.001*
	HCV	20	27.17	10.93	4.963	45.24	a	
	Liver cirrhosis	22	28.87	11.61	6.438	62.43	a	
IL-1 (ng/ml)	HBV	35	11.13	9.58	1.667	49.49	b	.001*
	HCV	20	17.609	7.14	1.667	29.82	a	

	Liver cirrhosis	22	15.62	5.29	2.914	29.55	a	

*Significant at 0.05 level, Similar letter (a) means non-significant differences

4.2.2. Differences in Mean level of LPS, IL-1, and IL-10 According to Sex.

This study showed that there were significant higher mean level of IL-10 and LPS in HCV infected females. Inversely, HBV infected females had lower LPS, IL-1, and IL-10 levels than males but not for the significant level. Additionally, non-significant higher mean of LPS was found in females with liver cirrhosis, as shown in table 4-7.

The presence of differences in immune response (both innate and acquired immune response and may include cytokine production) between males and females has been documented. Females produce stronger response to viral infections render females capable of eliminating viruses more rapidly than males. However, Females develop more pronounced antibody responses than males making them more susceptible to various autoimmune and inflammatory diseases (Butterworth *et al*., 1967, Beeson, 1994, Pennell *et al.,* 2012, Klein & Flanagan, 2016) .

Studies in mice further reveald that cytokine responses of CD4T cells often differ between males and females with females reportedly exhibiting higher Th1 (i.e. IFN-γ), helper T cell type 2 (Th2, i.e. IL-4), and regulatory T cell (i.e. IL-10) responses than males, depending on stage of infection or type of antigen encountered (Barrat *et al*., 1997 , Roberts *et al.*, 2001 , Pinzan *et a*l., 2010)

The results of this study could be explained by the presence of higher chronicity rate among female in HBV infected patients as compared to male. Inversely, chronicity rate was higher in HCV infected male than female, as shown in table 4-4B.

It has been documented that during persistent viral infection many cell types produce IL-10 (Rouse & Masopust, 2006, Belkaid & Tarbell, 2009). which is immunosuppressive cytokine and can limit immune response and prevent viral clearance (Özgüler *et al*., 2015). Persistent HCV infection leads to chronic hepatitis, which is mainly due to the inability of the immune system to eliminate the virus. Dysregulated cytokine production is related to the chronicity of hepatitis C, the cytokine acts by limiting the marked pro-inflammatory response and damage caused by inflammation. In infectious processes, there is a direct correlation between lower IL-10 production and greater disease severity (Santiago *et al*., 2021).

Table (4-7): Differences in Mean levels of LPS, IL-1, and IL-10 according to Sex.

Type of Disease	Sex	LPS (U/I) Mean ± SD	IL-1 (ng/ml) Mean ± SD	IL-10 (ng/ml) Mean ± SD
HBV	Male	117.71 ± 130	12.71 ± 12.52	18.09 ± 17.92
	Female	98.70 ± 98.38	10.194 ± 7.52	17.77 ± 14.38
P value		0.61	0.46	0.95
HCV	Male	163.03 ± 116.815	15.09 ± 9.56	20.99 ± 12
	Female	278.47 ± 123.11	19.28 ± 4.72	31.29 ± 8.28
P value		0.05	0.2	0.035*
Liver Cirrhosis	Male	285.99 ± 114.73	15.6 ± 5.37	28.18 ± 10.81
	Female	316.01± 157.94	15.64 ± 5.48	29.69 ± 13.05
P value		0.61	0.98	0.77

*Significant at 0.05 level

4.2.3. Differences in Mean level of LPS, IL-1, and IL-10 According to Age.

This study found that there was a significant lower mean level of IL-10 in HCV infected patients with more than 40 years old. However, LPS, IL-1, and IL-10 serum levels were found to decrease but not for the significant level in HBV infected patients with age more than 40 years, whereas, in liver cirrhotic patients the mean level was increased, as shown in table 4-8.

The adult population has up until now been the center of the global hepatitis response, which is most at risk for HBV and HCV morbidity and mortality. To achieve the goal of eradicating HBV and HCV infection as a global public health problem, all

impacted populations, especially children and adolescents, must be taken into consideration (Indolfi *et al*., 2019) .

It has been documented that exaggerated proinflammatory cytokine responses can be observed with aging, and reduced levels of the anti-inflammatory cytokine IL-10 may contribute to these responses (Meador *et al*., 2008). This might possibly due to the liver function in detoxification and elimination of toxic materials and microbial products that reach the liver even during the HBV and HCV infection whereas in liver cirrhosis, complete loss of liver function may occur. Subsequently, the systemic inflammation associated with the presence of LPS in blood will vary. The presence of lower level of LPS is concomitant with the lower inflammatory response and lower cytokines level and this is proved by the presence of lower IL-1 and IL-10 in HBV and HCV infected patients with age more than 40 years and higher mean was observed in liver cirrhotic patients within the same age group, as shown in table 4-8. The result of the current study was in agreement with previous studies (Von Baehr *et al*., 2000 , Li *et al*., 2013) .

It has been documented that "Cirrhosis due to any etiology disrupts the homeostatic role of liver in the body. Cirrhosis-associated immune dysfunction leads to alterations in both innate and acquired immunity, due to defects in the local immunity of liver as well as in systemic immunity. Cirrhosis-associated immune dysfunction is a dynamic phenomenon, comprised of both increased systemic inflammation and immunodeficiency, and is responsible for 30% mortality. Immune paralysis is characterized by increase in anti-inflammatory cytokines and suppression of proinflammatory cytokines. There is also presence of increased gut permeability, reduced gut motility and altered gut flora, all of which leads to increased bacterial translocation. This increased bacterial translocation and consequent endotoxemia leads to increased blood stream bacterial infections that cause systemic inflammatory

response syndrome, sepsis, multiorgan failure and death. The gut microbiota of cirrhotic patients has more pathogenic microbes than that of non-cirrhotic individuals, and this disturbs the homeostasis and favors gut translocation" (Noor & Manoria, 2017) .

Table (4-8): Differences inMean level of LPS, IL-1, and IL-10 according Age

	Sex	LPS (U/I) Mean ± SD	IL-1 (ng/ml) Mean ± SD	IL-10 (ng/ml) Mean ± SD
HBV	≤40 years	109.83 ± 113.0	11.84 ± 10.6	19.08 ± 16
	>40 years	100.12 ± 109.14	10.06 ± 7.9	16.26 ± 15.4
P value		0.79	0.59	0.58
HCV	≤40 years	276.42 ± 130.29	20.07 ± 5.89	32.23 ± 9.7
	>40 years	178.35 ± 116.64	14.59 ± 7.68	20.99 ± 9.38
P value		0.09	0.08	0.01*
Liver Cirrhosis	≤40 years	279.95 ± 158.39	12.46 ± 5.05	24.11 ± 11.21
	>40 years	307.02 ± 127.95	16.8 ± 5.01	30.65 ± 11.6
P value		0.68	0.08	0.24

*significant at 0.05 level

4.2.4. Differences in Mean level of LPS, IL-1, and IL-10 According to BMI

No significant differences in the mean level of LPS, IL-1, and IL-10 was found among HBV, HCV, and cirrhotic patients according to BMI. However, overweight patients with HBV and cirrhosis tend to have higher mean level than normal weight patients. The result of the current study was in agreement with previous studies (Charles *et al*., 2011, Hegazy *et al*., 2020). The risk of weight gain was highest in young people with cirrhosis, and moderate alcohol consumption depending on important clinical characteristics (age, presence of cirrhosis, weight at treatment initiation, and alcohol use habits). DAA treatment, there is a link between excessive weight gain and both advanced fibrosis and cirrhosis (Do *et al*., 2020) .

Table (4-9): Differences in Mean level of LPS, IL-1, and IL-10 according to BMI

Type of Disease	BMI	LPS (U/I) Mean ± SD	IL-1(ng/ml) Mean ± SD	IL-10 (ng/ml) Mean ± SD
HBV	Normal	85.27 ± 77	10.02 ± 6.86	16.04 ± 11.77
	Overweight	117.07 ± 124.43	11.63 ± 10.69	18.65 ± 17.10
P value		0.4	0.65	0.64
HCV	Normal	286.26 ± 157.88	17.92 ± 8.01	29.9 ± 13.25
	Overweight	196.31 ± 101.30	17.39 ± 6.86	25.35 ± 9.25

P value		0.13	0.87	0.37
Liver Cirrhosis	Normal	255.57 ± 138.64	13.46 ± 4.97	24.39 ± 9.97
	Overweight	324.82 ± 128.68	16.85 ± 5.23	31.43 ± 12.04
P value		0.25	0.15	0.17

4.2.5. Correlation of LPS with IL-10 and IL-1 among the three studied groups.

The current study revealed that there was positive significant correlation between LPS with IL-10, and IL-1 among the three studied groups of patients, as shown in table (4.10), It been found that LPS positivity correlated with IL-1 production in concentration dependent miner (Loppnow *et al*., 1990) .

Interleukin levels are directly linked to HBV infection and influence how inflamed the liver is in HBV patients, exacerbating liver damage and resulting in a poor prognosis (Zhang *et al.,* 2022). Inflammation is also encouraged by increasing the body's exposure to microbial substances, gram-negative LPS, for instance. It is believed that this exposure both results from and accelerates the progression of liver disease. It is thought that this exposure is both a consequence of and an aggravating factor in liver disease progression (Townsend *et al.,* 2020). It has been documented that *in vitro* LPS stimulation result in different level of cytokine production; more TNFα and less IL-10 were produced (Chistyakov *et al*., 2018) .

Previous study found that injection of high doses of LPS may leads to the development of systemic inflammatory response. The key element of signal transmission in gram-negative infections is the activation of TLR on the surface of mononuclear phagocytes, neutrophilic granulocytes, dendritic cells, endothelial cells, hepatocytes, and epithelial cells in the intestinal, respiratory, and urogenital tracts. The binding of LPS with TLR4 triggers an intracellular cascade, which leads to the activation of inhibitor of NF-κB (I-κB kinase), and further translocation of NF-κB through the nuclear membrane initiates the transcription of proinflammatory cytokine genes: IL-1, IL-6, IL-8, and TNF. Activation of NF-κB is necessary for the full expression of proinflammatory mediators,

such as cytokines, adhesion molecules, and chemokines, and as a result, their hyper production leads to the development of multiple organ dysfunction associated with inflammation (Kosyreva *et al*., 2018) .

Table (4.10): Correlation between LPS and IL-10 and IL-1 within HBV, HCV and Liver cirrhosis

LPS (U/I)		IL-1 (ng/ml)	IL-10 (ng/ml)
HBV	Pearson Correlation	.968**	.957**
	Sig. (2-tailed)	.000	.000
	No	34	35
HCV	Pearson Correlation	.774**	.792**
	Sig. (2-tailed)	.000	.000
	No	20	20
Liver cirrhosis	Pearson Correlation	.784**	.805**
	Sig. (2-tailed)	.000	.000
	No	22	22
**. Correlation is significant at the 0.01 level (2-tailed).			

Additionally, significant positive correlation was found between IL-1 and IL-10 among the studied groups, as show in table (4.11), The result of the current study is in agreement with previous study (Sikora *et al*., 2008) .

One of the most well-known proinflammatory cytokines is IL-1. The individuals with HCV had higher serum IL-1 levels, which is crucial for viral eradication and the immunological system of the host. Its documented that in cirrhotic patients and a persistent hepatitis C infection the level of IL-1 was markedly increased (Tawfik *et al*., 2018). In chronic HBV infection, inflammation that causes liver damage is linked to immune response activation, which is necessary for virus elimination from hepatocytes. The genetic makeup of IL-10 in the host is a danger sign for liver damage

and the onset of cirrhosis when hepatitis B is persistent. It's interesting to note that people with HBV infection are not the only ones who can get liver disease from IL-10, and HCV infection has also been associated with interactions between the IL-10 promoter genotype and liver outcome (Rybicka *et al.*, 2020).

Table (4.11): Correlation between IL-10 and IL-1 within HBV, HCV and Liver cirrhosis

	Type of Disease		IL-1(ng/ml)
IL-10 (ng/ml)	HBV	Pearson Correlation	.948**
		Sig. (2-tailed)	.000
		No	34
	HCV	Pearson Correlation	.845**
		Sig. (2-tailed)	.000
		No	20
	Liver cirrhosis	Pearson Correlation	.936**
		Sig. (2-tailed)	.000
		No	22
	**. Correlation is significant at the 0.01 level (2-tailed)		

4.2.6 Correlation of LPS, IL-1, and IL-10 with viral load of HBV and HCV.

This study found that significantly, there was a negative correlation between IL-1 level and viral load in patients with HCV infection. No other significant correlation was found, as shown in table (4.12). The present study was in agreement with the previous study (Wani *et al*., 2014)

On The other hand, this outcome was consistent with earlier research (Askoura *et al*., 2022). It has been shown that IL-1 can influence host immune responses and viral clearance; It can have a variety of biological effects on different cell types, and

IL-1 depletion may make the virus more persistent (IL-1 is expressed by sinusoidal cells in chronic hepatitis C (Al-Khafaji & Al-Kelaby, 2020) .

Table (4.12): Correlations between Viral load with LPS, IL-1, and IL-10

Viral load		LPS (U/I)	IL-10 (ng/ml)	IL-1 (ng/ml)
HBV	Pearson Correlation	-.254	-.216	-.349
	Sig. (2-tailed)	.254	.335	.132
	No	22	22	20
HCV	Pearson Correlation	-.500	-.637	-.736*
	Sig. (2-tailed)	.171	.065	.024
	No	9	9	9

*. Correlation is significant at the 0.05 level (2-tailed).

4.3 Association of LPS, IL-1, and IL-10 with the disease duration and disease chronicity

According to disease duration, the current study revealed that 46 (53.4%) of patients had the disease for less than or equal to 1 year. No significant differences were found among the three groups. However, higher frequency (25,58.1%) of patients with HBV had the disease for more than one year where as higher frequency of patients with HCV and Cirrhosis were found to have the disease for less than or equal to 1 year (14,70% and (14,60%) respectively as shown in table (4.13).

