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Study the Correlation of Galectin-9 and Interleukin-33 with some Biochemical Variables for Chronic Hepatitis B Patients in AL-Fallujah City

A Thesis

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Hiba Awad Khudhair

Department of Chemistry – College of Science

(Study the Correlation of Galectin-9 and Interleukin-33 with some Biochemical Variables for Chronic Hepatitis B Patients in AL-Fallujah City)

Dedication

To whom words cannot describe him, my guide who faces everything with love and wisdom … ***My father***

To the most precious thing in existence, you have made me who I am today you have all the love and gratitude … ***My mother***

To those who believed in me even when I didn't believe in myself, gave me everything with love to the diamonds that beautiful my life … ***My husband and son***

To good and shy hearts full of kindness, love and respect… ***My brothers and their families***

To the bright pearls that light up my life, they are my best friends … ***My sister and her family***

To everyone who wishes me the best.

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Summary

Chronic hepatitis B (CHB) is a common disease that leads to hepatocellular carcinoma (HCC) and liver cirrhosis. Main aim of this study is to determine serum levels of Galectin-9 (Gal-9) and Interleukin-33 (IL-33) and explore the possible association that might present between them in CHB patients in AL-Fallujah City.

Eighty subjects were included in this study, they were divided into two groups; controls group which involved 40 persons (20 males and 20 females), and patients group involved 40 patients (20 males and 20 females), the sample collection has been attended the Fallujah teaching hospital and some private laboratories in Al-Fallujah city, for the sampling period was from September 2020 to January 2021 and the ages of all subjects was ranged from 20-55 year. Serum levels of Gal-9 and IL-33 were estimated by enzyme linked immunosorbent assay (ELISA) technique. However, color enzymatic methods were used to determine liver enzymes for specific variants of viral hepatitis.

The results showed high serum levels of Gal-9 (pg/mL) with significant differences in CHB patients ($P < 0.0001$) than in control group. The serum levels of IL-33 (ng/mL) was significantly higher in CHB patients ($P < 0.0001$) in comparison with control, also the serum levels of ALT, AST, ALP, bilirubin and albumin were significantly higher in CHB patients than controls ($P < 0.001$), while the serum levels of globulin decreased slightly in the patients group in comparison to the controls group ($P < 0.001$). The study also revealed that, there were no significant differences in serum levels of TSP in both groups ($P = 0.77$).

The current study showed that the correlation for Gal-9 with IL-33 is very strongly positive ($r = 0.919$), strong positive correlations were noticed of Gal-9 with T.BIL ($r = 0.731$), ALT ($r = 0.725$) and also

positive correlations with AST (r = 0.683), D.BIL (r = 0.656), IN.BIL (r = 0.650), Albumin (r = 0.581), ALP (r = 0.468), BMI (r = 0.323). However, negative correlation was notice with Globulin (r = -0.563) with p-value less than 0.05 for all these parameters. In addition to no correlation was observed between Gal-9 with DBP, W/N, ROP, W/T, SBP, age, W/H and TSP .

The correlation between IL-33 with variables being studied was as following: very strong positive correlations were noticed with Gal-9 (r = 0.919) and strong positive correlations were noticed of IL-33 with T.BIL (r = 0.743), ALT (r = 0.729). Also positive correlations with D.BIL (r = 0.680), IN.BIL (r = 0.647), AST (r = 0.644), Albumin (r = 0.530), ALP (r = 0.426), BMI (r = 0.254) while negative correlation with Globulin (r = -0.513) with p-value less than 0.05 for all these parameters. However no correlation was noticed between IL-33 with DBP, W/N, ROP, age, W/T, TSP, SBP and W/H.

The results of receiver operating characteristic (ROC) curve showed the following descending arrangement which illustrates the differential ability between patients and healthy controls for the variables studied, Gal-9, IL33, ALT and AST (1), T.BIL (0.9588), Albumin (0.922), D.BIL (0.9197), IN.BIL (0.9084), Globulins (0.8559), BMI (0.7894), ALP (0.7678), W/T (0.7079), W/H (0.6294), DBP (0.7285), W/N (0.5668), TSP (0.5644), ROP (0.5614), SBP (0.5232), Age (0.5138).

It was concluded that low serum levels of Gal-9 and IL-33 are pathological factors and may be used as an indicators for diagnosis and prediction of CHB occurrence.

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Abbreviations	Details
ACLF	Acute on Chronic Liver Failure
ALF	Acute Liver Failure
ALP	Alkaline phosphatase
ALT	alanine aminotransferase
AMs	Anthropometric measurements
anti-HBc	antibodies to Hepatitis B core protein
anti-HBe	antibodies to Hepatitis B e antigen
anti-TNF	antibodies to Tumor Necrosis Factors
ASC	Asymptomatic HBV Carrier status
AST	Aspartate aminotransferase
AUC	Area Under Curve
BIL	Bilirubin
BMI	Body Mass Index
CccDNA	Covalently Closed Deoxy ribonucleic acid
CD	Cluster of Differentiation
CHB	Chronic Hepatitis B
CHC	Chronic Hepatitis C
D.BIL	Direct Bilirubin
DBP	Diastolic Blood Pressure
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immumosorbent Assay
FH	Fulminant Hepatic failure
Gal-9	Galectin-9
HAV	Hepatitis A virus
HBcAg	Hepatitis B Core Antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBSP	HBV Splice-generated Protein
HBV	Hepatitis B Virus
HC	Hip Circumference
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HDV	Hepatitis D Virus
HEV	Hepatitis E Virus
HIV	Human Immunodeficiency Virus
HRP	Horse Reddish Peroxidase
HT ≥3	Hepatotoxicity grade 3 or 4

IFN	Interferon
IL	Interlukin
ILs	Interleukins
IN.BIL	Indirect Bilirubin
KC	Kupffer Cells
LDH	Lactate Dehydrogenase
L-HBsAg	Large- Hepatitis B surface Antigen
MDH	Malate Dehydrogenase
MetS	Metabolic Syndrome
M-HBsAg	Medium -Hepatitis B surface Antigen
mTregs	memory Tregs
NC	Neck Circumference
O.D	Optical Density
OBI	Occult HBV Infection
Pg RNA	Pre- genomic Ribonucleic Acid
PLC	primary Liver Cancer
Pol	Viral Polymerase
RBC's	Red Blood Cells
rc DNA	Relaxed circular Deoxyribonucleic Acid
Rop	Rate of Pulses
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
SBP	Systolic Blood Pressure
SC	Subcutaneous
SD	Standard Deviation
SE	Standard Error
S-HBsAg	Small- Hepatitis B surface Antigen
ST2	Suppression of Tumorigenicity 2
SVPs	Subviral Particles
T.BIL	Total Bilirubin
TC	Thoracic Circumferences
Th	T helper cells
Tim-3	T cell immunoglobulin mucin domain 3
TNF	Tumor Necrosis Factor
VR	Virological Response
TSP	Ttotal Serum Proteins
W/H	Waist to Hip ratio
W/N	Waist to Neck ratio
W/T	Waist to Thoracic ratio

WAT	White Adipose Tissue
WC	Waist Circumference
WHO	World Health Organization

1. Introduction and Literature review

1.1 The Hepatitis B Virus

Hepatitis B is a potentially life-threatening infection of the liver caused by the hepatitis B virus (HBV). [1] HBV is a member of the hepadnaviridae family, a family of small enveloped viruses, that mainly cause hepatotropic infections. [2] Hepatitis viruses pose significant risks to human health, there are a number of different hepatitis viruses (not all of them identified), but the primary viruses are the hepatitis A virus (HAV), HBV, hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV). [3]

However, HAV and HEV spread through the oro-faecal route, HBV and HCV viruses are transmitted through exposure to blood, sexual intercourse and from the infected pregnant mother to the unborn child, although they are transmitted by blood, HDV can only cause infection in people of active HBV or in carriers. [4] HBV is a significant common health problem threats all over the world as nearly 300 million people have chronic hepatitis B virus (CHB) infections. [5]

A Study conducted in 2013 showed that between 1990 and 2013, it was a 63% increase in the global viral hepatitis deaths, passing from the tenth (in 1990) to seventh (in 2013) leading cause of death worldwide. [6]

The world health organization (WHO) issued a resolution in 2016 for the control of viral hepatitis after a diagnosis as a worldwide health problem, in order to significantly reduce the incidence of CHB infection by 90 % and the death by 65% of people infected with CHB by the year 2030. [7]

Effective necessitates that are a multifaceted approach, as well as provider education, particularly at the level of primary care practitioners, and required to obtain comprehensive medical

evaluations, that is an adequate financial support dependent are all critical to meeting the WHO goal of eradicating viral hepatitis by year 2030. [8]

Cytokines are important modulators of inflammation, that play a role in both acute and chronic inflammation through a complex and sometimes contradictory network of interactions, cytokines can be classified based on the nature of the immune response, into: pro and ante-inflammatory , interleukin-1(IL-1) and tumor necrosis factor (TNF) are two important pro-inflammatory cytokines. [9] Cytokines, which are immune system secretory proteins, are responsible for initiating signaling cascades by binding to their cognate receptors and are important in virus pathogenesis and interfering with the fate of HBV infection. [10]

Interleukins (ILs) have an impact on the determination of HBV continuing contagion and the extent of liver injury, the causes of persistent HBV infection are unknown, however, they may be linked to host immune elements, ILs are known to play important roles in host immune responses. [11] Therefor, many studies have been conducted on viral hepatitis B disease and linked to other factor in order to find quick ways to diagnose or treat it.