The current study was in agreement with previous study (Caradonna *et a*l., 2002 , Marhetti *et al.*, 2011). The longer the duration of the disease, the more severe fibrosis and the greater the development of HCC (Zampino *et al.*, 2015) .The burden of advanced liver disease varies widely across countries, This burden is dependent upon several factors including chronic HCV prevalence, age distribution (and duration of infection) (Dore *et al.*, 2014). In an analysis of immune cells from patients with chronic HBV infection, found that the duration of HBsAg exposure, rather than the quantity of

HBsAg, was associated with the level of anti-HBV immune response, duration of HBV infection exerts on the breadth of HBV specific T cells suggest that earlier treatment intervention should be considered (Le Bert *et al.*, 2020).

The presence of higher percentage of HBV infected patients with diseases duration for more than one year in comparison to HCV and liver cirrhotic patients could be attributed to high fatality rate among certain populations of chronic HCV and liver cirrhosis patients .It has been documented that's the proportion of death ,attributed to HCV infection is on rise and must deaths occur among those with end – stage liver disease (Ly *et al*., 2012). The progression to which occur over a long period of time (Thein *et al*., 2008) .

Table (4.13): Duration of the disease

Duration of the disease		Type of infection			Total
		HBV No(%)	HCV No(%)	Liver cirrhosis No(%)	
	≤1 year	18(41.8)	14(70)	14(60)	46(53.4)
	>1 year	25(58.1)	6(30)	9(39.1)	40(46.5)
Total		43 (50)	20(17.5)	23(26.7)	86
Chi Square *P-value*					0.081

As table (4.14) showed, the current study revealed that there were no significant differences in LPS, IL-1, and IL-10 level according to disease Duration. The current study was in agreement with previous study (Broekman *et al*., 2015). Immunopathology can degenerate into a state of chronic low-level inflammation generally maintained by chronic antigenic stimuli (such as those provided by

persisting hepatotropic viruses), which presents the main substrate for HCC development (Timperi & Barnaba, 2020).

Table (4.14) Differences in the Mean levels of LPS, IL-10, IL-1 according to disease duration

Duration categories			LPS(U/I)	IL-1 (ng/ml)	IL-10 (ng/ml)
HBV	≤ 1years	Mean	116.65	13.150875	22.582375
		No (%)	18(41.8)	16(37.2)	16(37.2)
		SD	118.319	11.6835905	17.2245175
	≥1years	Mean	102.15	9.998471	14.839900
		No(%)	17(39.5)	17(39.5)	20(46.5)
		SD	107.913	7.4419344	14.2394451
ANOVA test *P- value*			0.708	0.359	0.149
HCV	≤1years	Mean	230.09	17.979643	27.708786
		No(%)	14(70)	14(70)	14(70)
		SD	137.547	6.1496263	10.8585971
	≥1years	Mean	242.60	17.308200	26.827800
		No(%)	5(25)	5(25)	5(25)
		SD	140.746	10.7451932	13.2527809
ANOVA test *P- value*			.864	.865	.884
Liver Cirrhosis	≤1 years	Mean	328.91	16.914000	30.744077
		No(%)	13(56.5)	13(56.5)	13(56.5)
		SD	143.152	5.5816091	13.3104297
	≥1 years	Mean	257.36	13.752667	26.170889
		No(%)	9(39.1)	9(39.1)	9(39.1)
		SD	112.418	4.4879500	8.6397475
ANOVA test *P- value*			.255	.174	.377

SD (Standard deviation)

The current study found that individuals with HBV, HCV, and liver cirrhosis did not have significantly different mean levels of LPS, IL-1, and IL-10 within disease chronicity. However, the mean level of LPS, IL-1, and IL-10 were greater in acute HBV than chronic HBV. Whereas, higher mean of LPS, and IL-10 were found with liver cirrhosis than in acute and chronic HBV, HCV infection, as shown in table (4.15).

This results was in agreement with previous studies (Schlaak *et al.*, 1999 ,Gómez-Hurtado *et al*., 2011) .

Recent research documented that was BT form intestine plays important role in advanced chronic liver disease. LPS may subsequently induce immune response in liver and other organs promote systemic inflammation as key pathophysiological mechanism in cirrhosis (Simbrunner *et al.*, 2023) Indeed, recent studies on gut microbiota in cirrhosis have shown that microbiota changed when the underlying disease worsened (Bajaj *et al.*, 2014). In advanced liver cirrhosis there appears to be an increase in dysbiosis, with a greater abundance of gram-negative taxa (Enterobacteriaceae, Bacteroidaceae). The consistent pattern of microbiota change and its association with the severity of cirrhosis cross-sectionally and longitudinally indicates that the underlying severity of cirrhosis may be a stronger determinant of microbial abundance (Giannelli *et al.,* 2014)

Dietary modifications may also affect the stability of gut microbiota in cirrhotic patients over time. For instance, restriction of dietary protein was considered a mainstay in the therapy of hepatic encephalopathy for a long time. It has been recognized that protein energy malnutrition is frequent in advanced liver disease and may adversely affect patient outcome (Merli *et al.,* 2013) .The use of antibiotics during hospitalization is also frequent and might interact with the composition of gut microbiota (Pérez-Coba *et al.*, 2013) .

Table (4.15) Differences in LPS,IL-1, and IL-10 mean level according to disease chronicity

Diagnosis			LPS (U/I)	IL-1 (ng/ml)	IL-10 (ng/ml)
HBV	Acute	Mean	137.08	14.333500	23.105200
		No	11(31.42)	10(30.30)	10(27.7)
		Std. Deviation	136.542	13.8428678	19.7568215
	Chronic	Mean	97.02	10.306652	16.425538

		No	24(68.56)	23(69.69)	26(72.22)
		Std. Deviation	99.477	7.3174164	14.1426428
Total		Mean	109.61	11.526909	18.281000
		No	35(100)	33(100)	36(100)
		Std. Deviation	111.954	9.7076369	15.8885285
ANOVA test *p-value*			.333	.280	.265
HCV	Acute	Mean	241.79	17.480750	26.362500
		No	4(22.22)	4(22.22)	4(22.22)
		Std. Deviation	88.030	3.4012640	3.3805232
	Chronic	Mean	238.03	17.826786	27.597857
		No	14((77.77)	14(77.77)	14(77.77)
		Std. Deviation	149.920	8.4082803	12.9825949
Total		Mean	238.86	17.749889	27.323333
		No	18(100)	18(100)	18(100)
		Std. Deviation	136.226	7.4918294	11.4536218
ANOVA test *p-value*			.936	.938	.856
Liver cirrhosis Chronic		Mean	299.64	15.620727	28.873227
		No	22 (100)	22 (100)	22 (100)
		Std. Deviation	133.495	5.2921143	11.6177144

4.4 Liver damage markers

4.4.1 Frequencies of HBV, HCV, and Liver cirrhotic patients according to normal range of liver damage markers.

The current study revealed that high frequency of HBV, HCV, and liver cirrhotic patients had abnormal value of ALT, AST, ALK, TSB, and PT, as shown in table 4.16.

This results were in agreement with previous studies (Zainal *et al*., 2012 , Al-Salih *et al.*, 2021). Increases in the levels of both ALT and AST strongly suggested hepatocellular injury. AST is released from the damaged muscle tissues, red blood

cells, and hepatocytes and ALT is released by hepatocytes during liver injury, usually reflecting the degree of liver damage (Al-Kanaan *et al*., 2020). It has been reported that neither ALT nor AST reflects the degree of inflammation seen on liver biopsy in patients with chronic hepatitis C (Anderson *et al*., 2000).

Biomarkers are equally useful in the evaluation and assessment of hepatic function and disease severity because HBV infection may alter the serum levels of certain hepatic enzymes and compounds such as ALK, AST, ALT, TSB (Abulude *et al*., 2017). Impaired Blood clotting may be due to poor protein production in the liver of patients compared with healthy peoples. Elevation in the level of total bilirubin in the patients is due to problems in the gall bladder itself which can seriously affect the liver (Jasim, 2013) .

Table 4.16. Frequencies of HBV, HCV, and Liver cirrhosis patients according to liver damage markers

Liver damage marker		HBV	HCV	Liver cirrhosis	Total
ALT(U/L)	Normal	21 (60)	6 (17.14)	8 (22.85)	35(30.8)
	Abnormal	24 (44.44)	15 (27.77)	15 (27.77)	54 (61.36)
AST (U/L)	Normal	19 (61.29)	5 (16.12)	7 (22.58)	31 (35.22)
	Abnormal	26 (44.82)	16 (27.58)	16 (27.58)	58 (65.90)
ALK (U/L)	Normal	23 (57.5)	10 (25)	7 (17.5)	40 (45.45)
	Abnormal	22(44.89)	11 (22.44)	16 (32.65)	49 (55.68)
TSB (mg/dl)	Normal	20 (52.63)	9 (23.68)	9 (23.68)	38 (43.18)
	Abnormal	25(49.01)	12 (23.52)	14 (27.45)	51 (57.95)
PT (seconds)	Normal	0	1(100)	0	1 (1.13)
	Abnormal	45(51.13)	20 (22.72)	23 (26.13)	88 (100)

Alanine transaminase (ALT), Aspartate transaminase (AST)., ALK: Alkaline Phosphatase, TSB: Total Serum Bilirubin, PT; Prothrombin time

4.4 .2 Differences in mean level of markers for liver damage in the three studies groups

As a result of immunological activation and nonspecific hepatocyte death brought on by the mononuclear cell recruitment to the liver during HBV infection, liver damage occurs. The liver damage enzyme ALT is a good indicator of the likelihood of an antiviral response (Johnson Valiente *et al*., 2022).

The liver has a big impact on the body's metabolism, digestion, detoxification, and elimination of toxins. A liver function test may typically measure the enzymes ALT and AST, ALK, TSB, PT, and Alb. These tests can help in locating a probable liver injury site (Lala *et al.*, 2021) . Liver injury and damage can cause a rise in liver enzyme levels, while hepatic aging, defects, and decreased hepatic blood flow can cause a reduction in liver enzyme levels (Kim *et al*., 2019)

According to this study, there were no significant variations in the mean levels of ALT, AST, ALK, PT, and TSB among HBV with HCV and liver cirrhotic patients, as shown in table (4.17), Similar results from earlier investigations have been reported (Peterson *et al*., 2003 , Effenberger *et al.*, 2023) .

As liver disease worsens, ALT and AST values rise, likely as a result of direct hepatocellular injury and membrane leakage. Increased ALK levels have been connected to numerous parenchymal liver illnesses, including hepatitis. ALK is found in the hepatic sinusoids and biliary canaliculi cell membranes of the liver. As Result, levels increase when there is sinusoidal blockage, intrahepatic and extrahepatic biliary obstruction, or when there is infiltrative liver disease. PT was noticeably longer in HBeAg positive individuals because these enzymes are found in hepatocytes and can "leak" into the blood if the hepatocytes are damaged. The sick condition of the liver, which is in charge of generating clotting factors, can be used to explain this type of alteration (Al-Kanaan *et al*., 2020) .

Increased serum TSB as a result of liver conditions such cirrhosis and viral hepatitis that cause anomalies in bilirubin metabolism (e.g., decreased hepatocyte absorption, impaired bilirubin conjugation, and decreased hepatocyte synthesis of bilirubin). An imbalance between synthesis and conjugation, followed by elimination, is typically the cause of elevated serum bilirubin levels (Fazaa *et al*., 2022) .

Albumin is a globular protein with liver protein synthetic function, Serum ALB level is major indexes for liver function examination (Lyu, 2020). Albumin function have shed new light on the diagnosis, evaluation and treatment of patients with liver diseases, especially those in the early stage of liver diseases, such as viral hepatitis (Sun *et al.,* 2019).

Table (4.17) Differences between HBV, HCV, Live cirrhosis regarding liver function test.

Types of diseases		Mean	SD	Min	Max	p-value
ALT (U/L)	HBV	141.20	361.7	8	1668	.769
	HCV	93.49	137.26	18	533	
	Liver cirrhosis	87.88	143.36	11	600	
AST (U/L)	HBV	112.44	289.66	14	1316	.901
	HCV	79.75	79.25	17	307	
	Liver cirrhosis	96.33	109.48	17	411	
Al k (U/L)	HBV	113.67	75.56	55	364	.669
	HCV	141.72	140.3	58	539	
	Liver cirrhosis	121.67	54.03	23	200	
PT (Seconds)	HBV	15.31	2.39	13	20	.214
	HCV	14.12	.96	13	15	
	Liver cirrhosis	18.18	6.14	14	35	
Alb Mg/dl	HBV	0	0	0	0	-
	HCV	0	0	0	0	
	Liver cirrhosis	3.18	.641	2.30	4	
TSB Mg/dl	HBV	1.29	2.86	.20	14	.288
	HCV	.85	.69	.20	2.4	
	Liver cirrhosis	2.43	3.51	.30	13.8	

SD (Standard deviation), Alanine transaminase (ALT), Aspartate transaminase (AST)., ALK: Alkaline Phosphatase, TSB: Total Serum Bilirubin, PT; Prothrombin time, Albumin (Alb)

4.4.3 AST/ ALT Ratio Among HBV, HCV, and Liver cirrhosis patients

It was documented that the ratio of AST to ALT is utilized to determine the cause of liver disease that is underlying (Katzke *et al.,* 2020, Liu *et al.,* 2021c). If the ratio is less than 1, this is an indication for Non-alcoholic fatty liver disease. If the ratio is equal to 1, this is a sign for acute viral hepatitis or drug related liver toxicity. Whereas, if the ratio is higher than 1, this indicate a liver cirrhosis. Finally, if the ratio is more than 2:1, this indicate alcohol liver diseases (Zou *et al.*, 2020, Ewid *et al.*, 2020, Agarwal, 2021). The current study revealed that there were no significant differences in the mean level of AST/ ALT ration among the three studied groups, as shown in table (4.18).

Table (4.18). Mean level of AST/ALT Ratio among the three studied groups

Type of disease	Mean	No	Std. Deviation
HBV	1.2393	28(48.27)	.68031
HCV	1.0508	12(20.68)	.48078
Liver cirrhosis	1.3839	18(31.03)	.54927
ANOVA *test P-Value*			0.343

This study showed that 15/28 (53.5%), 4/12 (33.3%), and 10/18 (55.5%) of HBV, HCV and liver cirrhotic patients, respectively, had AST/ ALT ration more than 1, as shown in table (4.19). Similar finding were documented in previous research (Scheller *et al.,* 2021,Chicco & Jurman, 2021) .

The AST: ALT ratio is one of the indirect serum fibrosis tests. Since the normal value is less than 1, a result higher than 1 indicates advanced fibrosis or cirrhosis. If a patient receives an early diagnosis, their prognosis is probably better. Having a clinical suspicion of liver disease is necessary for making the diagnosis, Especially among communities at risk. Imaging and biochemical testing are part of the early studies. The

existence of liver fibrosis can be predicted by combining serum indicators and clinical characteristics (Gupta & Walker, 2021).

(4.19) Distribution of HBV, HCV, Liver cirrhosis according to AST to ALT Ratio

AST/ALT Ratio		Type of Disease			Total
		HBV No (%)	HCV No (%)	Liver cirrhosis No (%)	
	Missing	17 (37.77)	9(42.85)	5(21.73)	31(34.84)
	less than 1	12(26.66)	6(28.57)	4(12.5)	22(24.7)
	equal to 1	0	1(4.76)	1(4.34)	2(2.24)
	More than 1	15(33.33)	4(19.0)	10(43.47)	29(32.58)
	More than 2 to 1	1 (2.22)	1(4.76)	3(13.0)	5(5.61)
Total		45(50.56)	21(23.59)	23(25.84)	89

4.4.4 Comparison the mean level of LPS, IL-1, and IL-10 according to markers of liver damage.

Comparison the mean level of LPS, IL-1, and IL-10 according to the normal/abnormal range of the ALT, AST, ALK, TSB, and PT among the three studied groups of patients revealed significant differences in IL-10 with AST and ALK in HBV infected patients and significant difference in LPS mean according to TSB in HCV infected patients and significant difference in IL-1 according to ALK in liver cirrhotic patients as shown in table 4.20A-C. No previous study was found that study the differences in mean level of LPS, IL-1, and IL -10 according to normal and abnormal values of liver enzymes.

Table (4.20) A. Differences in mean level of LPS, IL-1, and IL-10 according to Biochemical test in HBV infected patients

Biochemical test		LPS mean ± SD U/L	IL-1 mean ± SD mg/dl	IL-10 mean ± SD mg/dl
ALT(U/L)	Normal range	82.63±75.29	9.66±5.82	13.76±10.61
	Abnormal range	127.93±133.42	12.36±11.9	22.02±18.81
	P-value	0.215	0.414	0.104
AST(U/L)	Normal range	76.83±65.051	8.63±4.29	12.2±8.38
	Abnormal range	128.03±132	13±11.92	23.02±18.84
	P-value	0.16	0.18	0.03*
ALK(U/L)	Normal range	75.58±68	8.57±5.68	12.35±9.73
	Abnormal range	141.56±138.73	14.16±12.3	25.51±19
	P-value	0.06	0.08	0.008
TSB(mg /dl)	Normal range	84.63±71.454	9.15±6.07	14.6±10.48
	Abnormal range	123.97±133.74	12.79±11.67	20.55±18.65
	P-value	0.28	0.26	0.24
PT(seconds)	Abnormal	105.89±110.02	11.13±9.58	17.89±15.63

Table (4-20) B. Differences in mean level of LPS, IL-1, and IL-10 according to Biochemical test in HCV infected patients

Biochemical test		LPS mean ± SD U/L	IL-1 mean ± SD mg/dl	IL-10 mean ± SD mg/dl
ALT(U/L)	Normal range	228.62±159.15	17.61±7.7	26.54±12.7
	Abnormal range	233.87±123.86	17.6±7.19	27.45±10.6
	P-value	0.93	0.99	0.87
AST (U/L)	Normal range	276.58±205.18	20.2±10.09	29.36±16.24
	Abnormal range	217.53±101.67	16.74±6.07	26.44±9.2
	P-value	0.39	0.36	0.61
ALK (U/L)	Normal range	198.78±118.24	15.02±7.96	22.37±11.2
	Abnormal range	259.71±139.99	19.72±5.93	31.10±9.43
	P-value	0.31	0.14	0.07
TSB (mg/dl)	Normal range	153.70±88.5	14.19±7.76	22.14±12.05
	Abnormal range	284.68±131.09	19.88±5.96	30.53±9.13
	P-value	0.02*	0.08	0.09
PT(seconds)	Abnormal	234.22±134.33	17.76±7.3	26.64±10.96

Table (4-20) C. Differences in mean level of LPS, IL-1, and IL-10 according to Biochemical test in liver cirrhotic patients.

Biochemical test		LPS mean ± SD U/I	IL-1 mean ± SD ng/ml	IL-10 mean ± SD ng/ml
ALT(U/L)	Normal range	297.03±185.85	16.43±7.36	32.27±15.85
	Abnormal range	301.12±100.9	15.15±3.92	26.92±8.45
	P-value	0.94	0.59	0.31
AST(U/L)	Normal range	286.82±198.3	16.53±7.94	33.16±16.9
	Abnormal range	305.62±98.77	15.19±3.78	26.87±8.15
	P-value	0.76	0.59	0.24
ALK(U/L)	Normal range	234.11±163.43	12.47±5.03	24.02±11.27
	Abnormal range	330.22±110.15	17.09±4.88	31.13±11.43
	P-value	0.18	0.05	0.18
TSB(mg/dl)	Normal range	269.29±189.09	14.84±7.44	28.35±16.08
	Abnormal range	320.64±78.61	16.16±3.36	29.22±7.95
	P-value	0.38	0.57	0.86
PT(seconds)	Abnormal	299.64±	15.62±	28.87±

4.4.5 Correlation of LPS, IL-1, and IL-10 with Markers of liver damage

This study showed that there was significant positive correlation of LPS, IL-1, and IL-10 with alkaline phosphatase. No significant correlation was found with ALT, AST, TSB, PT, and Albumin, as shown in table (4.21).

The present study was in agreement with previous study (Vimali *et al*., 2022). ALK test may be ordered as part of a normal examination or if the patient exhibits signs of liver damage or a bone issue (Lowe *et al*., 2022). ALK also has a crucial anti- Inflammatory function. Detoxification of bacterial LPS and free nucleotides are two examples of the protective functions of intestinal alkaline phosphatase. It is thought to work through dephosphorylation of LPS reducing its activity (Engelmann *et al.,* 2020, Le-Vinh *et al*., 2022) .

Table (4.21): Correlation between LPS, IL-1, and L10 with Markers of liver damage

		ALT (U/L)	AST (U/L)	ALK (U/L)	TSB (mg/dl)	PT (Second)	Alb (mg/dl)
IL-10 (ng/ml)	Pearson Correlation	.060	.090	.323*	.139	-.098	.425
	Sig. (2-tailed)	.664	.512	.023	.357	.664	.341
	No	55	55	49	46	22	7
LPS (U/l)	Pearson Correlation	.007	.084	.395**	.173	.125	.028
	Sig. (2-tailed)	.962	.550	.006	.244	.579	.953
	No	55	53	47	47	22	7
IL-1 (ng/ml)	Pearson Correlation	.060	.104	.369*	.148	-.065	.552
	Sig. (2-tailed)	.673	.463	.012	.330	.780	.199
	No	52	52	46	45	21	7

**. Correlation is significant at the 0.01 level (2-tailed). Alanine transaminase (ALT), Aspartate transaminase (AST)., ALK: Alkaline Phosphatase, TSB: Total Serum Bilirubin, PT; Prothrombin time, Albumin (Alb)

Conclusion and Recommendations

Conclusion and Recommendations

Conclusion:

The current study concludes the following:

1. Higher mean age of patients with liver cirrhosis and higher frequencies of HBV, HCV infection within the age range of 20 -39 years and the presence of significant difference in the mean age of acute and chronic infection could reflect the impact of age on infection.
2. Increase rate of acute hepatitis infection among young adult and increase percentage (75%) of patients whom they suffer from chronic infection
3. Higher frequency of infection occurs among female in case of HBV and HCV infection while lower frequency occurs in case of cirrhosis.
4. The most common routes of infection associated with HBV, HCV, Liver cirrhosis were Tooth extract and surgical Procedures.
5. Significant decrease in LPS, IL-1, IL-10 mean levels were observed in HBV-infected patients in comparison to the two other groups which may reflect the role of this virus in manipulating the immune response to achieve persistent infection.
6. Increase bacterial translocation which is represented by LPS serum level associated with increase systemic inflammatory response which is reflected by increase in cytokine level.
7. Chronic HBV is associated with decrease LPS titer while chronic HCV is associated with higher LPS titer and Liver cirrhosis is associated with higher LPS than HBV, HCV infection.
8. Significant positive correlation of LPS, IL-1, and IL-10 with Alkaline phosphatase may support the role of these parameters in liver damage.

Recommendations

The current study recommends:

I. Making a Case-Control study to investigate the following

1. The difference in the mean levels of LPS, IL-10, and 1L-1 between patients and control groups
2. The differences in Microbiome of HBV, HCV, Liver cirrhosis patients and compare it with the microbiome of healthy individuals.

II. Make larger scale study that deals with study:

1. The association of LPS and IL-1, and IL-10 with viral load and viral genotypes.
2. The association of LPS with other inflammatory and anti-inflammatory cytokines during different phases of viral infection.

III. Make a study that assess health awareness among population regarding infections and modes of transmission and also spreading the importance of sterilization of surgical and dental instruments in proper way to prevent infections from spreading.

The References

References

Abdel-Gawad, M., Nour, M., El-Raey, F., Nagdy, H., Almansoury, Y., & El-Kassas, M. (2023). Gender differences in prevalence of hepatitis C virus infection in Egypt: a systematic review and meta-analysis. *Scientific Reports*, *13*(1), 1–10. https://doi.org/10.1038/s41598-023-29262-z

Abdulqadir, M. O., Rashid, P. M. A., Hussain, A. H., Rahman, H. S., & Ezzaddin, S. A. (2023). Genetic characterization of hepatitis B virus genotypes among patients with chronic infection in Sulaimaniyah city, Iraq. *PeerJ*, *11*, e14454.

Abdulrazzaq, D. N. A., Ahmed, M. M., Hasan, F. T., & Hasson, D. F. C. (2022). The Impacts of Viral Hepatitis on Liver Enzymes and Bilrubin. *International Journal of Chemistry, Mathematics and Physics*, *6*(6), 01–06. https://doi.org/10.22161/ijcmp.6.6.1

Abulude, O. A., Ahmed, I., & Sadisu, F. U. (2017). Assessment of hepatitis B viral infection as a predictor of hepatic enzymes and compounds alteration among antenatal patients. *Medical Sciences*, *5*(4), 24.

Abusaiba, T. H. H. (2020). Prevalence of hepatitis virus B and C in patients in Al Najaf Governorate, Iraq. *International Journal of Pharmaceutical Research*, *12*(3), 1297–1303.

Adel Dawood, A. (2022). Hepatitis B Virus, Genotypes and Subtypes. *Hepatitis B*, *June*. https://doi.org/10.5772/intechopen.100446

Agbozo, W. K., Dzudzor, B., Nyarko, E. N. Y., Lartey-Abrahams, K., Mensah, R. N. A., & Tachi, K. (2022). Sociodemographic and medical characteristics of liver cirrhosis deaths in a Ghana-ian tertiary hospital. *Ghana Medical Journal*, *56*(4), 259–267.

Agarwal, S. K. (2021). Alcohol and Noncommunicable Diseases: Part I Cardiovascular Diseases, Obesity, Respiratory Diseases, Depression, Liver Diseases. *Clinical Medicine Insights*, *2*(3), 169–184.

Al-Kanaan, B. M. A., Al-Ouqaili, M. T. S., & Al-Rawi, K. F. A. (2020). Comparative study of the molecular, biochemical, and other parameters in Iraqi hepatitis B patients. *Drug Invention Today*, *14*(6), 870–876.

Al-Khafaji, Y. A. R., & Al-Kelaby, K. K. A. (2020). Genetic Diversity and Pro inflammatory Mediators il-1β and il-6 in Sera of Hepatitis c Virus Infected Patients in Babylon Province, Iraq

Al-Khazraji, K. A., Hashim, M. K., Hashim, M. K., Abdulla, M. K., Khudhair, I. H., & Abbas, W. K. (2021). Etiologies of Liver Cirrhosis and Their Clinical Presentation among Inpatients in Medical City Complex - Baghdad Teaching Hospital. *Global Journal of Health*

References

Science, *13*(5), 64. https://doi.org/10.5539/gjhs.v13n5p64

Al-Madany, N. A., & Sarhat, E. R. (2018). Determination of Some Biochemical Parameters of Patients with Hepatitis B in Kirkuk City Online) *Kirkuk University Journal /Scientific*. *Studies (KUJSS)*, *13*(2), 2616–6801. www.uokirkuk.edu.iq/kujss

Al-rubaey, N. K. F., Al-zamily, K. Y., Abd, R. K., & Al-azzawi, J. A. (2019). Serological Diagnosis of Hepatitis A Virus Among Children with Acute Viral Hepatitis in AL-Kut City. *April*, 244–248.

Al-Sadeq, D. W., Taleb, S. A., Zaied, R. E., Fahad, S. M., Smatti, M. K., Rizeq, B. R., Al Thani, A. A., Yassine, H. M., & Nasrallah, G. K. (2019). Hepatitis B virus molecular epidemiology, host-virus interaction, coinfection, and laboratory diagnosis in the MENA region: An update. *Pathogens*, *8*(2), 63.