In 2017, Abdolhossein Zare and others conducted such a research to study to effect of IL-27 gene expression and HBV infection. [10]

In 2018 Na-Na Tao and others studied the role of IL-35 in stimulating HBV transcription and replication. [12]

In 2019 Osama B. Al-Saffara and others have studied the association of IL-1 single nucleotide polymorphisms in viral hepatitis of Iraqi patients. [13]

In 2020, Caixia Xia and others have studied IL-6 influence on the development of hepatitis B virus-related liver cirrhosis in

the Han Chinese population. [14]

1.1.1 Hepatitis B Virus Infection A Global Health Problem

Hepatitis B Virus is prevalent globally, and the prevalence of HBV infections is greatly different among different regions. [15] Hepatocellular carcinoma (HCC), end-stage liver disease, liver transplant, and death are all complications of CHB infection, there is a risk of these health problems, that vary according to mode of transmission and disease period. [16] The serological marker of CHB infectivity, Hepatitis B surface antigen (HBsAg), are used to determine the endemicity of HBV in a given geographical area. [17]

HBV endemicity can be classified into a high (where more than 8% of the population is HBsAg positive), intermediate (2–7%) and low (2%).[18] According to the WHO, around 257 million people worldwide were affected by this infection in 2015. [19]

1.1.2 Hepatitis B Viral Epidemiology in Iraq

Hepatitis is one of the most common chronic infectious diseases in the world, particularly in developing countries, it also is one of the serious health problems in Iraq and neighboring countries. [20]

The endemicity of HBV in Iraq was considered to be at low / intermediate level. [21] Infection rates may rise in the absence of an effective prevention program, early detection of HBV infection is critical for avoiding the negative consequences of the infection and preventing the virus from spreading. [22]

Iraq was considered to be of intermediate endemicity to HBV, as reflected by 3% seroprevalence of HBsAg in the normal population(2005-2006). [22] In 2020, 3–4.5% of people in Iraq will be infected with HBV, including 2–3% of seemingly healthy blood donors. [24]

1.2 Clinical Aspects with Hepatitis B virus Infection

Hepatitis B virus Infection with clinical aspects ranges from subclinical hepatitis to symptomatic and jaundice hepatitis depending on the patient's age at infection, the immune status of the host, and stage of disease recognition. (25)

To understand the clinical aspects of HBV infection, the causal relationship between HBV infection and liver disease must be understood, CHB patients do not all have persistently elevated aminotransferases, However, patients in the immune-tolerant phase have persistently normal alanine aminotransferase (ALT) levels, and a subset of hepatitis B antigen (HBeAg) negative, CHB patients may have intermittently normal ALT levels, therefore, the long-term longitudinal follow-up must be given great importance. (26)

Figure (1-1) depicts the progression of HBV infection, it can result in acute, self-clearing, or CHB infection, the development of a CHB infection correlates positively with younger age, a chronic infection typically has a long course in which the virus replicates at high levels, followed by immune-mediated viral replication control associated with liver inflammation. (27)

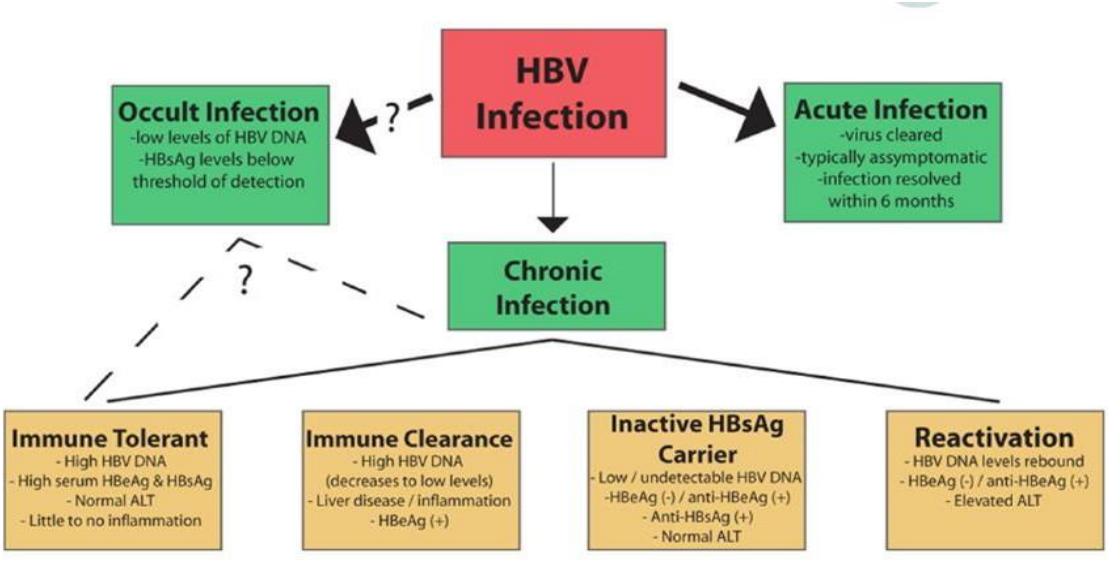


Figure (1-1) Depicts the advance of the infection with HBV. (27)

1.2.1 Acute Infections

The incubation period ranges from one to four months. [28] Acute HBV infection can be asymptomatic or cause symptoms of acute hepatitis, most adults who become infected with the virus have been recovered, however, 5% to 10% are unable to clear the virus and become chronically infected. [29]

Acute hepatitis was defined as acute illness with a simple of clinical symptoms for example (fever, headache, malaise, nausea, vomiting, loss of appetite, dark urine, and abdominal pain), it also can occur with jaundice or serum ALT at least twice as high as the upper limit of normalty without a history of chronic liver disease. [30]

The levels of ALT and aspartate aminotransferase (AST) may increase during the acute phase, ALT is usually higher than AST, bilirubin levels may be normal in a significant proportion of patients, persistent elevation of the ALT serum for over 6 months indicates progression to chronic hepatitis. [31] Acute HBV infection is a leading cause of acute liver failure (ALF) in many countries around the world. [32]

Also it is referred to as fulminant hepatic failure (FH), HBV is the cause of 2–32 % of FH cases worldwide. FH was initially defined as acute hepatitis complicated by ALF, with hepatic encephalopathy occurring less than 8 weeks after the onset of jaundice in a patient with no history of liver disease. [33]

1.2.2 Chronic Infections

Hepatitis B virus infection is one of the most common and serious infectious diseases in the world, CHB has high morbidity characteristics and they are difficult to cure. [34] CHB usually occurs in people who have been infected at an early age, the percentage of

carriers decreases, while the frequency of chronic diseases increases.[28]

Chronic infections develop in 80–90% of infants who are infected during their first year of life and 30–50% of children who are infected before 6 years of age, adults who are infected may develop chronic infections in less than 5% of otherwise healthy people, cirrhosis and/or liver cancer may develop in 20-30% of adults with chronic infection. [35]. Approximately 257 million chronic carriers and approximately 900,000 deaths annually from cirrhosis and HCC .[36]

The natural history and pathology of CHB infection involve a dynamic interaction between two virus infections and host immune cells which can thus be very variable, a significant proportion develop cirrhosis and HCC, while others have carcinoma with long term disease activity that does not necessitate antiviral treatment. [37] The phases of CHB infection are not necessarily sequential. [38] It can be categorized into five clinical phases. [39]

The first phase is immune tolerance. [37] The second phase of immune clearance HBeAg-positive, third phase is inactive HBV carrier, fourth phase is HBeAg-negative chronic hepatitis, and fifth phase is HBsAg-negative phases. [39]

- The first phase (immune tolerance): patients with HBeAg-positive, have high hepatitis B virus deoxyribonucleic acid (HBV DNA), but normal ALT levels, despite a recent research indicates that HBV-specific T-cell responses during this phase are not significantly different from those respone during the active phase, this phase should be changed its name “low inflammatory”. [40] Generally, it is asymptomatic and HBV-specific cluster of differentiation 8 (CD 8+ T-cells). [39]

- The second phase of immune clearance is called HBeAg-positive chronic hepatitis, after years of CHB infection, HBV-specific T-cells become increasingly activated and the infection develops to an inflammatory. [39] The immune clearance HBeAg-positive is characterized at the end of this second phase due to immune-mediated liver necroinflammation, fibrosis, and fluctuating serum ALT concentrations, HBeAg losses and anti-HBe antibodies are occur. [35]
- Third Phase (inactive HBV carrier).[39] HBeAg-negative chronic infection, previously referred to as the inactive carrier phase, it is characterized by the presence of serum antibodies to Hepatitis B e antigen (anti-HBe), undetectable or low (> 2000 IU/mL) HBV DNA and normal ALT levels, some of the patients in this phase may show a level of HBV DNA (< 2,000 IU/mL (usually) > 20,000 IU/mL) with persistently normal ALT and hepatic necroinflammatory activity and fibrosis were both low. [38] Before classifying a patient as an inactive HBV carrier, a year of follow-up with ALT levels at least every 3–4 months and serum HBV DNA levels is required for the majority of patients, the inactive HBV carrier state confers a favorable long-term outcome with a very low risk of cirrhosis or HCC due to immunological control of the infection.[41]
- Fourth phase: HBeAg-negative chronic hepatitis .[39] It is characterized by a lack of serum HBeAg usually with detectable anti-HBe and a persistent or fluctuating moderate to high serum HBV DNA (often lower than in HBeAg-positive patients) and fluctuating or a persistently elevated ALT value.[38] remain at high risk for fibrosis progression. [36]

- Fifth phase: HBsAg-negative phase is characterized by serum negative HBsAg and positive Hepatitis B Core Antigen (HBcAg) antibodies, HBsAg loss before cirrhosis begins ,and it is associated with a minimal risk of cirrhosis, HCC and an improvement in survival, however, if cirrhosis has developed prior to HBsAg, patients remain at risk of HCC, therefore HCC surveillance should continue immunosuppression wich may lead to reactivation of HBV in these patients. [38] In general, HBV-related end-stage liver disease (including cirrhosis and HCC) occurs decades post-exposure. [39]

1.2.3 Occult Hepatitis B virus Infection

Occult HBV infection (OBI) is defined as the absence of HBsAg as well as the presence or absence of other HBV antibodies (anti-HBc and anti-HBs) and the presence of HBV DNA in liver (with or without presence in serum). [42] HBsAg is absent due to the present of viral DNA in the liver despite it is not present in the blood. [43]

Occult HBV infection is in resource-limited environments. Serum samples are usually detected by OBI, as liver biopsy tests are not routinely available, when detectable, the amount of HBV DNA in the serum is generally very low (< 200 IU/mL). [44] OBI is a heterogeneous condition with different clinical implications, it is clearly associated with the risk of cirrhosis, HCC, and HBV reactivation with immunosuppressive therapy. [45]

Occult HBV infection or subclinical infection is now recognized as an increased risk to HCC, the risk is most noticeable in these situations over 50 years of age. [46]

1.2.3.1 Clinical Implications of Occult Infections

Occult Infections has important clinical significance because HBV can be transmitted through transfusion, organ transplantation

and perinatal route, in patients with low immunity or those who are having chemotherapy OBI infection can reactivate and lead to acute hepatitis which may develop hepatic fibrosis , it is also a risk factor that can develop HCC. [47]

There are three main clinical implications for OBIs: transmission, reactivation and liver disease.

•First: Transmission

This includes transmission of HBV through blood products and organ transplantation from OBI donors or hemodialysis. [48, 49] Although the risk of transmission of HBV via transfusion has decreased significantly, there is still some risk based on the prevalence of the population, the types of donors and the screening tests available in blood banks. [50] Donor screening before blood transfusion, prophylaxis for high-risk organ transplantation recipients, and dialysis-specific infection-control programs should be considered to reduce the risk of transmission. [49]

• Second: Reactivation

Occult HBV infection spontaneous reactivation of HBV rare in normal circumstances in most the subjects of OBI, the level of serum HBV DNA remains imperceptible but if it is detectable, it usually remains very low throughout the period of subsequent follow-up. However, there are circumstances in which HBV can be detected to reactivate this low viraemic state. [48] OBI reactivation occurs frequently in people who have undergone immunosuppressive therapy. For example, patients who have received organ transplantation, particularly liver, kidney and bone marrow, and those who have received systematic chemotherapy, radiotherapy OBI reactivation occurs. [47]

• Third: Liver disease

Occult HBV infection was discovered in patients with chronic liver disease (5–40%) severity of liver disease (e.g., cirrhosis, high ALT levels) and coinfection with HCV (22–73%) or human immunodeficiency virus (HIV) (10–45%), OBI is implicated in the development of HCC in patients with CHC infection and OBI patients, as well as more advanced histological grade tumors and earlier HCC diagnosis compared to non-OBI patients. [51]

It can be also found in patients co-infected with the HCV in which HCV core protein interferes with the replication of HBV and its protein synthesis or co-infected with the HIV due to cell immunodeficiency (decreased CD4+ cell numbers). [43]

1.3 Hepatitis B Virus Structure

Hepatitis B virus is an enveloped DNA virus that belongs to the family of hepadnaviridae. [52] HBV virions were named Dane particles after the researchers who led the team that first succeeded in identifying virus particles which have been visualized by electron microscope.[2] At least three types of HBV particles are detected in the serum of infected patients which are spherical structures of 42 nm in diameter, those with a diameter of 22 nm and variable length filament structures of 22 nm, the particles of 42 nm are infectious virions. [53]

Figure (1-2), showing the structure of the virion, Infectious circulating viral particles contain a small circular, partially double-stranded DNA for about 3200 nucleotides. [54] Enclosed by a capsid, comprised of Hepatitis B Core Antigen (HBcAg) and surrounded by a lipid envelope containing large (L-HBsAg), middle (M-HBsAg) and small (S-HBsAg), the virus also expresses two non-particulate proteins X protein for a transcriptional trans-activator protein and HBeAg viral polymerase (Pol) reverse transcriptase of a diameter. [55]

The 22 nm particles, which are much more abundant in the patient serum, include subviral particles (SVPs) lack nucleocapsid and are therefore non-infectious, other non-infectious particles are currently known to be caused by infection, including enveloped particles that lack of a viral genome, viral RNAs, and enveloped particles that do not have a viral genome.(56)

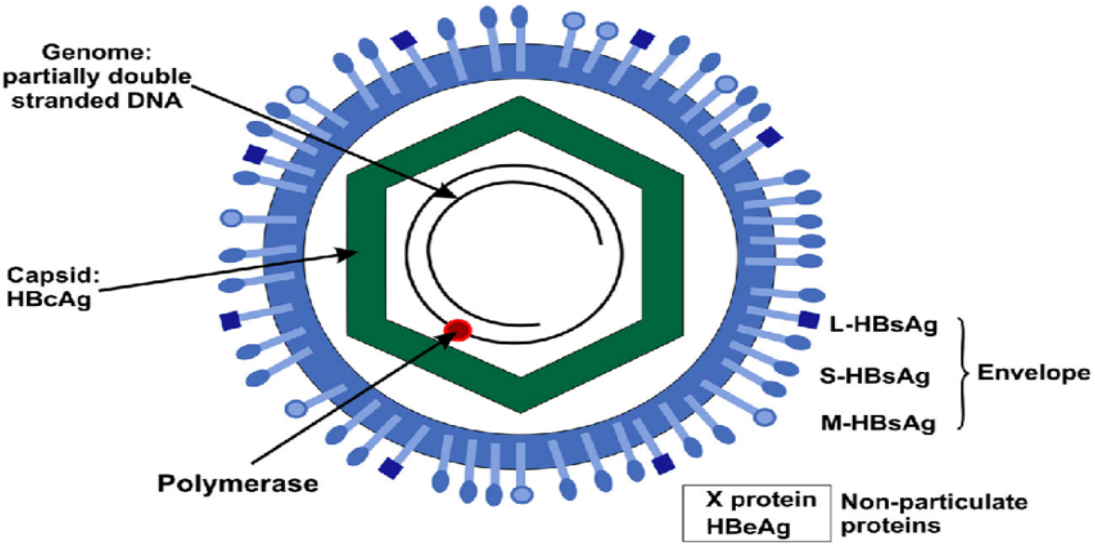


Figure (1-2) Schematic Representation of HBV Particles. (55)

1.4 Replication of the Hepatitis B Virus

Hepatitis B Virus infects hepatocytes on a long-term basis, it replicates itself by reversing the transcription of an ribonucleic acid (RNA) intermediate known as pregenome. (57)

The virus replicates and assembles exclusively in hepatocytes in the host and virions are released non-cytopathically via the cellular secretory pathway a route, HBV nucleocapsid is transported to the nucleus, where it is released.(58) The genome of relaxed- circular Deoxyribonucleic Acid (rcDNA) is covalently converted in the nucleoplasm, histones wrap cccDNA to form a eukaryotic eukary structure with episomal chromatinization, it is then used as a

transcription template for all viral transcripts translated into various viral proteins. [38]

Spliced pre- genomic ribonucleic acid (pgRNA) can form a novel protein called the HBV splice-generated protein (HBSP), it is linked to increased viral replication by increased core protein expression and HBeAg secretion. [59] Serum HBeAg has proven to be a useful marker for monitoring viral replication because its presence is associated with high levels of viral replication and its absence is associated with a decrease in viral replication. [60]

1.5 Factors Associated with Hepatitis B Virus Infection

Hepatitis B Virus infection and all related complications, including CHB, FH, ALF. [61] CHB patients with recurrent hepatic inflammation, particularly those with cirrhosis, are at risk of advanced hepatic disease or even acute on chronic liver failure (ACLF). [62]

CHB infection is linked to a number of diseases, including asymptomatic HBV carrier status (ASC), liver cirrhosis, and HCC, and it is a major cause of liver-related morbidity and mortality. [63] Significantly higher incidence rates of cirrhosis and HCC have been observed in HBsAg carriers compared to non-carriers, in addition, older age, male sex, HBeAg positivity, genotype C, and increased levels of ALT, HBV DNA, and HBsAg are significantly associated with increased risk of cirrhosis and HCC. [64]

Liver fibrosis is a common outcome of a sustained process of wound healing caused by a variety of causes, including CHB. [65] TNF antagonists are the first-line therapy for many autoimmune diseases ,they are linked to reactivation of the HBV, the rate of HBV reactivation and hepatotoxicity grade 3 or 4 (HT $\geq$3) for patients treated with an anti-TNF agent for an autoimmune disease were determined. [66]

1.6 Incidence of Hepatitis B Virus Infection

Hepatitis B Virus can survive contaminated environmental surfaces for up to 7 days and is transmissible when it comes into contact with non -intact skin or mucosa HBV is spreaded through percutaneous or mucosal contact with infected blood or bodily fluids [31] HBV infection has an incubation period of 45–150 days. [67]

Three modes of HBV transmission, including perinatal, sexual and blood transmission, perinatal or mother-to-infant transmission plays an important role in spreading HBV.[68] Since 1970, all blood donations have been screened for HBsAg, lowering the risk of transfusion-transmitted HBV infection, screening for HBs-Ag is still regarded as an important tool for blood screening by WHO.[69]

Also was found HBV patients gave a history of visiting dentists and undergoing previous surgery, in addition, toothbrushes, razors, and some other house-holding instruments can transmit HBV within the family, the prevalence of risk factors differs from a society to another according to the norms and traditions of that society, however, HBV infection is a preventable disease and can be prevented by 3 doses of vaccination.[22]

1.7 Risk Factors for Hepatitis B Virus Infection

The presence of metabolic risk factors, such as abdominal obesity and hypertension has shown a significant impact on the progression and even all-cause death rates in CHB. [70]

1.7.1 Body Mass Index

The Body Mass Index (BMI) is identified as the weight in kilograms divided by the height in meters squared (kg/m^2), the WHO consider a BMI of less than 18.5 as an underweight and BMI of 18.5 to 24.9 as normal weight while BMI of $\geq$ 25.0 up to 30.0 was

classified as overweight and BMI of ≥ 30.0 as an obese. [71] The increased BMI has been shown to worsen the outcome of CHB disease, obesity (BMI ≥ 30 kg/m^2) is associated with an increased risk of both HCC and liver-related mortality. [17]

1.7.2 Hypertension

Hypertension defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg, the global hypertension epidemic is largely uncontrolled, and hypertension continues to be the leading cause of noncommunicable disease deaths worldwide. [72] also, HBV infection is associated with increased risk of metabolic syndrome (MetS) many of the chronic diseases that are components of the MetS, such as diabetes, hypertension, stroke, cardiovascular disease, and fatty liver disease. [73]