Al-Salih, M., Abed, R. E., & Samsudin, S. (2021). Biochemical Assessment As Markers For Diagnosis And Evaluation Hepatitis B Virus (HBV). *Al-Qadisiyah Journal of Pure Science*, *26*(4), 156–168. https://doi.org/10.29350/qjps.2021.26.4.1359

Alexopoulou, A., Agiasotelli, D., Vasilieva, L. E., & Dourakis, S. P. (2017). Bacterial translocation markers in liver cirrhosis bactericidal/permeability increasing protein, soluble form of receptor for lipopolysaccharide. *Annals of Gastroenterology Gastroenterol*, *30*(305), 486–497. www.annalsgastro.gr

Alieva, N. M., & Tolipova, M. A. (2022). Influence of viral liver diseases on the state of the oral cavity.(literature review). *Innovative Development in Educational Activities*, *1*(5), 264–270.

Alnimer, L., & Noureddin, M. (2023). Non-invasive imaging biomarkers for liver steato-sis in non-alcoholic fatty liver disease: present and future. *Clinical and Molecular Hepatology*, *29*(2), 394–397. https://doi.org/10.3350/cmh.2023.0104

Alqahtani, S. A., & Colombo, M. (2020). Viral hepatitis as a risk factor for the development of hepatocellular carcinoma. *Hepatoma Research*, *2020*. https://doi.org/10.20517/2394-5079.2020.49

Anderson, F. H., Zeng, L., Rock, N. R., & Yoshida, E. M. (2000). An assessment of the clinical utility of serum ALT and AST in chronic hepatitis C. *Hepatology Research*, *18*(1), 63–71. https://doi.org/10.1016/S1386-6346(99)00085-6

Askoura, M., Abbas, H. A., Al Sadoun, H., Abdulaal, W. H., Abu Lila, A. S., Almansour, K.,

Alshammari, F., Khafagy, E.-S., Ibrahim, T. S., & Hegazy, W. A. H. (2022). Elevated levels of

References

IL-33, IL-17 and IL-25 indicate the progression from chronicity to hepatocellular carcinoma in hepatitis C virus patients. *Pathogens*, *11*(1), 57.

Assefa, A., Kiros, T. and Delelegn, B. (2023) 'Seroprevalence and Associated Factors of HBV and HCV among Pregnant Women Attending Antenatal Care at Debre Tabor Comprehensive Specialized Hospital, Northwest Ethiopia: A Cross-Sectional Study', *International Journal of Microbiology*, 2023. doi: 10.1155/2023/228267.

Aydin, M. M., & Akcali, K. C. (2018). Liver fibrosis. *Turkish Journal of Gastroenterology*, *29*(1), 14–21. https://doi.org/10.5152/tjg.2018.17330

Bajaj, J. S., Heuman, D. M., Hylemon, P. B., Sanyal, A. J., White, M. B., Monteith, P., Noble, N. A., Unser, A. B., Daita, K., & Fisher, A. R. (2014). Altered profile of human gut microbiome is associated with cirrhosis and its complications. *Journal of Hepatology*, *60*(5), 940–947.

Bakir, A. A., Othman, S. M., Rahman, L. Q., & Asaad, Y. A. (2023). Prevalence of HCV Genotypes in Correlation with Viral Load in Northern Region of Iraq. *Bahrain Medical Bulletin*, *45*(1), 1250–1254.

Bao, Y. L., Wang, L., Pan, H. T., Zhang, T. R., Chen, Y. H., Xu, S. J., Mao, X. L., & Li, S. W. (2021). Animal and Organoid Models of Liver Fibrosis. *Frontiers in Physiology*, *12*(May). https://doi.org/10.3389/fphys.2021.666138

Barrat, F., Lesourd, B., Boulouis, H.-J., Thibault, D., Vincent-Naulleau, S., Gjata, B., Louise, A., Neway, T., & Pilet, C. (1997). Sex and parity modulate cytokine production during murine ageing. *Clinical & Experimental Immunology*, *109*(3), 562–568.

Beeson, P. B. (1994). Age and sex associations of 40 autoimmune diseases. *The American Journal of Medicine*, *96*(5), 457–462.

Belkaid, Y., & Tarbell, K. (2009). Regulatory T cells in the control of host-microorganism interactions. *Annual Review of Immunology*, *27*, 551–589.

Bodsworth, N. J., Cooper, D. A., & Donovan, B. (1991). The influence of human immunodeficiency virus type 1 infection on the development of the hepatitis B virus carrier state. *Journal of Infectious Diseases*, *163*(5), 1138–1140.

Broekman, M. M. T. J., Roelofs, H. M. J., Hoentjen, F., Wiegertjes, R., Stoel, N., Joosten, L. A., de Jong, D. J., & Wanten, G. J. A. (2015). LPS-stimulated whole blood cytokine production is not related to disease behavior in patients with quiescent Crohn's disease. *PLoS One*, *10*(7), e0133932.

References

Butterworth, M., McClellan, B., & Aklansmith, M. (1967). Influence of sex on immunoglobulin levels. *Nature*, *214*(5094), 1224–1225.

Caradonna, L., Mastronardi, M. L., Magrone, T., Cozzolongo, R., Cuppone, R., Manghisi, O. G., Caccavo, D., Pellegrino, N. M., Amoroso, A., & Jirillo, E. (2002). Biological and clinical significance of endotoxemia in the course of hepatitis C virus infection. *Current Pharmaceutical Design*, *8*(11), 995–1005.

Charles, B. A., Doumatey, A., Huang, H., Zhou, J., Chen, G., Shriner, D., Adeyemo, A., & Rotimi, C. N. (2011). The roles of IL-6, IL-10, and IL-1RA in obesity and insulin resistance in African-Americans. *Journal of Clinical Endocrinology and Metabolism*, *96*(12), 2018–2022. https://doi.org/10.1210/jc.2011-1497

Chen, Y., & Tian, Z. (2019). HBV-induced immune imbalance in the development of HCC. *Frontiers in Immunology*, *10*(AUG), 1–11. https://doi.org/10.3389/fimmu.2019.02048

Chen, S., Guo, X., Yu, S., Zhou, Y., Li, Z., & Sun, Y. (2016a). Metabolic syndrome and serum liver enzymes in the general chinese population. *International Journal of Environmental Research and Public Health*, *13*(2). https://doi.org/10.3390/ijerph13020223

Chen, Y., & Tian, Z. (2019). HBV-induced immune imbalance in the development of HCC. *Frontiers in Immunology*, *10*(AUG), 1–11. https://doi.org/10.3389/fimmu.2019.02048

Chen, Y. X., Lai, L. N., Zhang, H. Y., Bi, Y. H., Meng, L., Li, X. J., Tian, X. X., Wang, L. M., Fan, Y. M., Zhao, Z. F., Han, D. W., & Ji, C. (2016b) . Effect of artesunate supplementation on bacterial translocation and dysbiosis of gut microbiota in rats with liver cirrhosis. *World Journal of Gastroenterology*, *22*(10), 2949–2959. https://doi.org/10.3748/wjg.v22.i10.2949

Cheng, Y.-L., Lee, C.-Y., Huang, Y.-L., Buckner, C. A., Lafrenie, R. M., Dénommée, J. A., Caswell, J. M., Want, D. A., Gan, G. G., Leong, Y. C., Bee, P. C., Chin, E., Teh, A. K. H., Picco, S., Villegas, L., Tonelli, F., Merlo, M., Rigau, J., Diaz, D., … Mathijssen, R. H. J. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *11*(tourism), 13. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics

Chicco, D., & Jurman, G. (2021). An ensemble learning approach for enhanced classification of patients with hepatitis and cirrhosis. *IEEE Access*, *9*, 24485–24498.

Chistyakov, D. V., Azbukina, N. V., Astakhova, A. A., Goriainov, S. V., Chistyakov, V. V., & Sergeeva, M. G. (2018). Sex-mediated differences in lps induced alterations of TNFα, IL-10 expression, and prostaglandin synthesis in primary astrocytes. *International Journal of*

References

Molecular Sciences, *19*(9), 1–14. https://doi.org/10.3390/ijms19092793

Cho, H. J., & Cheong, J. Y. (2021). Role of immune cells in patients with hepatitis b virus-related hepatocellular carcinoma. *International Journal of Molecular Sciences*, *22*(15). https://doi.org/10.3390/ijms22158011

Choi, W.-M., Kim, G.-A., Choi, J., Han, S., & Lim, Y.-S. (2022). Increasing on-treatment hepatocellular carcinoma risk with decreasing baseline viral load in HBeAg-positive chronic hepatitis B. *The Journal of Clinical Investigation*, *132*(10).

Chowdhury, A. B., & Mehta, K. J. (2023). Liver biopsy for assessment of chronic liver diseases: a synopsis. *Clinical and Experimental Medicine*, *23*(2), 273–285. https://doi.org/10.1007/s10238-022-00799-z

de Mattos, Â. Z., Debes, J. D., Boonstra, A., Yang, J. D., Balderramo, D. C., Sartori, G. D. P., & de Mattos, A. A. (2021). Current impact of viral hepatitis on liver cancer development: The challenge remains. *World Journal of Gastroenterology*, *27*(24), 3556–3567. https://doi.org/10.3748/wjg.v27.i24.3556

Delphin, M., Desmares, M., Schuehle, S., Heikenwalder, M., Durantel, D., & Faure-Dupuy, S. (2021). How to get away with liver innate immunity? A viruses' tale. *Liver International*, *41*(11), 2547–2559. https://doi.org/10.1111/liv.15054

Dirchwolf, M., & Ruf, A. E. (2015). Role of systemic inflammation in cirrhosis: From pathogenesis to prognosis. *World Journal of Hepatology*, *7*(16), 1974.

Do, A., Esserman, D. A., Krishnan, S., Lim, J. K., Taddei, T. H., Hauser, R. G., Tate, J. P., Re, V. Lo, & Justice, A. C. (2020). Excess weight gain after cure of hepatitis C infection with direct-acting antivirals. *Journal of General Internal Medicine*, *35*, 2025–2034.

Dore, G. J., Ward, J., & Thursz, M. (2014). Hepatitis C disease burden and strategies to manage the burden (Guest Editors Mark Thursz, Gregory Dore and John Ward). *Journal of Viral Hepatitis*, *21*, 1–4. https://doi.org/10.1111/jvh.12253

Duah, A., Duah, F., Ampofo-Boobi, D., Addo, B. P., Osei-Poku, F., & Agyei-Nkansah, A. (2022). Acute kidney injury in patients with liver cirrhosis: prevalence, predictors, and in-hospital mortality at a district hospital in Ghana. *BioMed Research International*, *2022*.

Ebrahimzadeh, A., Azarkar, Z., Ziaee, M., Sharifzadeh, G., & Bijari, B. (2016). Prevalence and Risk Factors of Hepatitis C Infection (HCV) in Birjand, Iran, 2014. *International Journal of Infection*, *3*(1), 1–5. https://doi.org/10.17795/iji-34102

Effenberger, M., Waschina, S., Bronowski, C., Sturm, G., Tassiello, O., Sommer, F., Zollner,

References

A., Watschinger, C., Grabherr, F., & Gstir, R. (2023). A gut bacterial signature in blood and liver tissue characterizes cirrhosis and hepatocellular carcinoma. *Hepatology Communications*, *7*(7).

Engelmann, C., Adebayo, D., Oria, M., De Chiara, F., Novelli, S., Habtesion, A., Davies, N., Andreola, F., & Jalan, R. (2020). Recombinant alkaline phosphatase prevents acute on chronic liver failure. *Scientific Reports*, *10*(1), 389.

Ewid, M., Sherif, H., Allihimy, A. S., Alharbi, S. A., Aldrewesh, D. A., Alkuraydis, S. A., & Abazid, R. (2020). AST/ALT ratio predicts the functional severity of chronic heart failure with reduced left ventricular ejection fraction. *BMC Research Notes*, *13*, 1–6.

Fan, C., Zhang, X., Zhang, P., Zhao, J., Shen, H., Zhang, Y., Wu, X., Jia, Z., & Wang, Y. (2020). LPS stimulation during HCV infection induces MMP/TIMP1 imbalance in macrophages. *Journal of Medical Microbiology*, *69*(5), 759.

Farshadpour, F., Taherkhani, R., & Bakhtiari, F. (2021). Prevalence and Predominant Genotype of Hepatitis C Virus Infection and Associated Risk Factors among Pregnant Women in Iran. *BioMed Research International*, *2021*. https://doi.org/10.1155/2021/9294276

Faure-Dupuy, S., Delphin, M., Aillot, L., Dimier, L., Lebossé, F., Fresquet, J., Parent, R., Matter, M. S., Rivoire, M., & Bendriss-Vermare, N. (2019). Hepatitis B virus-induced modulation of liver macrophage function promotes hepatocyte infection. *Journal of Hepatology*, *71*(6), 1086–1098.

Fazaa, A. H., Atya, A. K., & Kredy, H. M. (2022). Evaluation of Liver Function Tests and Their Correlation with HBV Viral Load in Patients with Hepatitis B Virus, Thi-Qar, Iraq. *HIV Nursing*, *22*(2), 1112–1116.

Fortini, I., Arouca, E. M. G., Tengam, F. M., & Nitrini, R. (2019). Chronic HCV infection and neuropsychiatric dysfunction. *Eneurologicalsci*, *17*.

Fourati, S., Feld, J. J., Chevaliez, S., & Luhmann, N. (2018). Approaches for simplified HCV diagnostic algorithms. *Journal of the International AIDS Society*, *21*(S2), 17–25. https://doi.org/10.1002/JIA2.25058

Gao, B. (2016). Basic liver immunology. *Cellular and Molecular Immunology*, *13*(3), 265–266. https://doi.org/10.1038/cmi.2016.09

Ghosh, S. S., Wang, J., Yannie, P. J., & Ghosh, S. (2020). Intestinal barrier dysfunction, LPS translocation, and disease development. *Journal of the Endocrine Society*, *4*(2), 1–15. https://doi.org/10.1210/jendso/bvz03

References

Giannelli, V., Di Gregorio, V., Iebba, V., Giusto, M., Schippa, S., Merli, M., & Thalheimer, U. (2014). Microbiota and the gut-liver axis: bacterial translocation, inflammation and infection in cirrhosis. *World Journal of Gastroenterology: WJG*, *20*(45), 16795.

Ginès, P., Krag, A., Abraldes, J. G., Solà, E., Fabrellas, N., & Kamath, P. S. (2021). Liver cirrhosis. *The Lancet*, *398*(10308), 1359–1376.

Gómez-Hurtado, I., Zapater, P., Bellot, P., Pascual, S., Pérez-Mateo, M., Such, J., & Francés, R. (2011). Interleukin-10-mediated heme oxygenase 1-induced underlying mechanism in inflammatory down-regulation by norfloxacin in cirrhosis. *Hepatology*, *53*(3), 935–944. https://doi.org/10.1002/hep.24102

Grüngreiff, K., Reinhold, D., & Wedemeyer, H. (2016). The role of zinc in liver cirrhosis. *Annals of Hepatology*, *15*(1), 7–16.

Gupta, S., & Walker, S. (2021). Testing for cirrhosis. *Australian Prescriber*, *44*(6), 197–199. https://doi.org/10.18773/austprescr.2021.05

Hadler, S. C., & Judson, F. N. (1991). O′ Malley PM, Altman NL, Penley K, Buchbinder S, et al. Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. *J Infect Dis*, *163*(3), 454–459.

Hagan, L. M., Kasradze, A., Salyer, S. J., Gamkrelidze, A., Alkhazashvili, M., Chanturia, G., Chitadze, N., Sukhiashvili, R., Shakhnazarova, M., Russell, S., Blanton, C., Kuchukhidze, G., Baliashvili, D., Hariri, S., Ko, S., Imnadze, P., Drobeniuc, J., Morgan, J., & Averhoff, F. (2019). Hepatitis C prevalence and risk factors in Georgia, 2015: Setting a baseline for elimination. *BMC Public Health*, *19*(Suppl 3), 1–12. https://doi.org/10.1186/s12889-019-6784-3

Hammood, A. J., Gharbi, W. A., & Razzaq, S. A. A. (2022). Estimation of Liver Enzymes in Patients Infected with Hepatitis B Virus in Baghdad Hospitals. *21*(2), 1–8.

Haq, I., Zahir, F., Haq, M., Zaman, R., Khan, A. M. K., Rehman, N., Ahmad, I., Jan, H., Nasar, Z., & Arif, H. (2022). Molecular and Epidemiological Evaluation of Liver Function Diagnosis between Different Genotypes of HBV and HCV in Non-Responders of HBV-HCV Co-Infected Patients with Healthy Controls. *Pakistan Journal of Medical & Health Sciences*, *16*(03), 827.

Hasa, E., Hartmann, P., & Schnabl, B. (2022). Liver cirrhosis and immune dysfunction. *International Immunology*, *34*(9), 455–466. https://doi.org/10.1093/intimm/dxac030

Hashmi, A. H., Farooq, A., Kausar, M., Husnain, M. A., & Imran, M. (2023). *Genotype*

frequency and pattern of transmission and of Hepatitis C virus (HCV) from the Capital city and proximate areas of the Pakistan.

Hegazy, M. A., Mogawer, S. M., Alnaggar, A. R. L. R., Ghoniem, O. A., & Samie, R. M. A. (2020). Serum LPS and CD163 biomarkers confirming the role of gut dysbiosis in overweight patients with NASH. *Diabetes, Metabolic Syndrome and Obesity*, *13*, 3861–3872. https://doi.org/10.2147/DMSO.S249949

Heim, M. H., & Thimme, R. (2014). nnate and adaptive immune responses in HCV infections Basic. *Journal of Hepatology*, *61*(1), S14–S25. https://doi.org/10.1016/j.jhep.2014.06.035

Herrscher, C., Roingeard, P., & Blanchard, E. (2020). Hepatitis B Virus Entry into Cells. *Cells*, *9*(6), 1–15. https://doi.org/10.3390/cells9061486

Hong, Y. A., Yee, S., Bagchi, P., Juon, H. S., Kim, S. C., & Le, D. (2022). Social media-based intervention to promote HBV screening and liver cancer prevention among Korean Americans: Results of a pilot study. *Digital Health*, *8*. https://doi.org/10.1177/20552076221076257

Howell, J., Pedrana, A., Schroeder, S. E., Scott, N., Aufegger, L., Atun, R., Baptista-Leite, R., Hirnschall, G., 't Hoen, E., Hutchinson, S. J., Lazarus, J. V., Olufunmilayo, L., Peck, R., Sharma, M., Sohn, A. H., Thompson, A., Thursz, M., Wilson, D., & Hellard, M. (2021). A global investment framework for the elimination of hepatitis B. *Journal of Hepatology*, *74*(3), 535–549. https://doi.org/10.1016/j.jhep.2020.09.013

Hsiao, P. J., Hsiao, C. J., Tsai, F. R., Hou, Y. L., Chiu, C. C., Chiang, W. F., Wu, K. L., Li, Y. K., Lin, C., Chan, J. S., Chang, C. W., & Chu, C. M. (2022). From Bench to Bedside: Clinical and Biomedical Investigations on Hepatitis C Virus (HCV) Genotypes and Risk Factors for Albuminuria. *Bioengineering*, *9*(10). https://doi.org/10.3390/bioengineering9100509

Huang, J., Lok, V., Ngai, C. H., Chu, C., Patel, H. K., Thoguluva Chandraseka, V., Zhang, L., Chen, P., Wang, S., Lao, X. Q., Tse, L. A., Xu, W., Zheng, Z. J., & Wong, M. C. S. (2021). Disease Burden, Risk Factors, and Recent Trends of Liver Cancer: A Global Country-Level Analysis. *Liver Cancer*, *10*(4), 330–345. https://doi.org/10.1159/000515304

Hussein, N. R., Musa, D. H., Hawezy, D., Ahmed, F., Khalid, F. K., Naqid, I. A., & Assafi, M. (2021). A study on the prevalence and the risk factors of Hepatitis B Virus infection in Kurdistan Region, Iraq: a multicenter study. *Journal of Contemporary Medical Sciences*, *7*(5), 282–285.

Hyams, K. C. (1995). Risks of chronicity following acute hepatitis B virus infection: a review. *Clinical Infectious Diseases*, *20*(4), 992–1000.

References

Iida-Ueno, A., Enomoto, M., Tamori, A., & Kawada, N. (2017). Hepatitis B virus infection and alcohol consumption. *World Journal of Gastroenterology*, *23*(15), 2651–2659. https://doi.org/10.3748/wjg.v23.i15.2651

Indolfi, G., Easterbrook, P., Dusheiko, G., El-Sayed, M. H., Jonas, M. M., Thorne, C., Bulterys, M., Siberry, G., Walsh, N., & Chang, M.-H. (2019). Hepatitis C virus infection in children and adolescents. *The Lancet Gastroenterology & Hepatology*, *4*(6), 477–487.

Inoue, T., & Tanaka, Y. (2016). Hepatitis B virus and its sexually transmitted infection-an update. *Microbial Cell*, *3*(9), 420.

Ismail, A. M. A., Tolba, A. M. N., & Felaya, E.-S. E. E.-S. (2021). Effect of aerobic exercise training on leptin and liver enzymes in non-diabetic overweight hepatitis C men. *Postepy Rehabilitacji*, *35*(2), 17

Israr, M., Ali, F., Nawaz, A., Idrees, M., Khattak, A., Ur Rehman, S., Azizullah, A., Ahmad, B., Bano, S. A., & Iqbal, R. (2021). Seroepidemiology and associated risk factors of hepatitis B and C virus infections among pregnant women attending maternity wards at two hospitals in Swabi, Khyber Pakhtunkhwa, Pakistan. *Plos One*, *16*(8), e0255189.

JALIL, A. T., DILFY, S. H., KAREVSKIY, A., & NAJAH, N. (2020). Viral hepatitis in Dhi-Qar Province: demographics and hematological characteristics of patients. *International Journal of Pharmaceutical Research*, *12*(1), 2081–2087.

Jasim, A. M. (2013). Some hematological and biochemical changes on patients with chronic liver diseases and cirrhosis among random samples of Iraqi people. *Journal of Al-Ma'moon College*, *22*.

Jin, J. (2020). Screening for Hepatitis C Virus Infection. *Jama*, *323*(10), 1008. https://doi.org/10.1001/jama.2020.1761

Jin, S., Wetzel, D., & Schirmer, M. (2022). Deciphering mechanisms and implications of bacterial translocation in human health and disease. *Current Opinion in Microbiology*, *67*, 102147.

Johannesson, J. M., Fridriksdottir, R. H., Löve, T. J., Runarsdottir, V., Hansdóttir, I., Löve, A., Thordardottir, M., Hernandez, U. B., Olafsson, S., & Gottfredsson, M. (2022). High rate of hcv reinfection among recently injecting drug users: results from the trap hep c program-a prospective nationwide, population-based study. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*.

Johnson Valiente, A., Liem, K. S., Schwarz, K. B., Rosenthal, P., Murray, K. F., Mogul, D., Teckman, J., Rodriguez-Baez, N., Schwarzenberg, S. J., Feld, J. J., Wong, D. K., Lewis-

References

Ximenez, L. L., Lauer, G., Hansen, B. E., Ling, S. C., Janssen, H. L. A., & Gehring, A. J. (2022). The Inflammatory Cytokine Profile Associated With Liver Damage Is Broader and Stronger in Patients With Chronic Hepatitis B Compared to Patients With Acute Hepatitis B. *Journal of Infectious Diseases*, *225*(3), 470–475. https://doi.org/10.1093/infdis/jiab373

Jung, Y. K., & Yim, H. J. (2017). Reversal of liver cirrhosis: current evidence and expectations. *The Korean Journal of Internal Medicine*, *32*(2), 213.

Kadhem, S. B., Jumaa, Z. M. E. M. G., & Rhaymah, M. S. (2019). Prevalence of Viral Hepatitis Infections in Misan Province, Iraq, 2013 through 2017. *Journal of Pharmaceutical Sciences and Research*, *11*(4), 1263–1268.

Kalas, M. A., Chavez, L., Leon, M., Taweesedt, P. T., & Surani, S. (2021). Abnormal Kalas, M. A., Chavez, L., Leon, M., Taweesedt, P. T., & Surani, S. (2021). Abnormal liver enzymes: A review for clinicians. World Journal of Hepatology, 13(11), 1688–1698. https://doi.org/10.4254/wjh.v13.i11.1688liver enzymes: A review for clinic. *World Journal of Hepatology*, *13*(11), 1688–1698. https://doi.org/10.4254/wjh.v13.i11.1688

Kaneko, N., Kurata, M., Yamamoto, T., Morikawa, S., & Masumoto, J. (2019). The role of interleukin-1 in general pathology. *Inflammation and Regeneration*, *39*(1), 1–16. https://doi.org/10.1186/s41232-019-0101-5

Kang, C. K., Brennan, P. N., & Dillon, J. F. (2022). How to Effectively Monitor Aging Patients with Chronic Hepatitis B: A Review. *Clinical Interventions in Aging*, *17*(December), 1811–1820. https://doi.org/10.2147/CIA.S366255

Kanwal, F., Khaderi, S., Singal, A. G., Marrero, J. A., Loo, N., Asrani, S. K., Amos, C. I., Thrift, A. P., Gu, X., & Luster, M. (2022). Risk factors for HCC in contemporary cohorts of patients with cirrhosis. *Hepatology*

Karim, S. M. F., Rahman, M. R., Shermin, S., & Sultana, R. (2015). Correlation between Aminotransferase Ratio (AST/ALT) and Other Biochemical Parameters in Chronic Liver Disease of Viral Origin. *Delta Medical College Journal*, *3*(1), 13–17. https://doi.org/10.3329/dmcj.v3i1.22234

Kassa, Y., Million, Y., Gedefie, A., & Moges, F. (2021). Alteration of gut microbiota and its impact on immune response in patients with chronic HBV infection: A review. *Infection and Drug Resistance*, *14*, 2571–2578. https://doi.org/10.2147/IDR.S305901

Katzke, V., Johnson, T., Sookthai, D., Hüsing, A., Kühn, T., & Kaaks, R. (2020). Circulating liver enzymes and risks of chronic diseases and mortality in the prospective EPIC-Heidelberg case-cohort study. *BMJ Open*, *10*(3), 1–15. https://doi.org/10.1136/bmjopen-

References

2019-033532

Kaźmierczak, J., Caraballo Cortes, K., Bukowska-Ośko, I., & Radkowski, M. (2016). Virus-Specific Cellular Response in Hepatitis C Virus Infection. *Archivum Immunologiae et Therapiae Experimentalis*, *64*(2), 101–110. https://doi.org/10.1007/s00005-015-0364-8

Khalid, F. K., Rasheed, N. A., Hussein, N. R., & Naqid, I. A. (2022). A study of HBV infection and its risk factors in pregnant women in Zakho city, Iraq. *PLoS ONE*, *17*(8 August), 1–8. https://doi.org/10.1371/journal.pone.0273362

Khayrulla, F., Mukhaye, A., Zaynitdin, K., & Shukhrat, Z. (2021). Immunological aspects of the pathogenesis of chronic hcv infection. *Annals of the Romanian Society for Cell Biology*, *25*(3), 4164–4173.

Khudhair, H. A. A., Albakaa, A. A. H., & Hussein, K. R. (2020). Detection the Prevalence of Hepatitis C Virus among Iraqi People. *BioRxiv*.

Kim, H. seok, Yang, J. D., El-Serag, H. B., & Kanwal, F. (2019). Awareness of chronic viral hepatitis in the United States: An update from the National Health and Nutrition Examination Survey. *Journal of Viral Hepatitis*, *26*(5), 596–602. https://doi.org/10.1111/jvh.13060

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews Immunology*, *16*(10), 626–638.

Kosyreva, A. M., Makarova, O. V, Kakturskiy, L. V, Mikhailova, L. P., Boltovskaya, M. N., & Rogov, K. A. (2018). Sex differences of inflammation in target organs, induced by intraperitoneal injection of lipopolysaccharide, depend on its dose. *Journal of Inflammation Research*, 431–445.