1.8 Anthropometric Measurements

The anthropometric measurements (AMs) is known as body weight and body measurements and is used in specific epidemiological studies or clinical settings. [74] AMs include unite of measure weight (kg), height (m), waist circumference (cm) and hip circumference (cm). [75]

1.8.1 Hip Circumference

The hip circumference (HC) is measured in centimeters using a tape measure at the widest point between the hip and buttock. [70] It is one of AMs used in liver diseases associated with patients with HBV such as fibrosis. [75]

1.8.2 Waist Circumference

An elastic measuring tape is used to measure waist circumference (WC) in centimeters at the midpoint between the lower rib cage margin and the top of the iliac crest. [70] WC is a simple and

dependable anthropometric test used as a replace for centric obesity in epidemiological studies .[75] Central obesity was defined as a WC ≥ 90 cm for men and ≥ 80 cm for women. [76] WC may be an independent predictor of primary liver cancer (PLC) risk in men, particularly in those with HBsAg negativity. [77]

1.8.3 Thoracic Circumference

Thoracic circumference was measured with a tape measure horizontally placed around the thorax .[78] In 2020, a study showed the relationship between CHB patients who have thoracic circumferences (TC) greater than 75 cm and liver fibrosis. [79]

1.8.4 Neck Circumference

Neck circumference (NC) is the girth below the thyroid cartilage protrusion, NC measurement is easy and highly reproducible and has little variation. [80] NC is a marker of upper body subcutaneous (SC) adipose tissue distribution, it is a relatively new method of differentiating between normal and abnormal fat distribution. [81] The NC > 37 cm in men and NC > 34 cm in women are probably the best cutoff points to determine subjects with central obesity. [82] It indicates to high correlations between NC and BMI which is linked to several diseases include nonalcoholic fatty liver disease and abnormal liver function tests.[81]

1.9 Clinical Significance of Galectin-9 in Hepatitis B Virus Infection

Galectins are a phylogenetically conserved family of soluble β-galactoside binding proteins, consisting of 15 different types, each with a specific function. [83] Galectin-9 (Gal-9) can bind to a variety of cell surface receptors and influence intracellular molecules, depending on the cell type, Gal-9 can perform a variety of functions.[84] It binds

to T cell immunoglobulin mucin domain 3 (Tim-3), a type I glycoprotein is found in dysfunctional T cells during chronic viral infections, Gal-9 exerts critical immunomodulatory effects by inducing apoptosis or suppressing effector functions via its receptor, Tim-3. [85]

Galectin-9 is a preserved S-type lectin that plays multiple immune modulatory roles in both innate and adaptive immune responses. [86] Gal-9 has been shown to reduce the abundance of immune effector cells T helper1 (Th1) and T helper 17 (Th17) by binding to T-cell immunoglobulin and Tim-3 and inducing programmable cell death. [87] Gal-9 is highly expressed in the liver and has a wide range of innate and adaptive biological functions that are instrumental in maintaining hepatic homeostasis, in the setting of viral hepatitis, increased expression of Gal-9 stimulates the expansion of regulatory T cells and the contraction of effector T cells, favoring viral persistence. [88]

In 2017, Lucy Golden-Mason and Hugo R. Rosen have found that Gal-9 levels in HBV-infected liver biopsies are high, and serum levels of this protein are significantly higher than in uninfected controls. [88]

In 2021, Preeti Moar, and Ravi Tandon have found that Gal-9 levels in CHB infection serum are elevated, which correlated positively with HBV-associated liver inflammation, Gal-9 expression was also increased in Kupffer cells (KC) of the liver sinusoidal network.[89]

1.10 Clinical Significance of Interleukin-33 in Hepatitis B Virus Infection

Interlukin-33 (IL-33) is a member of the IL-1 family of cytokines with an increasing number of target cells and a plethora of biological functions. [90] IL-33 binds to its receptor suppression of Tumorigenicity 2 (ST2). [91] IL-33 stimulated the production of pro-inflammatory and T helper-2 (Th2)-associated cytokines, serum IL-33 was associated with liver damage in patients with CHB. [92] Based on the disease, IL-33 has pro- and anti-inflammatory functions, IL- 33 has been shown to be a pathogenic marker in patients with acute liver failure, CHB, HCV and liver cirrhosis, it has also been proposed that IL-33 be used as a pro-fibrotic factor in chronic liver disease because it is linked to liver fibrosis.[93]

In 2015, Octavie Rostan and others have studied the crucial and diverse role of the Interleukin-33/ST2 axis in infectious diseases and they found elevated serum levels of IL-33 in chronically infected HBV patients. [94]

In 2017, Zijian Sun, and other have found That CHB patients with high serum ALT concentrations showed higher serum IL-33 and ST2 levels.[95]

In 2020, Wei Yuan with other have found that the IL-33/sST2 axis could be used to assess progression and mortality in CHB patients experiencing a hepatic flare. [60]

1.11 Liver Enzyme Function

Liver synthesizes several nonessential amino acids and serum enzymes such as AST, ALT, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP). [96] Some studies have shown that liver

function markers, including albumin, bilirubin, ALT, AST and AST/ALT marked variation among different HBV patients. [97]

1.11.1 Alanine Aminotransferase

Alanine aminotransferase, (EC: 2.6.1.2). [98] It is the most commonly used enzyme for detecting hepatocellular damage, it is found in many tissues, but it is most active in the liver. [99] ALT concentrations are found to be lower than AST concentrations in all cells except hepatic cells; thus, its elevation is particularly related to liver disease. [100]

Chronic hepatitis B virus infection acquired at birth or early childhood typically progresses through an early stage of disease characterized by normal serum ALT and high titer viremia. [101] CHB was characterized by seropositivity of HBsAg for ≥ 6months with repeated abnormalities in serum ALT. [60] Patients with ALT levels that are 0.5–1 times higher than the normal upper limit are at risk of developing cirrhotic complications and HCC. [102] Elevations in ALT are traditionally linked to hepatocellular injury; however, there have also been reports of severe muscle damage linked to an increase in ALT activity. [103]

1.11.2 Aspartate Aminotransferase

Aspartate aminotransferase (EC 2.6.1.1). [98] AST is less specific for liver damage. [100] AST is found throughout tissues , with high concentrations in the heart, liver, kidney, and skeletal muscle as a result, it should not be used as a sole indicator of liver damage unless other supporting enzymes are measured, elevations in AST may be less pronounced in most species than increases in ALT with minimal to mild liver injury. [99]

Aspartate aminotransferase is a biomarker commonly used for the assessment of hepatocyte damage, AST levels are increased in

acute and chronic viral hepatitis, alcoholic hepatitis and cirrhosis. [104] Increased AST activities are commonly associated with effects on the liver and skeletal muscle, but they can also be seen in hemolytic conditions. [103]

1.11.3 Alkaline phosphatase

Alkaline phosphatases (EC 3.1.3.1) are widely present in nature, and are found in many organisms, it is an enzyme which catalyzes the hydrolysis of phosphate monoesters, ALP is membrane-bound and widely found in liver for about 55%; in bone 45%; in osteoblasts, gut 5%. [105] ALP is a well-established biomarker for a number of diseases, studies have shown that elevated ALP serum concentrations are often associated with biliary obstruction, osteopathy such as osteoplastic bone tumors and osteomalacia, leukemia or lymphoma, however, decreased levels of ALP may also occur when certain metabolic disorders such as Wilson's disease or hematological diseases such as aplastic anemia and chronic myelogenous leukemia occur. [106] Serum ALP is increased in HBV. [107]

1.12 Biochemical Markers Related to Liver Diseases

Routine liver biochemical tests includes tests of hepatic function (e.g., serum albumin, serum bilirubin), abnormal liver biochemical test are often the first clues to liver disease. [108]

1.12.1 Bilirubin

Bilirubin (BIL) $C_{33}H_{36}N_4O_6$ is a primary end product of heme catabolism. [109] It is released in an unconjugated form as an indirect bilirubin (IN.BIL), which is bound to albumin in the plasma and transported to the liver, where bilirubin is conjugated with glucuronic acid in hepatocytes, a process catalyzed by glucuronyl transferase. [110]

Conjugated bilirubin direct bilirubin (D.BIL), is excreted into the bile and enters the duodenum, some of the bilirubin is hydrolyzed in the small bowel to produce unconjugated bilirubin and glucuronic acid, as well as urobilinogen, the majority of urobilinogen is secreted in the stool. [111] But some of urobilinogenis is reabsorbed and returned to the liver via the portal vein, enters circulation and secreted by kidney. [110] Higher levels of serum (D.BIL) are seen in viral hepatitis, hepatocellular damage and hyperbilirubinemia in acute viral hepatitis is directly proportional to the degree of hepatocyte histological injury and the duration of the disease.[112]

1.12.2 Total protein

The estimation of total serum proteins (TSP) in the body is useful in distinguishing between normal and damaged liver function because the important role of liver produces the majority of proteins such as albumin and globulin.[113] TSP represents the sum of albumin and globulin, it may be elevations duo to chronic infection, collagen vascular disease, liver dysfunction wile it could be decreased due to malnutrition, liver diseases etc. [114] TSP levels should be between 6.0 and 8.3 g/dL.[113]

1.12.3 Albumin

Albumin is the main protein in the blood and is produced by the liver. [115] Low serum albumin concentration indicates that the liver is not functioning properly, serum albumin levels are not reduced in acute liver failure because it takes several weeks of impaired albumin production for the serum albumin level to fall. [116] The normal range of albumin is 3.4-5.4 g/dL .[115]

In chronic liver disease, serum albumin concentrations are usually normal until cirrhosis and significant liver damage develop, serum albumin levels in advanced liver disease may be less than 3.5 g/dL.