Krishnan, S. (2019). Liver diseases-an overview. *March*. https://doi.org/10.20959/wjpps20191-13036

Kumar, V., Gala, D., Shah, M., Kumar, N., Gayam, V. R., & Bandaru, P. (2023). *Outcomes of Portal Vein Thrombosis in Smokers With and Without Cirrhosis and Predictors of Mortality : A Nationwide Assessment*. *15*(4), 8–12. https://doi.org/10.7759/cureus.37658

Lala, V., Goyal, A., & Minter, D. A. (2021). Liver function tests. In *StatPearls [internet]*. StatPearls Publishing.

Lattanzi, B., Baroncelli, S., De Santis, A., Galluzzo, C. M., Mennini, G., Michelini, Z., Lupo, M., Ginanni Corradini, S., Rossi, M., Palmisano, L., & Merli, M. (2018). Microbial translocation and T cell activation are modified by direct-acting antiviral therapy in HCV-

infected patients. *Alimentary Pharmacology and Therapeutics*, *48*(10), 1146–1155. https://doi.org/10.1111/apt.14994

Lazarus, J. V, Safreed-Harmon, K., & Sperle, I. (2013). Global policy report on the prevention and control of viral hepatitis: In WHO Member States. *Global Alert and Response*, i–208.

Le-Vinh, B., Akkuş-Dağdeviren, Z. B., Le, N. N., Nazir, I., & Bernkop-Schnürch, A. (2022). Alkaline phosphatase: a reliable endogenous partner for drug delivery and diagnostics. *Advanced Therapeutics*, *5*(2), 2100219.

Le Bert, N., Gill, U. S., Hong, M., Kunasegaran, K., Tan, D. Z. M., Ahmad, R., Cheng, Y., Dutertre, C. A., Heinecke, A., Rivino, L., Tan, A., Hansi, N. K., Zhang, M., Xi, S., Chong, Y., Pflanz, S., Newell, E. W., Kennedy, P. T. F., & Bertoletti, A. (2020). Effects of Hepatitis B Surface Antigen on Virus-Specific and Global T Cells in Patients With Chronic Hepatitis B Virus infection. *Gastroenterology*, *159*(2), 652–664. https://doi.org/10.1053/j.gastro.2020.04.019

Li, C., Ji, H., Cai, Y., Ayana, D. A., Lv, P., Liu, M., & Jiang, Y. (2013). Serum interleukin-37 concentrations and HBeAg seroconversion in chronic HBV patients during telbivudine treatment. *Journal of Interferon and Cytokine Research*, *33*(10), 612–618. https://doi.org/10.1089/jir.2013.0001

Licata, A., Russo, G. T., Giandalia, A., Cammilleri, M., Asero, C., & Cacciola, I. (2023). Impact of Sex and Gender on Clinical Management of Patients with Advanced Chronic Liver Disease and Type 2 Diabetes. *Journal of Personalized Medicine*, *13*(3). https://doi.org/10.3390/jpm13030558

Liu, H., Ding, C., Hu, L., Li, M., Zhou, W., Wang, T., Zhu, L., Bao, H., & Cheng, X. (2021c). The association between AST/ALT ratio and all-cause and cardiovascular mortality in patients with hypertension. *Medicine*, *100*(31).

Liu, M., Li, L., Zhao, J., Ungvari, G. S., Ng, C. H., Duan, Z., Zheng, S.-J., & Xiang, Y.-T. (2022a). Gender differences in demographic and clinical characteristics in patients with HBV-related liver diseases in China. *PeerJ*, *10*, e13828.

Liu, T., Song, C., Zhang, Y., Siyin, S. T., Zhang, Q., Song, M., Cao, L., & Shi, H. (2022b). Hepatitis B virus infection and the risk of gastrointestinal cancers among Chinese population: A prospective cohort study. *International Journal*

Liu, X., Yin, S., Chen, Y., Wu, Y., Zheng, W., Dong, H., Bai, Y., Qin, Y., Li, J., & Feng, S. (2018). LPS-induced proinflammatory cytokine expression in human airway epithelial cells

and macrophages via NF-κB, STAT3 or AP-1 activation. *Molecular Medicine Reports*, *17*(4), 5484–5491.

Liu, Y.-P., & Yao, C.-Y. (2015). Rapid and quantitative detection of hepatitis B virus. *World Journal of Gastroenterology*, *21*(42), 11954.

Lowe, D., Sanvictores, T., Zubair, M., & John, S. (2022). Alkaline phosphatase. In *StatPearls [internet]*. StatPearls Publishing.

Loppnow, H., Libby, P., Freudenberg, M., Krauss, J. H., Weckesser, J., & Mayer, H. (1990). Cytokine induction by lipopolysaccharide (LPS) corresponds to lethal toxicity and is inhibited by nontoxic Rhodobacter capsulatus LPS. *Infection and Immunity*, *58*(11), 3743–3750. https://doi.org/10.1128/iai.58.11.3743-3750.1990

Ly, K. N., Xing, J., Klevens, R. M., Jiles, R. B., Ward, J. W., & Holmberg, S. D. (2012). The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. *Annals of Internal Medicine*, *156*(4), 271–278.

Lyu, Z. (2020). Albumin-to-Alkaline Phosphatase Ratio Associates with Good Prognosis of Hepatitis B Virus-Positive HCC Patients. 2377–2384.

Ma, Q., Dong, X., Liu, S., Zhong, T., Sun, D., Zong, L., Zhao, C., Lu, Q., Zhang, M., & Gao, Y. (2020). Hepatitis B e antigen induces NKG2A+ natural killer cell dysfunction via regulatory T cell-derived interleukin 10 in chronic hepatitis B virus infection. *Frontiers in Cell and Developmental Biology*, *8*, 421.

Manns, M. P., Buti, M., Gane, E. D., Pawlotsky, J.-M., Razavi, H., Terrault, N., & Younossi, Z. (2017). Hepatitis C virus infection. *Nature Reviews Disease Primers*, *3*(1), 1–19.

Marchetti, G., Cozzi-Lepri, A., Merlini, E., Bellistrì, G. M., Castagna, A., Galli, M., Verucchi, G., Antinori, A., Costantini, A., & Giacometti, A. (2011). Microbial translocation predicts disease progression of HIV-infected antiretroviral-naive patients with high CD4+ cell count. *Aids*, *25*(11), 1385–1394.

Mbaga, D. S., Kenmoe, S., Bikoï, J. N., Takuissu, G. R., Amougou-Atsama, M., Okobalemba, E. A., Ebogo-Belobo, J. T., Bowo-Ngandji, A., Oyono, M. G., & Magoudjou-Pekam, J. N. (2022). Global prevalence of occult hepatitis C virus: A systematic review and meta-analysis. *World Journal of Methodology*, *12*(3), 179.

McMahon, B. J., Alward, W. L. M., Hall, D. B., Heyward, W. L., Bender, T. R., Francis, D. P., & Maynard, J. E. (1985). Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *Journal of Infectious Diseases*, *151*(4), 599–603.

References

Meador, B. M., Krzyszton, C. P., Johnson, R. W., & Huey, K. A. (2008). Effects of IL-10 and age on IL-6, IL-1β, and TNF-α responses in mouse skeletal and cardiac muscle to an acute inflammatory insult. *Journal of Applied Physiology*, *104*(4), 991–997.

Mehta, P., & Reddivari, A. K. R. (2022). Hepatitis. In *StatPearls [Internet]*. StatPearls Publishing.

Merli, M., Giusto, M., Lucidi, C., Giannelli, V., Pentassuglio, I., Di Gregorio, V., Lattanzi, B., & Riggio, O. (2013). Muscle depletion increases the risk of overt and minimal hepatic encephalopathy: results of a prospective study. *Metabolic Brain Disease*, *28*, 281–284.

Midgard, H., Weir, A., Palmateer, N., Re III, V. Lo, Pineda, J. A., Macías, J., & Dalgard, O. (2016). HCV epidemiology in high-risk groups and the risk of reinfection. *Journal of Hepatology*, *65*(1), S33–S45.

Mehmood, S., Raza, H., Abid, F., Saeed, N., Rehman, H. M., Javed, S., & Khan, M. S. (2020). National prevalence rate of hepatitis B and C in Pakistan and its risk factors. Journal of Public Health (Germany), 28(6), 751–764. https://doi.org/10.1007/s10389-019-01081-5

Mohammed, H. K. (2022). Study of Physiological and Some Biochemical Parameters in Patients Infected with Chronic Hepatitis-B Virus. *HIV Nursing*, *22*(2), 2039–2044.

Mohr, A. E., Crawford, M., Jasbi, P., Fessler, S., & Sweazea, K. L. (2022). Lipopolysaccharide and the gut microbiota: considering structural variation. *FEBS Letters*, *596*(7), 849–875. https://doi.org/10.1002/1873-3468.14328

Moman, R. N., Gupta, N., & Varacallo, M. (2017). *Physiology, albumin*.

Moon, M. S., Quinn, G., Townsend, E. C., Ali, R. O., Zhang, G. Y., Bradshaw, A., Hill, K., Guan, H., Hamilton, D., Kleiner, D. E., Koh, C., & Heller, T. (2019). Bacterial Translocation and Host Immune Activation in Chronic Hepatitis C Infection. *Open Forum Infectious Diseases*, *6*(7), 1–5. https://doi.org/10.1093/ofid/ofz255

Nikolaou, P., Sciuto, E. L., Zanut, A., Petralia, S., Valenti, G., Paolucci, F., Prodi, L., & Conoci, S. (2022). Ultrasensitive PCR-Free detection of whole virus genome by electrochemiluminescence. *Biosensors and Bioelectronics*, *209*, 114165.

Nishikawa, H., Enomoto, H., Nishiguchi, S., & Iijima, H. (2021). Sarcopenic obesity in liver cirrhosis: Possible mechanism and clinical impact. *International Journal of Molecular Sciences*, *22*(4), 1–13. https://doi.org/10.3390/ijms22041917

Noor, M. T., & Manoria, P. (2017). Immune dysfunction in cirrhosis. *Journal of Clinical and Translational Hepatology*, *5*(1), 50.

References

Osasona, O. G., Oguntoye, T., Eromon, P., Abdulkareem, L., Arowosaye, A. O., Ariyo, O. E., George, U. E., Yusuf, M., Adewumi, O. M., Happi, C., & Folarin, O. A. (2023). Atypical serologic profiles of hepatitis B virus infection across clinical cohorts of patients in Southwestern Nigeria. *Journal of Immunoassay and Immunochemistry*, *44*(2), 176–191. https://doi.org/10.1080/15321819.2023.2168556

Othman, R. A., & Abbas, Y. A. (2020). Prevalence of hepatitis B and C in Thi-Qar prov ince - Iraq from 2015-2019. European Journal of Molecular & Clinical Medicine, 7(2), 43–48.

Owiti, J. A., Greenhalgh, T., Sweeney, L., Foster, G. R., & Bhui, K. S. (2015). Illness perceptions and explanatory models of viral hepatitis B & C among immigrants and refugees : a narrative systematic review. 1–17. https://doi.org/10.1186/s12889-015-1476-0

Owusu-Dommey, A., & Ladd-Wilson, S. (2021). In ves tigative Guidelines. *February*, 1–9.

Özgüler, M., Akbulut, H. H., & Akbulut, A. (2015). Evaluation of interleukin-10 levels in patients diagnosed with chronic hepatitis. *The West Indian Medical Journal*, *64*(2), 71.

Papadakos, S. P., Dedes, N., Kouroumalis, E., & Theocharis, S. (2022). The Role of the NLRP3 Inflammasome in HCC Carcinogenesis and Treatment: Harnessing Innate Immunity. *Cancers*, *14*(13), 3150.

Pennell, L. M., Galligan, C. L., & Fish, E. N. (2012). Sex affects immunity. *Journal of Autoimmunity*, *38*(2–3), J282–J291.

Pérez-Cobas, A. E., Gosalbes, M. J., Friedrichs, A., Knecht, H., Artacho, A., Eismann, K., Otto, W., Rojo, D., Bargiela, R., & von Bergen, M. (2013). Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. *Gut*, *62*(11), 1591–1601.

Peterson, J. R., Hsu, F. C., Simkin, P. A., & Wener, M. H. (2003). Effect of tumour necrosis factor α antagonists on serum transaminases and viraemia in patients with rheumatoid arthritis and chronic hepatitis C infection. *Annals of the Rheumatic Diseases*, *62*(11), 1078–1082. https://doi.org/10.1136/ard.62.11.1078

Petitclerc, L., Sebastiani, G., Gilbert, G., Cloutier, G., & Tang, A. (2017). Liver fibrosis: Review of current imaging and MRI quantification techniques. *Journal of Magnetic Resonance Imaging*, *45*(5), 1276–1295. https://doi.org/10.1002/jmri.25550

Pinter, M., Trauner, M., Peck-Radosavljevic, M., & Sieghart, W. (2016). Cancer and liver cirrhosis: implications on prognosis and management. *ESMO Open*, *1*(2), e000042.

Pinzan, C. F., Ruas, L. P., Casabona-Fortunato, A. S., Carvalho, F. C., & Roque-Barreira, M.-C. (2010). Immunological basis for the gender differences in murine Paracoccidioides

brasiliensis infection. *PloS One*, *5*(5), e10757.

Popa, S. L., Ismaiel, A., Abenavoli, L., Padureanu, A. M., Dita, M. O., Bolchis, R., Munteanu, M. A., Brata, V. D., Pop, C., Bosneag, A., Dumitrascu, D. I., Barsan, M., & David, L. (2023). Diagnosis of Liver Fibrosis Using Artificial Intelligence: A Systematic Review. *Medicina (Lithuania)*, *59*(5), 1–15. https://doi.org/10.3390/medicina59050992

Pouri, A. A., Ghojazadeh, M., Shirmohammadi, M., Eftekhar-Sadat, A.-T., & Somi, M. H. (2020). Seroepidemiology and Risk Factors of Hepatitis B Virus Infection: A Population-Based Azar Cohort Study. *Iranian Journal of Public Health*, *49*(11), 2152.

Radonjić, T., Dukić, M., Jovanović, I., Zdravković, M., Mandić, O., Popadić, V., Popović, M., Nikolić, N., Klašnja, S., Divac, A., Todorović, Z., & Branković, M. (2022). Aging of Liver in Its Different Diseases. *International Journal of Molecular Sciences*, *23*(21). https://doi.org/10.3390/ijms232113085

Rahimzadeh, G., Safar, M. J., Rezai, S., Rezai, M. S., & Movahedi, F. S. (2022). Seroepidemiology of HBV, HCV, HIV, HTLV, and CMV in pregnant women referring to Sari Birth Cohort. *Advanced Biomedical Research*, *11*.

Rajpurohit, S., Musunuri, B., Mohan, P. B., & Lakshmi, R. V. (2023). Predictors for oesophageal candidiasis in patients with liver cirrhosis. 79–85. https://doi.org/10.53854/liim-3101-11

Rakhmatullaeva, O. U., Shomurodov, K. E., Hadjimetov, A. A., Sadiqova, X. K., & Nazarov, Z. Z. (2021). The Position of the Cytokine Profile and Cytolysis Enzymes in Patients with Viral Hepatitis before Tooth Extraction. *Annals of the Romanian Society for Cell Biology*, 6558–6567.

Razavi, H. (2020). Global Epidemiology of Viral Hepatitis. *Gastroenterology Clinics of North America*, *49*(2), 179–189. https://doi.org/10.1016/j.gtc.2020.01.001

Riaz, M., Khalid, H., & Kiran, N. (2022). Prevalence of Hepatitis B and C Viral Infections in Chronic Liver Disorder. *Pakistan Journal of Medical & Health Sciences*, *16*(10), 396–398. https://doi.org/10.53350/pjmhs221610396

Ridker, P. M. (2016). From C-Reactive Protein to Interleukin-6 to Interleukin-1: Moving Upstream to Identify Novel Targets for Atheroprotection. *Circulation Research*, *118*(1), 145–156. https://doi.org/10.1161/CIRCRESAHA.115.306656

Roberts, C. W., Walker, W., & Alexander, J. (2001). Sex-associated hormones and immunity to protozoan parasites. *Clinical Microbiology Reviews*, *14*(3), 476–488.

References

Robinson, M. W., Harmon, C., & O'Farrelly, C. (2016). Liver immunology and its role in inflammation and homeostasis. *Cellular and Molecular Immunology*, *13*(3), 267–276. https://doi.org/10.1038/cmi.2016.3

Roger, S., Ducancelle, A., Le Guillou-Guillemette, H., Gaudy, C., & Lunel, F. (2021). HCV virology and diagnosis. *Clinics and Research in Hepatology and Gastroenterology*, *45*(3), 101626.

Rojas, J. M., Avia, M., Martín, V., & Sevilla, N. (2017). IL-10: a multifunctional cytokine in viral infections. *Journal of Immunology Research*, *2017*.

Rouse, B. T., & Masopust, D. (2006). Waking up T cells to counteract chronic infections. *Trends in Immunology*, *27*(5), 205–207.

Rybicka, M., Woziwodzka, A., Sznarkowska, A., Romanowski, T., Stalke, P., Dręczewski, M., Verrier, E. R., Baumert, T. F., & Bielawski, K. P. (2020). Genetic variation in IL-10 influences the progression of hepatitis B infection. *International Journal of Infectious Diseases*, *96*, 260–265.

Ryerson, A. B., Schillie, S., Barker, L. K., Kupronis, B. A., & Wester, C. (2020). Vital Signs: Newly Reported Acute and Chronic Hepatitis C Cases — United States, 2009–2018 .*MMWR. Morbidity and Mortality Weekly Report*, *69*(14), 399–404. https://doi.org/10.15585/mmwr.mm6914a2

Sadik, N. A. H., Shaker, O. G., Ghanem, H. Z., Hassan, H. A., & Abdel-Hamid, A.-H. Z. (2015). Single-nucleotide polymorphism of Toll-like receptor 4 and interleukin-10 in response to interferon-based therapy in Egyptian chronic hepatitis C patients. *Archives of Virology*, *160*, 2181–2195.

Sagnelli, C., Pisaturo, M., Calò, F., Martini, S., Sagnelli, E., & Coppola, N. (2019). Reactivation of hepatitis B virus infection in patients with hemo-lymphoproliferative diseases, and its prevention. *World Journal of Gastroenterology*, *25*(26), 3299–3312. https://doi.org/10.3748/wjg.v25.i26.3299

Saitta, C., Pollicino, T., & Raimondo, G. (2019). Obesity and liver cancer. In *Annals of Hepatology* (Vol. 18, Issue 6, pp. 810–815). Elsevier Espana S.L. https://doi.org/10.1016/j.aohep.2019.07.004

Sajja, K. C., Mohan, D. P., & Rockey, D. C. (2014). Age and ethnicity in cirrhosis. *Journal of Investigative Medicine*, *62*(7), 920–926.

Salman, A. D., Ali, I. A., & Hwaid, A. H. (2022). Prevalence of occult hepatitis B infection in Diyala province *, Iraq*. *5*(2), 685–692.

References

Samo, A. A., Laghari, Z. A., Baig, N. M., & Khoso, G. M. (2021). Prevalence and Risk Factors Associated with Hepatitis B and C in Nawabshah, Sindh, Pakistan. *American Journal of Tropical Medicine and Hygiene*, *104*(3), 1101–1105. https://doi.org/10.4269/ajtmh.20-1228

Sampath, V. (2018). Bacterial endotoxin-lipopolysaccharide; structure, function and its role in immunity in vertebrates and invertebrates. *Agriculture and Natural Resources*, *52*(2), 115–120.

Sant'Anna, T. B., & Araujo, N. M. (2023). Hepatitis B Virus Genotype D: An Overview of Molecular Epidemiology, Evolutionary History, and Clinical Characteristics. *Microorganisms*, *11*(5), 1101.

Saraceni, C., & Birk, J. (2021). A review of hepatitis b virus and hepatitis c virus immunopathogenesis. *Journal of Clinical and Translational Hepatology*, *9*(3), 409–418. https://doi.org/10.14218/JCTH.2020.00095

Sato, K., Kennedy, L., Liangpunsakul, S., Kusumanchi, P., Yang, Z., Meng, F., Glaser, S., Francis, H., & Alpini, G. (2019). Intercellular communication between hepatic cells in liver diseases. *International Journal of Molecular Sciences*, *20*(9). https://doi.org/10.3390/ijms20092180

Santiago, A. M., da Silva Graça Amoras, E., Queiroz, M. A. F., da Silva Conde, S. R. S., Cayres-Vallinoto, I. M. V., Ishak, R., & Vallinoto, A. C. R. (2021). TNFA-308G> A and IL10-1082A> G polymorphisms seem to be predictive biomarkers of chronic HCV infection. *BMC Infectious Diseases*, *21*, 1–8.

Sayaf, K., Gabbia, D., Russo, F. P., & De Martin, S. (2022). The Role of Sex in Acute and Chronic Liver Damage. *International Journal of Molecular Sciences*, *23*(18), 1–21. https://doi.org/10.3390/ijms231810654

Scheller, L., Hilgard, G., Anastasiou, O., Dittmer, U., Kahraman, A., Wedemeyer, H., & Deterding, K. (2021). Poor clinical and virological outcome of nucleos(t)ide analogue monotherapy in HBV/HDV co-infected patients. *Medicine (United States)*, *100*(28), E26571. https://doi.org/10.1097/MD.0000000000026571

Sehgal, R., Bedi, O., & Trehanpati, N. (2020). Role of Microbiota in Pathogenesis and Management of Viral Hepatitis. *Frontiers in Cellular and Infection Microbiology*, *10*(August). https://doi.org/10.3389/fcimb.2020.00341

Schlaak, J. F., Tully, G., Löhr, H. F., Gerken, G., & zum Büschenfelde, K.-H. M. (1999). The presence of high amounts of HBV-DNA in serum is associated with suppressed

costimulatory effects of interleukin 12 on HBV-induced immune response. *Journal of Hepatology*, *30*(3), 353–358.

Serraino, R., Mazzitelli, M., Greco, G., Serapide, F., Scaglione, V., Marascio, N., Trecarichi, E. M., & Torti, C. (2020). Risk factors for hepatitis B and C among healthy population: A community-based survey from four districts of Southern Italy. *Infezioni in Medicina*, *28*(2), 223–226.

Sharifinejad, N., Zaki-Dizaji, M., Sepahvandi, R., Fayyaz, F., Dos Santos Vilela, M. M., Elghazali, G., Abolhassani, H., Ochs, H. D., & Azizi, G. (2022). The clinical, molecular, and therapeutic features of patients with IL10/IL10R deficiency: A systematic review. *Clinical and Experimental Immunology*, *208*(3), 281–291. https://doi.org/10.1093/cei/uxac040

Sharma, A., & Nagalli, S. (2021). Chronic liver disease. In *StatPearls [Internet]*. StatPearls Publishing.

Shin, E. C., Sung, P. S., & Park, S. H. (2016). Immune responses and immunopathology in acute and chronic viral hepatitis. *Nature Reviews Immunology*, *16*(8), 509–523. https://doi.org/10.1038/nri.2016.69

Sikora, J. P., Chlebna-Sokół, D., Andrzejewska, E., Chrul, S., Polakowska, E., Wysocka, A., & Sikora, A. (2008). Clinical Evaluation of Proinflammatory Cytokine Inhibitors (sTNFR I, sTNFR II, IL-1 ra), Anti-inflammatory Cytokines (IL-10, IL-13) and Activation of Neutrophils after Burn-induced Inflammation. *Scandinavian Journal of Immunology*, *68*(2), 145–152.

Simbrunner, B., Caparrós, E., Neuwirth, T., Schwabl, P., Königshofer, P., Bauer, D., Marculescu, R., Trauner, M., Scheiner, B., Stary, G., Mandorfer, M., Reiberger, T., & Francés, R. (2023). Bacterial translocation occurs early in cirrhosis and triggers a selective inflammatory response. *Hepatology International*, *0123456789*. https://doi.org/10.1007/s12072-023-10496-y

Skinner, C., Thompson, A. J., Thursz, M. R., Marchesi, J. R., & Vergis, N. (2020). Intestinal permeability and bacterial translocation in patients with liver disease, focusing on alcoholic aetiology: methods of assessment and therapeutic intervention. *Therapeutic Advances in Gastroenterology*, *13*, 1–16. https://doi.org/10.1177/1756284820942616

Soffientini, U., Beaton, N., Baweja, S., Weiss, E., Bihari, C., Habtesion, A., Patel, V., Paradis, V., Sharma, A., Luong, T. V., Hall, A., Nadar, A., Sarin, S., Chokshi, S., Williams, R., Py, B., Moreau, R., Jalan, R., & Mehta, G. (2021). The Lipopolysaccharide-Sensing Caspase(s)-4/11 Are Activated in Cirrhosis and Are Causally Associated With Progression to Multi-

References

Organ Injury. *Frontiers in Cell and Developmental Biology*, *9*(July), 1–13. https://doi.org/10.3389/fcell.2021.668459

Sun, L., Yin, H., Liu, M., Xu, G., Zhou, X., Ge, P., Ã, L. S., Ã, H. Y., Ã, M. L., Xu, G., Zhou, X., Ge, P., & Yang, H. (2019). Annals of Medicine Impaired albumin function : a novel potential indicator for liver function damage ? Impaired albumin function : a novel potential indicator for liver. *Annals of Medicine*, *51*(7–8), 333–344. https://doi.org/10.1080/07853890.2019.16930

Suriguga, S., Li, M., Luangmonkong, T., Boersema, M., De Jong, K. P., Oosterhuis, D., Gorter, A. R., Beljaars, L., & Olinga, P. (2022). Distinct responses between healthy and cirrhotic human livers upon lipopolysaccharide challenge: Possible implications for acute-on-chronic liver failure. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *323*(2), G114–G125. https://doi.org/10.1152/ajpgi.00243.2021

Tawfik, A. K., Amin, A. M., Yousef, M., El-Sayd, N. M., Elashry, H., Elkadeem, M., & Abd-Elsalam, S. (2018). IL-1α correlates with severity of hepatitis C virus-related liver diseases. *Journal of Inflammation Research*, *11*, 289–295. https://doi.org/10.2147/JIR.S166564

Thein, H., Yi, Q., Dore, G. J., & Krahn, M. D. (2008). Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. *Hepatology*, *48*(2), 418–431.

Timperi, E., & Barnaba, V. (2020). Viral hepatitides, inflammation and tumour microenvironment. *Tumor Microenvironment: State of the Science*, 25–43.

Townsend, E. C., Zhang, G. Y., Ali, R., Surana, P., Firke, M., Moon, M. S., Han, M. A. T., Gewirtz, M., Haddad, J. A., Kleiner, D. E., Koh, C., & Heller, T. (2020). Microbial Translocation in the Context of Hepatitis B Infection and Hepatitis D Infection. *Open Forum Infectious Diseases*, 1–9. https://doi.org/10.1093/ofid/ofaa496

Trehanpati, N., & Vyas, A. K. (2017). Immune Regulation by T Regulatory Cells in Hepatitis B Virus-Related Inflammation and Cancer. *Scandinavian Journal of Immunology*, *85*(3), 175–181. https://doi.org/10.1111/sji.12524

Tsukuda, S., & Watashi, K. (2020). Hepatitis B virus biology and life cycle. *Antiviral Research*, *182*, 104925.

Tucureanu, M. M., Rebleanu, D., Constantinescu, C. A., Deleanu, M., Voicu, G., Butoi, E., Calin, M., & Manduteanu, I. (2018). Lipopolysaccharide-induced inflammation in monocytes/macrophages is blocked by liposomal delivery of Gi-protein inhibitor. *International Journal of Nanomedicine*, 63–76.

References

Utero, I. (2017). crossm Vertical Transmission of Hepatitis C Virus : Variable Transmission Bottleneck and Evidence of Midgestation In Utero.

Vaz, J., Strömberg, U., Eriksson, B., Buchebner, D., & Midlöv, P. (2020). Socioeconomic and marital status among liver cirrhosis patients and associations with mortality: a population-based cohort study in Sweden. *BMC Public Health*, *20*(1), 1–13. https://doi.org/10.1186/s12889-020-09783-2

Veronese, P., Dodi, I., Esposito, S., & Indolfi, G. (2021). Prevention of vertical transmission of hepatitis B virus infection. *World Journal of Gastroenterology*, *27*(26), 4182.