[116] increased albumin may be due to dehydration or improper use of protein.[115]

1.12.3 Globulins

Globulin are proteins that include four major groups that can be identified: gamma globulins, beta globulins, alpha-2 globulins and alpha-1 globulins. [114] Globulin is one type of protective antibody produced by immune system that helps identify and fight infections, increased globulin levels may be due to chronic inflammation, kidney infection, stress, liver disease and parasite infestation, while decreased globulin levels may be due to anemia, depressed immune systems.[115]

The globulin values have been calculated by subtracting the albumin values from the corresponding total protein values. [117]

1.13 Aims of the Study

The main aim of this study was to evaluate serum levels of Gal-9 and IL-33 in CHB Patients in Al-Fallujah City and explore the correlation and strength of associations between these two variables with others studied parameters, and the ability to use of serum levels of Gal-9 and IL-33 as indicator for diagnosis of CHB.

2. Materials and Methods

2.1 Subjects

2.1.1 Chronic Hepatitis B virus Patients

Forty patients (20 females and 20 males) with CHB were subjected in the study. The age range was within (20 to 55 years), after ethical clearance, the study was carried out in those attending the Fallujah teaching hospital and some private laboratories in Al-Fallujah city between September 2020 to January 2021.Questionnaire sheet was completed for each subjects (**Appendix I**).

After full clinical exanimation by their consultant physicians, patients with other chronic diseases such as diabetes, joint diseases, as well as, those with chronic kidney diseases and also pregnant women were excluded from this study.

2.1.2 Controls

For the purpose of comparisons, 40 (20 female and 20 male) control subjects comparable to HBV patients in respect to age (20 to 55 years), were included in the study. The controls were selected among subjects who were apparently seems healthy in terms of not infected with hepatitis and non-diabetic, non-hypertensive, any other endocrine disorders or metabolic kidney diseases and were without acute illness or infection at time of sampling.

2.1.3 Characteristics of Patients and subjects

Control samples and patients were described in the terms of gender, age, CHB family history, BMI, waist to hip (W/H) ratio , waist to thoracic (W/T) ratio, waist to neck (W/N) ratio, SBP, DBP, rate of pulses (ROP).

2.2 Materials

2.2.1. Instruments that are used:

The sources of instruments in this study are presented in table (2-1).

Table (2-1): The instruments that are used in this study

Instruments	Company	Origin
Centrifuge	Hettich Universal	Germany
Deep freeze	Beko	Turkey
Spectrophotometer	Mindray BA-88 A	Chine
Blood pressure monitor	Rossmax	Switzerland
Water bath	Memmert	Germany
ELISA	MR-96A Mindray Elisa Microplate Reader	India
Normal refrigerator	LG	South Korea
Incubator	Memmert	Germany
Timer with alarm	Junghans	Germany

Table (2-2): The tools that are used in this study

Tools	Company	Origin
Pipette tips	Gilson	France
Eppendorff tubes	Eppendorff	Germany
Micropipettes	Gilson	France
Plastic disposable syringes:5ml	Meheco	China
Blood Collection gel tubes	AFMH	England

2.2.2 Materials that are used:

Materials that are used in this study are listed in tables (2-3) with its companies' names.

Table (2-3): The materials that are used in this study.

Materials	Company	Origin
ALT Kit	Agappe	Switzerland
AST Kit	Agappe	Switzerland
ALP Kit	Agappe	Switzerland
Total Proten Kit	Agappe	Switzerland

Albumin Kit	Agappe	Switzerland
Bilirubin Total & Direct Kit	Agappe	Switzerland
Human IL-33 ELISA Kit	Bioassay Technology Laboratory	China
Human Gal-9 ELISA Kit	Bioassay Technology Laboratory	China

2.3 Blood Sampling

About 5 mL of blood were taken from all subjects participate in this study by using disposable syringe, blood was left for 15 minutes for clotting at room temperature , the blood was centrifuged at 4000 xg for 15 minutes. The serum was divided into two parts, the first one was used for estimating liver function test and the second part was stored in two eppendorff tubes (250μL) and froze at (-20 °C) until use to estimate Gal-9and IL-33.

2.4 Laboratory Methods

2.4.1 Determination Serum Activity of Alanine Amino transferase.

Principle

Alanine aminotransferase stimulates the reverse transfer of an amino group of alanine to α-ketoglutarate forming pyruvate and glutamate and the pyruvate produced is reduced to lactate by lactate dehydrogenase(LDH) and NADH.

Kinetic determination of ALT is according to the following reaction

$$\text{L-Alanine + alpha –ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate +L-Glutamate}$$

$$\text{Pyruvate + NADH + } H^{+} \xrightarrow{LDH} \text{L-Lactate + } NAD^{+}$$

- **Kit Components**

Reagents Types	Materials	Concentration
SALT (S.L) R1	Tris buffer (PH 7.5) L-Alanine LDH	110 mmol /L 600 mmol/L > 1500 U/L
SALT (S.L) R2	Alpha- Ketoglutarate NADH	16 mmol /L 0.24 mmol /L

- **Assay Procedure**

1- Reagents and samples were left at temperature between 20-25 ˚C.

2- Reagent 2(R2) was added to Reagent 1(R1) and mixed gently to prepare working reagent.

3- The spectrophotometer was set to zero with distilled water (D.W).

4- This tube was prepared (4 volumes was mixed for R1 with 1 volume of R2 + sample).

Table (2 - 4): Procedure for ALT Determination

Laboratory Procedure for Semi Auto Analyzer	
Working reagent	1000 µL (800 µL R1+200 µL R2)
Sample	100 µL

5- The tube was incubated for 1 minute in a water bath at 37 ˚C.

6- The absorbance was read at 340 nm during 3 minute.

7- The serum activity of ALT was calculated according to equation:

ALT activity (U/L) = (ΔOD/min) ×1745

- 1745 proved factor for estimation of this assay on semi auto

Normal value for ALT	
ALT	Serum up to 49 U/L

2.4.2 Determination Serum Activity of Aspartate Amino transferase

Principle

Aspartate aminotransferase catalyzes the reversible transfer of an amino group from aspartate to α-ketoglutarate forming oxaloacetate

and glutamate. The oxaloacetate produced is reduced to malate by NADH and malate dehydrogenase (MDH).

Kinetic determination of AST is based upon the following reaction

$$\text{L-Aspartate} + \text{alpha –ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^{+} \xrightarrow{\text{MDH}} \text{L-Malate} + \text{NAD}$$

- **Kit Components**

Reagents Types	Materials	Concentration
AST (S.L)R1	Tris buffer (PH 7.8) L-Aspartate LDH MDH	88 mmol /L 260 mmol /L <1500 U/L <900 U/L
AST (S.L)R1	Alpha- Ketoglutarate NADH	12 mmoL/L -0.24 mmol/L

- **Assay Procedure**

1- Reagents and samples were left at temperature between 20-25 ˚C.

2- R2 was added to R1 and mixed gently to prepare working reagent.

3- The spectrophotometer was set to zero with D.W.

4-This tube was prepared (4 volumes was mixed for R1 with 1 volume of R2 + sample).

Table (2-5): Procedure for AST Determination

Laboratory Procedure for Semi Auto Analyzer	
Working reagent	1000 µL (800 µL R1+200 µL R2)
Sample	100 µL

5- The tube was incubated for 1 minute in a water bath at 37 ˚C.

6- The absorbance was read at 340 nm during 3 minute.

7- The serum activity of AST was calculated according to equation:

AST activity (U/L) = (ΔOD/min) × 1745

• 1745 proved factor for estimation of this assay on semi auto analyzer.

Normal value for AST	
AST	Serum up to 46 U/L

2.4.3 Determination Serum Activity of Alkaline Phosphatase

Principle

Alkaline phosphatase stimulates P-nitrophenyl phosphate hydrolysis at pH 10.2 and releases phosphate and p-nitrophenol. Kinetic determination of ALP according to the following reaction

P-nitrophenyl phosphate + $H_2O \xrightarrow{ALP}$ P-nitrophenol +Inorgnic phosphate

- **Kit Components**

Reagents Types	Materials	Concentration
alkaline phosphates RI	Diethanolamine buffer (PH 10.2) Magnesium Chloride	125 mmol /L -0.625 mmol /L
Alkaline phosphates R2	P-Nitro phennyl phosphate	50 mmol /L

- **Assay Procedure**

1- Reagents and samples were left at temperature between 20-25 ˚C.

2- R2 was added to R1 and mixed gently to prepare working reagent.

3- The spectrophotometer was set to zero with D.W.

4- This tube was prepared (4 volumes was mixed for R1 with 1 volume of R2 + sample).

Table (2-6): Procedure for ALP Determination

Laboratory Procedure for Semi Auto Analyzer	
Working reagent	1000 µL (800 µL R1+200 µL R2)
Sample	20 µL

5- The tube was incubated for 1 minute in a water bath at 37 ˚C.

6- The absorbance was read at 405 nm during 3 minute.

7- The serum activity calculated according to equation:

ALP Activity (U/L) = (ΔOD/min)× 2750

• 2750 proved factor for estimation of this assay on semi auto analyzer.

Normal value	
Women	64-306 U/L
Men	80-306 U/L
Children	180-1200 U/L

2.4.4 Determination Level of Total Serum Protein

Principle

Total protein colorimetric determination is based on the Biuret reaction principle (copper salt in an alkaline medium). When protein in plasma or serum samples is treated with cupric ions in alkaline solution, it forms a blue complex. The intensity of the blue color is directly proportional to the protein concentration**.**

- **Kit Components**

Reagents Types	Materials	Concentration
Total Protein Reagent	Potassium Iodide Potassium Sodium tartarate Copper Sulphate Sodium Hydroxide	6 mmol /L 21 mmol /L 6 mmol /L 58 mmol /L
Total Protein Standard	Total Protein Standard	6 g /dL

- **Assay Procedure**

1- Reagents and samples were left at temperature between 20-25 ˚C.

2- Standard of total protein was added to total protein reagent and mixed gently to prepare working reagent.

3- The spectrophotometer was set to zero with blank..

4- Three tubes were prepared as shown in table (2-7)

Table (2-7): Procedure for Total Protein Determination

Laboratory Procedure for Semi Auto Analyzer			
	Blank	**Standard**	**Sample**
Reagent	1000 µL	1000 µL	1000 µL
Standard	----------	20 µL	---------
Sample	----------	----------	20 µL

5- The tube was incubated for 10 minutes in a water bath at37 ˚C.

6- The absorbance was read at 546 nm.

7- Serum level of total protein was calculated according to equation:

$$\text{TSP Concentration (g/L)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 6$$

- 6 g /L Standard Concentration.

Normal value:	
TSP	6.2 – 8.0 g /dL

2.4.5 Determination Serum Level of Albumin Clinical

Principle

The reaction of albumin from serum or plasma with the dye bromocresol-green results in a color change proportional to albumin concentration.

- **Kit Components**

Reagents Types	Materials	Concentration
Albumin Reagent	Succinate Buffer (PH4.20) Bromocresol green	75 mmol/L 0.14 g/L
Albumin Standard	Albumin Standard concentration	3 g/dL

- **Assay Procedure**

1- Reagents and samples were left at temperature between 20-25 ˚C.

2- Standard of Albumin was added to Albumin reagent and mixed gently to prepare working reagent.

3- The spectrophotometer was set to zero with blank.

4- This tube was prepared (1 volume was mixed for ALP reagent with 1 volume of Standard + sample).

Table (2-8): Procedure for Albumin Determination

Laboratory Procedure for Semi Auto Analyzer			
	Blank	**Standard**	**Sample**
Reagent	1000 µL	1000 µL	1000 µL
Standard	----------	10 µL	----------
Sample	----------	---------	10 µL

5- The tube was incubated for (1) minute.

6- The absorbance was read at 630 nm.

7- Serum level of Albumin was calculated according to equation:

$$\text{Albumin Concentration (g/L)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 3$$

• 3 g /L Standard Concentration

Normal value:	
Albumin	3.5 – 5.5 g /dL

2.4. 6 Determination Serum Level of Total and Direct Bilirubin

Principle

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. T.BIL reacts with diazotized sulfanilic acid to form azobilirubin. The principle of D.BIL same of T.BIL but D.BIL reacts with diazotized sulfanilic acid to form azobilirubin.

- **Kit Components for Total Bilirubin**

Reagents Types	Materials	Concentration
Total Bilirubin Reagents	Sulfanilic acid Total Bilirubin	28.9 mmol /L 9 mmol /L
Total Bilirubin Activator	Sodium nitrite	2.0 g /L

- **Kit Components for Direct Bilirubin**

Reagents Types	Materials	Concentration
Direct Bilirubin Reagents	Sulfanilic acid Hydrochloric Acid	28.9 mmol /L 165 mmol /L
Direct Bilirubin Activator	Sodium nitrite	1.0 g /L

- **Assay Procedure**

1- Reagents and samples were left at temperature between 20-25 ˚C.

2- T.BIL activator was added to T.BIL reagents was mixed gently to prepare working reagent.

3- The spectrophotometer was set to zero with blank.

4- Tow tubes were prepared as shown in table (2.9) below:

Table (2-9): Procedure for Total Bilirubin Determination

Laboratory Procedure for Semi Auto Analyzer		
	Reagent Blank	**Test**
Total Bilirubin Reagent	1000 µL	1000 µL
Activator Total	20 µL	20 µL
Serum / Calibrator	----------	50 µL

5-The tube was incubated for (5) minute at room temperature (20-25°C).

6- The absorbance was read at 546/ 630 nm.

7-The serum activity of Bilirubin was calculated with factor according to equation:

$$\text{Total Bilirubin} = \text{OD of Test} - \text{OD of Reagent Blank} \times 29$$

Normal value	
T.BIL of Adults	up to 1.2 mg/dL
T.BIL of Infants	0.2 – 8 mg /dL
D.BIL	Up to 0.4 mg /dL

2.4.7 Determination Serum Levels of Galectin -9. (Catalog No: E2998Hu) Provided from Bioassay Technology Laboratory Company.

Serum level of Gal-9 was quantitatively determined by mean of using sandwich ELISA method.

- **Kit Components**

Components	Quantity
Standard Solution (3200pg /mL)	0.5 ml ×1
Pre-coated ELISA Plate	12 *8well strips ×1
Standard Diluent	3mL
Streptavidin -HRP	6 mL
Stop Solution	6 mL
Substrate Solution A	6 mL
Substrate Solution B	6 mL
Wash Buffer Concentrate (25x)	20 mL
Biotinylated human Gal-9 Antibody	1 mL
User Instruction	1
Plate Sealer	2pic
Zipper bag	1pic

- **Assay Procedure:**

Before performing the examination procedure, the kit components were left at room temperature (20 -25°C) for 30 minutes before using.

1- Serial concentrations (1600, 800, 400, 200, 100, and 0 pg/ml) for Gal-9 of the standard were made using the diluent.

2- Fifty µL of standard working solution were added to the standard well and 40 µL of samples were added to sample wells, then 10 µL of anti-Gal-9 antibody were added to sample wells, then 50 µL of streptavidin – Horse Reddish Peroxidase (HRP) were added to sample

wells, these well mixed and then the ELISA plate was covered with the sealer in the kit and was incubated for 60 minutes at 37 °C.

3- The sealer was removed, and the plate was washed five times with wash buffer before filling wells with at least 0.35 mL wash buffer for 30 seconds to 1 minute each time. All wells were aspirated and washed 5 times with wash buffer, overfilling wells with wash buffer, for automated washing. Paper towels or other absorbent materials were used to blot the plate.

4- Each well received 50 µL of substrate solution, followed by 50 µL of substrate solution B for 10 minutes at 37 °C in the dark, the plate was incubated and covered with a new sealer.

5- Fifty µL of the stop solution was added to every well.

6- The absorbance of each well was read simultaneously using an ELISA reader with a wavelength of 450 nm.

2.7.8 Determination Serum Levels of Interleukin-33.

(Catalog No: E0044Hu) Provided from Bioassay Technology Laboratory company

Serum level of IL-33 was quantitatively determined by mean of using sandwich ELISA method.

- **Kit Components**

Components	Quantity
Standard Solution	0.5 mL ×1
Pre-coated ELISA plate	12*8 well stripe ×1
Standard Diluent	3 ml × 1
Streptavidin-HRP	6 m × 1
Stop solution	6 ml ×1
Substrate Solution A	6 ml ×1
Substrate Solution B	6 ml ×1
Wash Buffer Concentrated(25x)	20ml ×1
Biotinylated human IL-33 Antibody	1 ml ×1

User Instruction	1
Plate Stealer	2 pic
Zipper bag	1 pic

- **Assay Procedure:**

Before performing the examination procedure, the kit components were left at room temperature (20 -25 °C) for 20 minutes before using them.

1- Serial concentrations (2400, 1200, 600, 300, 150,75 and 0 ng/L) for IL33 of the standard were made using the diluent.

2- Fifty µL of standard working solution were added to standard well and 40 µL of samples were added to sample wells, then 10 µL of anti-IL-33 antibody were added to sample wells, 50 µL of streptavidin – HRP were added to sample wells, well mixed and then the ELISA plate was covered with the sealer in the kit and was incubated for 60 minutes at 37°C.

3- The sealer was removed, and the plate was washed five times with wash buffer before filling wells with at least 0.35 mL wash buffer for 30 seconds to 1 minute each time. All wells were aspirated and washed 5 times with wash buffer, overfilling wells with wash buffer, for automated washing. Paper towels or other absorbent materials were used to blot the plate.

4- Each well received 50 µL of substrate solution, followed by 50 µL of substrate solution B for 10 minutes at 37°C in the dark, the plate was incubated and covered with a new sealer.

5- Fifty µL of the stop solution was added to every well.

6-The absorbance of each well was read simultaneously using an ELISA reader with a wavelength of 450 nm.

2.5 Statistical Analysis

• A statistical analysis of data was carried out using SPSS version 24 and Graph Pad prism and version 7.04.

• The statistical significance level was set to less than 0.05.

• Descriptive statistics consist of mean, standard deviation (SD), and standard error of mean (SEM) was calculated separately for each parameter

• The associations between ALT, AST, ALP, TSP, Albumin, Bilirubin with characteristics of both Gal-9 and IL-33 was studied courtesy of Pearson's correlation ($r = -1$ to 1).

• The receiver's operating characteristic (ROC) curve was created to investigate distinctive ability of the levels Gal-9 and IL-33 among healthy individuals and patients with CHB.

3. Results and Discussion

3.1 Anthropometric Analysis

This study shows a statistical analysis of 80 subjects, which included two groups consist of 40 CHB patients and 40 controls, their ages ranged from 20 to 55 years and each group was divided equally into females and males. The obtained results of this study were presented in **appendix II.**

3.1.1 Age

There was no significant statistical difference in mean age (years) between controls and CHB patient (P= 0.7729). The results showed that the mean age of CHB patients (37.4 ± 10.79) was slightly higher than control (36.7 ± 10.83), as shown in table **(3-1)** and **figure (1) in appendix III**

Table (3-1): Mean ± S.D of age in CHB Patients and Controls

Parameter	Healthy controls	CHB patients	p-value
	Mean ± S.D	Mean ± S.D	
Age (Years)	36.7 ± 10.83	37.4 ±10. 79	0.7729

Hepatitis B virus affects both men and women of all ages. [118] In this study, we found non-significant difference in mean age and these results agreed with many previous studies. [92, 120]

3.1.2 Body Mass Index, the Ratio of Waist/Hip, Waist/Thoracic, and Waist/Neck

The mean BMI (kg/m^2) was higher among CHB patients (31.28 ± 9.004) compared to controls (24.94 ± 1.835), BMI has shown significant differences (P< 0.0001), as shown in **table (3-2)** and **figure (2) in appendix III.**

Table (3-2): Mean ± S.D of BMI in CHB Patients and Controls

Parameter	Healthy controls	CHB patients	p-value
	Mean ± S.D	Mean ± S.D	
BMI (Kg/m^2)	24.94 ± 1.835	31.28 ± 9.004	<0.0001

The mean of W/H showed no statistically significant difference in control and CHB patients (P=0.4298), the results are as shown in **table (3-3)** and **figure (3) in appendix III** . W/T has shown significant differences (P= 0.0260), as illustrated in the table **(3-2)** and **figure (4) in appendix III,** W/N (P=0.1365) showed no statistically significant difference in control and CHB patients. The results are as shown in **table (3-3)** and **figure (5) in appendix III.** The results demonstrated that the mean W/H, W/T, W/N of CHB patients (0.9159 ± 0.1403), (0.9276 ± 0.09307), (2.39 ± 0.332), were slightly higher than the control (0.8928 ± 0.1101), (0.8881 ± 0.05466), (2.286 ± 0.2654).