Vimali, J., Yong, Y. K., Murugesan, A., Vishnupriya, K., Ashwin, R., Daniel, E. A., Balakrishnan, P., Raju, S., Rosmawati, M., Velu, V., Larsson, M., & Shankar, E. M. (2022). Plasma interleukin-7 correlation with human immunodeficiency virus RNA and CD4+ T cell counts, and interleukin-5 with circulating hepatitis B virus DNA may have implications in in viral control. *Frontiers in Medicine*, *9*(November), 1–16. https://doi.org/10.3389/fmed.2022.1019230.

Von Baehr, V., Döcke, W. D., Plauth, M., Liebenthal, C., Küpferling, S., Lochs, H., Baumgarten, R., & Volk, H. D. (2000). Mechanisms of endotoxin tolerance in patients with alcoholic liver cirrhosis: Role of interleukin 10, interleukin 1 receptor antagonist, and soluble tumour necrosis factor receptors as well as effector cell desensitisation. *Gut*, *47*(2), 281–287. https://doi.org/10.1136/gut.47.2.281

Wang, R., Tang, R., Li, B. O., Ma, X., Schnabl, B., & Tilg, H. (2021). Gut microbiome, liver immunology, and liver diseases. *Cellular & Molecular Immunology*, *18*(1), 4–17.

Wang, Yan-hua. (2021). Current progress of research on intestinal bacterial translocation. *Microbial Pathogenesis*, *152*, 104652.

Wang, Yuhan, Jie, W., Ling, J., & Yuanshuai, H. (2021). HCV core antigen plays an important role in the fight against HCV as an alternative to HCV-RNA detection. *Journal of Clinical Laboratory Analysis*, *35*(6), 1–9. https://doi.org/10.1002/jcla.23755

Wani, M. Y., Dhama, K., Latheef, S. K., Singh, S. D., & Tiwari, R. (2014). Correlation between cytokine profile, antibody titre and viral load during sub-clinical chicken anaemia virus infection. *Veterinarni Medicina*, *59*(1), 33–43. https://doi.org/10.17221/7244-VETMED

Wiegand, J., & Berg, T. (2013). The Etiology, Diagnosis and Prevention of Liver Cirrhosis. *Deutsches Ärzteblatt International*, *110*(5). https://doi.org/10.3238/arztebl.2013.0085

References

Wiest, R., Lawson, M., & Geuking, M. (2014). Pathological bacterial translocation in liver cirrhosis. *Journal of Hepatology*, *60*(1), 197–209. https://doi.org/10.1016/j.jhep.2013.07.044

Witanto, B., El-Khobar, K. E., Parewangi, M. L., Rasyak, M. R., Wibowo, D. P., Thedja, M. D., Yusuf, I., Massi, M., Patellongi, I., & Syafruddin, D. (2022). Host Factors in the Natural History of Chronic Hepatitis B: Role of Genetic Determinants. *International Journal of Hepatology*, *2022*.

World Health Organization. (2021). Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. In *Who* (Vol. 53, Issue 9). https://www.who.int/publications/i/item/9789240027077

Wu, J., Han, M., Li, J., Yang, X., & Yang, D. (2020). Immunopathogenesis of HBV infection. *Hepatitis B Virus Infection: Molecular Virology to Antiviral Drugs*, 71–107.

Yadav, R. (2022). Elucidating Structural, Functional and Phylogenetic Relationship of Large Envelope Protein of Hepatitis B V irus. *Indian Journal of Science and Technology*, *15*(10), 451–456. https://doi.org/10.17485/ijst/v15i10.491

Yang, B., Petrick, J. L., Thistle, J. E., Pinto, L. A., Kemp, T. J., Tran, H. Q., Gewirtz, A. T., Waterboer, T., Fedirko, V., & Jenab, M. (2019). Bacterial Translocation and Risk of Liver Cancer in a Finnish CohortBacterial Translocation and Liver Cancer. *Cancer Epidemiology, Biomarkers & Prevention*, *28*(4), 807–813.

Yang, J. D., Ahmed, F., Mara, K. C., Addissie, B. D., Allen, A. M., Gores, G. J., & Roberts, L. R. (2020). Diabetes is associated with increased risk of hepatocellular carcinoma in patients with cirrhosis from nonalcoholic fatty liver disease. *Hepatology*, *71*(3), 907–916.

Yang, J., Qi, J.-L., Wang, X.-X., Li, X.-H., Jin, R., Liu, B.-Y., Liu, H.-X., & Rao, H.-Y. (2023). The burden of hepatitis C virus in the world, China, India, and the United States from 1990 to 2019. *Frontiers in Public Health*, *11*(1). https://doi.org/10.3389/fpubh.2023.1041201

Ye, F., Zhai, M., Long, J., Gong, Y., Ren, C., Zhang, D., Lin, X., & Liu, S. (2022). The burden of liver cirrhosis in mortality: Results from the global burden of disease study. *Frontiers in Public Health*, *10*. https://doi.org/10.3389/fpubh.2022.909455.

Yip, T. C.-F., Lyu, F., Lin, H., Li, G., Yuen, P.-C., Wong, V. W.-S., & Wong, G. L.-H. (2023). Non-invasive biomarkers for liver inflammation in non-alcoholic fatty liver disease: present and future. *Clinical and Molecular Hepatology*, *29*(Suppl), S171.

Yu, X., Lan, P., Hou, X., Han, Q., Lu, N., Li, T., Jiao, C., Zhang, J., Zhang, C., & Tian, Z.

References

(2017). HBV inhibits LPS-induced NLRP3 inflammasome activation and IL-1β production via suppressing the NF-κB pathway and ROS production. *Journal of Hepatology*, *66*(4), 693–702.

Zainal, I. G., Safa, A. A., & Obead, W. K. (2012). Comparison of Glycoproteins levels with some biochemical parameters in Iraqi patients with chronic liver diseases. 89–93. www.tjeas.com

Zampino, R., Pisaturo, M. A., Cirillo, G., Marrone, A., Macera, M., Rinaldi, L., Stanzione, M., Durante-Mangoni, E., Gentile, I., Sagnelli, E., Signoriello, G., del Giudice, E. M., Adinolfi, L. E., & Coppola, N. (2015). Hepatocellular carcinoma in chronic HBV-HCV co-infection is correlated to fibrosis and disease duration. *Annals of Hepatology*, *14*(1), 75–82. https://doi.org/10.1016/s1665-2681(19)30803-8

Zang, W. W., Su, M. H., Ling, X. Z., Wang, R. M., Cao, B. C., Wu, Y. L., Deng, D. L., Wei, H. L., Liang, X. S., & Jiang, J. N. (2020). Risk factors for the development of liver cancer in patients with hepatitis B-related liver cirrhosis treated with long-term nucleos (t) ide analogues. *Zhonghua Gan Zang Bing Za Zhi= Zhonghua Ganzangbing Zazhi= Chinese Journal of Hepatology*, *28*(8), 679–685

Zhang, K., Lai, X., Song, J., He, L., Wang, L., Ou, G., Tian, X., Wang, L., Deng, J., & Zhang, J. (2021). A novel cell culture model reveals the viral interference during hepatitis B and C virus coinfection. *Antiviral Research*, *189*, 105061.

Zhang, L., Hu, J., Gou, C., Jin, H., Zhang, C., Liu, Y., Wang, Y., & Wang, X. (2022). Review Article Serum Interleukins as Potential Prognostic Biomarkers in HBV- Related Acute-on-Chronic Liver Failur*e*. *2022*.

Zou, Y., Zhong, L., Hu, C., & Sheng, G. (2020). Association between the alanine aminotransferase/aspartate aminotransferase ratio and new-onset non-alcoholic fatty liver disease in a nonobese Chinese population: a population-based longitudinal study. *Lipids in Health and Disease*, *19*(1), 1–10. https://doi.org/10.1186/s12944-020-01419-z

Appendix

Questionnaires

Name : **Age:**

Marital status :

Academic achievement :

living condition :

HCV: duration: load :

HBsAg: HBcAb: HBeAg: HBeAb: Load:

Diagnosis: acute: chronic: cirrhosis:

family history: contact: noncontact:

Risk Factors:

Dental: surgical: blood: plasma: endoscope: tattoo: hijamah: smoking:

History of chronic disease:

ALT: AST: ALK: TSB S. AIB:

BMI

Length Weight

Sera Occurrence of HBV, HCV

Causes of Liver Cirrhosis:

الخلاصة

يعتبر التهاب الكبد الفيروسي وهو عدوى تصيب الكبد يؤدي إلى التهابه وتلفه، ,واعدت مشكلة صحية عالمية لفترة طويلة جدًا ولا يزال يعتبر تهديدا خطيرا على صحة الإنسان. يمكن أن يتسبب التهاب الكبد الفيروسي ب او ج بالعدوى الحادة والمزمنة. يعد تشمع الكبد سببًا مهمًالمسبب المرضي والوفيات بين مرضى الكبد المزمن، ومن المسببات الرئيسية لتشمع الكبد عدوى فيروس التهاب الكبد ب و ج على الصعيد العالمي، من بين الأفراد المصابين بتشمع الكبد، كان نسبة المصابون بعدوى فيروس التهاب الكبد ب و عدوى ج.42%,21% على التوالي .

تم توثيق أنه مع تقدم مرض الكبد، تضعف قدرته على أداء وظائف المناعة، مما يسمح بنقل أكبر للميكروبات المشتقة من الأمعاء إلى الدورة الدموية الجهازية وزيادة التعرض للعدوى البكتيرية الجهازية. يُعتقد أن المنتجات الميكروبية، مثل LPSهي نتيجة تعزز تطور مرض الكبد.

هدفت الدراسة الحالية من تحديد ارتباط مستوى LPS في مصل المرضى المصابين بعدوى التهاب الكبد ب وج وتشمع الكبد ودراسة الارتباط بين مستوى LPS وشدة مرض الكبد.

أجريت دراسة مقطعية في كلية العلوم الطبية التطبيقية / قسم المختبرات السريرية من الفترة من تشرين الاول 2022إلى شبا ط 2023. تم جمع عينة الدم من 89 مريضًا مصابًا بفيروس التهاب الكبد ب وج وتشمع الكبد لاستخدامها في قياس مستوى LPS و1-ILو IL-10 بتقنية ELISA.

من أصل 45 (50.56٪) من المرضى أصيبوا بفيروس التهاب الكبد ب، أصيب 21 (23.59٪) بفيروس التهاب الكبد ج و 23 (25.84٪) مصابين بتشمع الكبد، وكان متوسط عمر المرضى المصابين بفيروس التهاب الكبد ب، وفيروس التهاب الكبد ج، وتشمع الكبد 39.84 ± 16.823. و42.76 ± 15.59 و49.87 ± 15.9 على التوالي.

لوحظ فرق كبير في متوسط عمر التهاب الكبد الفيروسي ب وتشمع الكبد. لوحظ على أعلى معدل تكرار لفيروس التهاب الكبد ب وفيروس التهاب الكبد ج وتشمع الكبد في نطاق العمر بين 20-39 عامًا. كان أكثر من 75٪ من المرضى يعانون من أمراض مزمنة ولوحظ فرق كبير في متوسط عمر العدوى الحادة والمزمنة.

لوحظ عدم وجود على فرق كبير بين مجموعات الدراسات الثلاث وفقًا لنسبة الجنس مع نسبة(29/16,1.8) ، (12/9,1.3) (10/13, 0.76) لمرضى التهاب الكبد الفيروسي ب و ج وتشمع الكبد، على التوالي.

كشفت الدراسة الحالية أن هناك انخفاضًا كبيرًا في متوسط مستوى LPS و 1-ILو IL-10بين التهاب الكبد الفيروسي ب ومجموعتا الدراستين الأخريين. أيضا، تم العثور على ارتباط معنوي موجب بين LPS مع IL-10، و1-IL بين المجموعات الثلاث المدروسة من المرضى، وبين 1-IL، IL-10.

الخلاصة

اشارت هذه الدراسة أنه لا توجد فروق ذات دلالة إحصائية في متوسط مستويات ALT و AST و ALK و PT و TSB بين مرضى التهاب الكبد ب مع مرضى التهاب الكبد ج وتشمع الكبد.
فيما يتعلق بالأمراض مزمنة، اشارت الدراسة الحالية أنه لا توجد فروق ذات دلالة إحصائية في مستويات LPS و IL-1 و IL-10 حسب مدة المرض. ومع ذلك، كان متوسط مستوى LPS و IL-1 و IL-10 أعلى خلال السنة الأولى من المرض وكان المتوسط أعلى في التهاب الفيروسي ب الحاد منه في التهاب الكبد الفيروسي ب المزمن.
ما يقرب من 53.5٪ و33.3٪ و55.5٪ من مرضى التهاب الكبد الوبائي وفيروس التهاب الكبد الوبائي وتليف الكبد لديهم نسبة AST / ALT أكبر من 1. أيضًا، كان هناك ارتباط إيجابي كبير بين LPS و IL-1 و IL-10 وALK .

إن المتوسط المنخفض بشكل ملحوظ لـ LPS و IL-1 و IL-10 في المرضى المصابين بفيروس التهاب الكبد ب يعكس تأثير التهاب الكبد الفيروسي ب أو مكونه في التلاعب بالاستجابة المناعية الالتهابية أثناء العدوى، مما يعيق إزالة مسببات الأمراض وينتج عنه عدوى مستمرة. يشير الارتباط المعنوي بين LPS وIL-10 و IL-1 إلى أن LPS قد يحفز الاستجابة الالتهابية ويضخمها بين المجموعات المدروسة.

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم التحليلات المرضية

ارتباط مستوى متعدد السكريد الدهني في المصل مع الانترلوكين-1 والانترلوكين -10مع شدة مرض التهاب الكبد الفيروسي نوع بي وسي مع تشمع الكبد

رسالة مقدمه

الى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

من قبل

نوار شامل طاهر

بكالوريوس تحليلات مرضية / 2008 كلية التقنيات الصحية والطبية - الجامعة التقنية الوسطى

بأشراف

أ .د. سهاد هادي محمد

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