Table (3-3): Mean ± S.D of (Waist / Hip ratio, Waist/ Thoracic ratio, Waist/Neck ratio) in CHB Patients and Controls

Parameter	Healthy controls	CHB patients	p-value
	Mean ± S.D	Mean ± S.D	
W/H	0.8928 ± 0.1101	0.9159 ± 0.1403	0.4298
W/T	0.8881 ± 0.05466	0.9276 ± 0.09307	0.0260
W/N	2.286 ± 0.2654	2.39 ± 0.332	0.1365

Body mass index (which measures overall obesity); and W/H measures abdominal obesity). [121] W/H was calculated as (waist circumference)/ (hip circumference). [122] W/T maybe more related to musculature. [123]

These results are agreed with a previous study that found increased BMI in CHB patients but obesity (BMI< 30 kg/m^2) has been linked to an increased risk of both HCC and liver-related mortality. [71] Obesity may impair an obese person's ability to mount an effective

immune response to an HBV infection due to increased body fat and increase the production of leptin (pro-inflammatory) . [124] And reduction the production of adiponectin (ant- inflammatory) both leptin and adiponectin secreted by white adipose tissue (WAT) adiponectin leads to increased predispose liver to various pathological processes, including inflammation, and fibrosis . [125] Obese patients are more likely to develop serious CHB-related diseases, making them vulnerable to excess morbidity and mortality caused by HBV. [124]

3.1.3 Blood Pressure and Rate of Pulse

There were no statistically significant difference in mean blood pressure (mmHg) in both SBP (P= 0.7703), DBP (P= 0.0543). The level of SBP was increased in control group (121.3 ± 4.965) compared with patients group (120.9 ± 4.931). The level of DBP was increased in CHB patients group (78.91±5.204) compared with healthy control group (76.63 ± 4.923) at same time. The results are as shown in **table (3-4)** and **figures (6, 7) in appendix III** .The ROP (1/min) showed no significant (P= 0.4629), in CHB patients group, the mean pulse rate was (76.11 ± 8.581), while in control group, was (74.5 ± 10.15), as shown in **table (3-4)** and **figure (8) in appendix III**

Table (3-4): Mean ± S.D of Blood Pressure and Rate of Pulses in CHB Patients and Controls

Parameter	Healthy controls	CHB patients	p-value
	Mean ± SD	Mean ± SD	
SBP(mmHg)	121.3 ± 4.965	120.9 ± 4.931	0.7703
DBP(mmHg)	76.63 ± 4.923	78.91 ± 5.204	0.0543
ROP(1/min)	74.5 ± 10.15	76.11 ± 8.581	0.4629

The results of this study are agreement with previous study, which established that HBsAg positivity is not associated with elevated blood pressure. [126]

3.2 Biochemical Investigations (Liver function test)

The statistical analysis in **table (3-5)** shows the examined of liver function, data of this study detect high significant differences in liver enzymes, the serum level of ALT (U/L) in CHB patients was higher than controls with mean for CHB patients (40.35 ± 10.75) and controls (15.53 ± 3.154) with (P >0.0001) for all variables except TSP. The serum levels of AST (U/L), ALP (U/L), T.BIL (mg/dL), D.BIL (mg/dL), IN.BIL (mg/dL), albumin (g/dL), in CHB patients were higher than controls with mean for CHB patients (37.03 ± 12.48), (238.6 ± 63.64), (1.143 ± 0.2836), (0.6125 ± 0.1682), (0.53 ± 0.1911), (5.248 ± 0.6135) respectively, and controls (16.7 ± 1.937), (185.5 ± 48.07), (0.595 ± 0.1709), (0.3275 ± 0.124), (0.2675 ± 0.08883), (4.348 ± 0.3544), with (P < 0.0001). The serum levels of globulins and TSB in controls were higher than CHB patients . There was no significant difference in TSP (P= 0.6843), but high significant differences in globulins (P < 0.0001), as showed in the table **(3-5)** with **figure (9, 10, 11, 12, 13, 14, 15, 16, 17) in appendix III.**

Table (3-5): Mean ± S.D of Biochemical Investigations in CHB patients and Controls

Parameter	Healthy controls	CHB patients	p-value
	Mean ± S.D	Mean ± S.D	
ALT (U/L)	15.53 ± 3.154	40.35 ± 10.75	<0.0001
AST (U/L)	16.7 ± 1.937	37.03 ± 12.48	<0.0001
ALP (U/L)	185.5 ± 48.07	238.6 ± 63.64	<0.0001
T.BIL (mg/dL)	0.595 ± 0.1709	1.143 ± 0.2836	<0.0001
D.BIL (mg/dL)	0.3275 ± 0.124	0.6125 ± 0.1682	<0.0001
IN.BIL(mg/dL)	0.2675 ± 0.08883	0.53 ± 0.1911	<0.0001
TSP (g/dL)	7.105 ± 0.4646	7.03 ± 1.065	0.6843
Albumin (g/dL)	4.348 ± 0.3544	5.248 ± 0.6135	<0.0001
Globulin (g/dL)	2.77 ± 0.3736	1.77 ± 0.7297	<0.0001

Liver function tests are critical for patients with HBV or any other liver disease, tests such as ALP, AST, and bilirubin that indicates the amount or level of enzymes produced by the liver, furthermore, when the level of liver enzymes is high, it could indicate either a damaged or inflamed liver, such findings can aid in identifying the part of the liver that is functionally abnormal. [127]

The high serum level of liver enzyme activity could be attributed to the destruction of liver cells, which resulted in a significant increase in enzymes, an increase in ALT levels is a specific indicator of liver damage, ALT and AST serum levels rise with the progression of liver disease in hepatitis which are most likely due to direct hepatocellular damage and membrane leakage. [128] Previous research has found that patients with normal or mildly elevated ALT are not necessarily free from liver injury or liver-associated mortality.[129] However, that serum ALT reflects the host immunity to viral challenge, virological response (VR) is often accompanied with ALT normalization, suggesting attenuated liver damage. [130] In all cells except hepatic cells, ALT concentrations are found to be lower than AST concentrations; thus, its elevation is particularly related to liver disease.[100] AST is less specific for liver dysfunction, because AST is also present in the heart, skeletal muscle, kidneys, brain, and red blood cells, an increase in ALT serum levels is more specific for liver damage.[131] Increased plasma aminotransferase activity is a sensitive indicator of cytoplasmic and/or mitochondrial membrane damage. Liver cells contain more AST than ALT, but ALT is confined to the cytoplasm and has a higher concentration than AST, leakage of cytoplasmic contents which causes a relatively greater increase in plasma ALT than AST activities in inflammatory or infectious conditions, such as viral hepatitis, to chronic persistent hepatitis, or to

early chronic active hepatitis, however raised plasma AST activity higher than ALT may be due to cirrhosis or severe chronic active hepatitis. [132]

High levels of ALP activity may play a role in a variety of parenchymal liver disorders, including hepatitis. [133] ALP is a well-known biomarker for a variety of diseases. [106] ALP is also produced by organs other than the liver, including the bone, placenta, kidneys, and intestine. [134] The results of this study compatible with previous study, which showed increased activity of liver enzyme (AST, ALT, ALP) and bilirubin in CHB patients than controls, but the values of TSP parameters were not significantly different. [133] These finding are agreed with a study in 2018 found that patients with viral hepatitis had significantly higher serum levels of ALT, AST, and ALP concentrations than controls. [107]

Bilirubin can be used as a biomarker of liver function because it rises with the severity of the disease. [134] Hyperbilirubinemia can be cleary seen in HBV which is directly of this abnormality could be attributed decreased hepatic clearance due to HBV, serum bilirubin is considered a true test of liver function, as it reflects the liver's ability to process and secrete bilirubin into bile. [135] The most abundant compounds in serum are thought to be total protein, it play important roles in enzymes, hormones, and antibodies, as well as osmotic pressure balance, the secondary cause of decreased serum total protein is decreased protein synthesis by the liver abnormal TSP and albumin levels are signs of liver dysfunction. [136]

Albumin is a protein that is only produced in the liver, it serves as a biomarker for liver synthetic function and health, because albumin levels can be reduced by a variety of illnesses, it has a low specificity as a marker of liver function. [134] More cells are destroyed

as the disease progresses, and serum AST activity may equal or exceed ALT activity; mild jaundice may develop, the plasma albumin concentration falls when there is significant hepatocellular destruction. [132] Albumin and globulin are the two major components of serum proteins. [137] Because these proteins are found in hepatocytes, they have the potential to release into the bloodstream when the hepatocytes are damaged. [23]

3.3 Serum Levels of Galectin-9

Serum levels of Gal-9 (**pg/mL**) was higher in CHB patients (618.6± 171.1) than controls (136.3± 35.48) with high significant differences ($p < 0.0001$), as illustrated in the **table (3-6)** with **figure (18) in appendix III.**

Table (3-6): Mean ± S.D of Galectine-9 CHB Patient and Controls

Parameter	Healthy controls	CHB patients	p-value
	Mean ± S.D	Mean ± S.D	
Gal-9 (pg/mL)	136.3±35.48	618.6± 171.1	<0.0001

Galectin-9 contains two distinct carbohydrate recognition domains connected by a linker peptide and is a natural ligand for the T cell immunoglobulin domain and Tim-3.[138]

In this study CHB patients showed significantly higher levels of Gal-9 than controls, the results of this study are compatible with reported that Gal-9 levels in HBV-infected liver biopsies are high and serum levels of this protein are significantly higher than controls. [88] There was an increase in circulating Gal-9 in CHB patients, which correlated with disease severity. [139]

The interaction of Gal-9 with T-cell immunoglobulin and Tim-3 ligand (Gal-9/Tim-3) can cause specific inhibition of T helper cells, specifically Th1 and Th17 immune responses. [140] In chronic viral

infection, TIM-3 appears to play a critical negative regulatory role in T cells.)87)

Tim-3 is significantly increased in CD8+T-cells in acute HBV infection, CD8+ T-cells are primarily involved in virus clearance by producing cytokines interferon (IFN)-γ and TNF-α and this is associated with the exhaustion of CD8+ T-cells exhibit exhausted phenotype suffering from loss of function in CHB patients. (63) Gal-9/Tim-3 limits liver damage or inhibits antiviral immune cell response, because it was found to correlate with the upregulation of memory tregs (mTregs), that can limit ongoing liver damage during the inflammatory phase of CHB and signaling pathway was found to regulate T cell dysfunctions in patients with HBV associated HCC resulting in T cell exhaustion in CHB. (140)

3.4 Serum Levels of Interleukin-33

Serum concentration of IL-33 (**ng/mL**) in CHB patients and controls were presented in **table (3-7)** and **(figure 19) in appendix III**. Serum level of IL-33 was significantly increased in CHB patients compared to controls (404.3 ±148) versus (59.84 ± 13.57), with P<0.001.

Table (3-7): Mean ± S.D of IL-33 CHB Patient and Controls

Parameter	Healthy controls	CHB patients	p-value
	Mean ± S.D	Mean ± S.D	
IL-33 (ng/mL)	59.84 ± 13.57	404.3 ± 148	<0.0001

Interleukins are a type of cytokine, which are diverse group of small soluble proteins, and play critical roles in the physiological and pathological processes of chronic viral hepatitis. (141) Changes in various interleukins activities that occur during the inflammatory response to these viruses cause varying degrees of liver impairment or destruction, previous study suggested that IL-33 may increase the

expression and can be associated with the development of HBV/HCV-related liver fibrosis. [142] In this study, CHB patients showed significantly higher levels of IL-33 than controls, results of this study are in agreement with previous study by Shu-Ling Huan and others which determine serum IL-33 and ST2 levels in the natural course of CHB infection, these findings confirmed that IL-33 may play an important role in the progression of CHB, and the data indicated that IL-33 may be a pathogenic factor in the pathogenic process of CHB patients. [92] Another study mentioned the role of IL-33/ST2 that has been studied during HBV and HCV infections in humans, they found the serum level of IL-33 in CHB patient was significantly higher than in healthy people. [94] This suggests the acute and massive liver damage happens as follow the release of IL-33 by injured hepatocytes may serve as a protective mechanism, whereas in chronic injury, IL-33 acts as a hepatic fibrosis-promoting factor. [143] Another study found that concentrations of serum IL-33 are significantly higher in HBV infected patients than healthy control and are significantly decreased after 12 weeks of treatment, this suggests that IL-33 may be a pathogenic factor in HBV-related liver injury in CHB patients. [144] Although IL-33 stimulates both Th1 and Th2 cytokines, it suppresses TNF-expression in the liver via direct effects and nuocyte activation, thus, in acute viral hepatitis, IL-33 acts as a potent immune stimulator and a hepatoprotective cytokine, and it could be a potentially promising therapeutic candidate for viral hepatitis management. [145]

3.5 Pearson Correlation Analysis

3.5.1 Correlation of Galectin-9 with Studied Parameters.

The correlations between Gal-9 and variables were investigated in this study. The results are presented in a **table (3-8)** and **figure (3-1)** as shown a very strong positive correlation was indicated between serum levels of Gal-9 with IL-33 ($r = 0.919$, $p< 0.001$) as shown in **table (3-8)**, **figure (3-1 A),** also the study found strong positive correlation between Gal-9 and serum levels of T.BIL ($r = 0.731$, $p< 0.001$) shown in **table (3-8)**, **figure (3-1 B),** ALT ($r = 0.725$, $p< 0.001$) as shown in **table (3-8)**, **figure (3-1 C),** the moderately strong positive correlation of Gal-9 and serum levels of AST ($r= 0.683$, $p< 0.001$) was detected as shown in **table (3.8)** with **figure (3-1 D)**, also moderately strong positive correlation of D.BIL, IN.BIL, and Albumin ($r= 0.656$, $P< 0.001$), ($r = 0.650$, $P< 0.001$) ($r = 0.581$, $p< 0.001$) were observed respectively as shown in **table (3.8)** (**figure (3-1 , E, F,G),** also the results in the **table (3-8)** showed a weak positive correlation of GaL-9 with ALP serum levels ($r= 0.468$, $P <0.001$), as shown in **figure (3-1 H)**, well observed with BMI ($r= 0.323$, $P= 0.005$) as shown in (**table 3-8), (figure 3-1 I**), but negative correlation of GaL-9 observed with serum levels of Globulins ($r= -0.563$, $P < 0.001$) as shown in **table (3-8)** and **figure (3-1 J)**.

While no significant correlation was observed between Gal-9 and DBP ($r= 0.165$, $P= 0.157$), W/N ($r= 0.114$, $P=0.331$), ROP($r= 0.094$, $P= 0.422$), W/T ($r= 0.085$, $P= 0.466$), SBP ($r= 0.030$, $P= 0.798$), Age ($r= 0.019$, $P=0.867$) , W/H ($r= -0.012$, $P= 0.921$), TSP ($r= -0.038$, $P= 0.739$).

Table (3-8): The Correlation of Galectin9 with the Studied Parameters

Gal-9 (pg/mL)	r	p-value
IL-33 (ng/mL)	0.919	<0.001
ALT (U/L)	0.725	<0.001
AST (U/L)	0.683	<0.001
ALP (U/L)	0.468	<0.001
T.BIL (mg/dL)	0.731	<0.001
D.BIL (mg/dL)	0.656	<0.001
IN.BIL (mg/dL)	0.650	<0.001
TSP (g/dL)	-0.038	0.739
Albumin (g/dL)	0.581	<0.001
Globulins (g/dL)	-0.563	<0.001
Age Years	0.019	0.867
BMI kg/m2	0.323	0.005
W/H	-0.012	0.925
W/T	0.085	0.466
W/N	0.114	0.331
SBP mmHg	0.030	0.798
DBP mmHg	0.165	0.157
ROP 1/Min	0.094	0.422

Galectin-9 is an immune suppressor that controls hepatitis, it has been reported that the transition from acute to chronic hepatitis is frequently accompanied by an increase in the proportion of regulatory T cells and a decrease in the proportion of effector T cells. [146] Regulatory T cells were found to be more active during the inflammatory phase of HBV infection, and they were linked to higher levels of serum ALT and Gal-9. [147]

This is consistent with the result of study, figure (3-1 A to J) showed The correlation between Gal-9 with studied parameters.

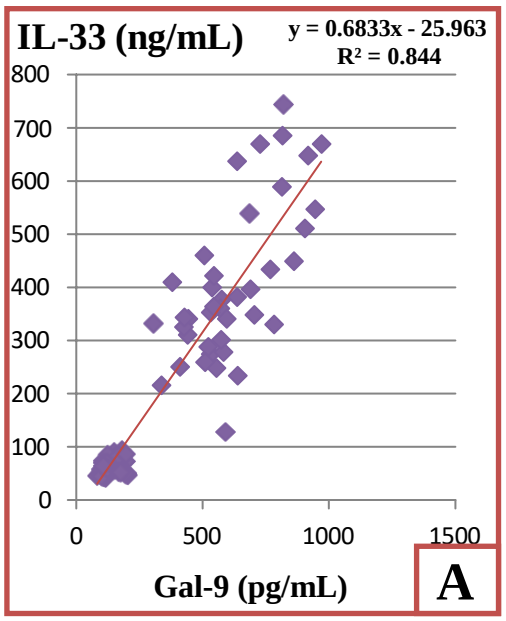


Figure (3-1 A): Correlation of Gal-9 with IL-33

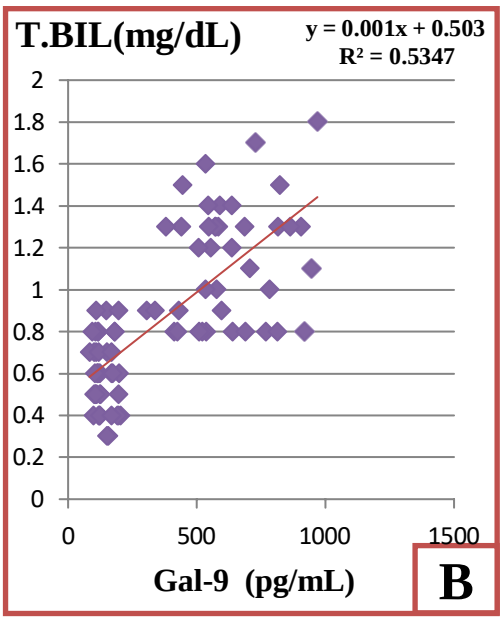


Figure (3-1 B): Correlation of Gal-9 with T.BIL

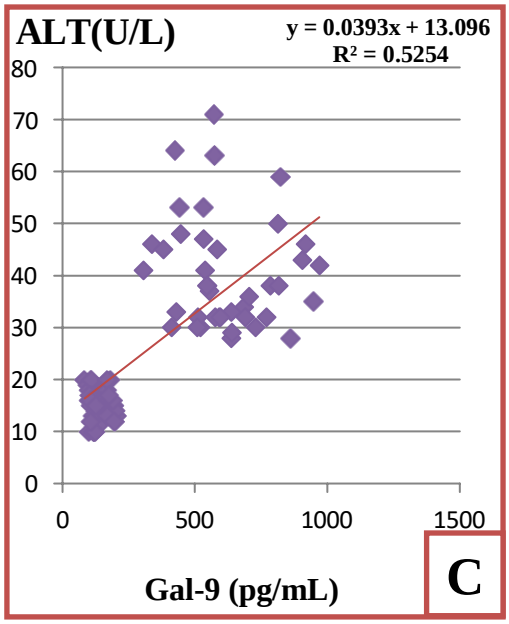


Figure (3-1 C): Correlation of Gal-9 with ALT

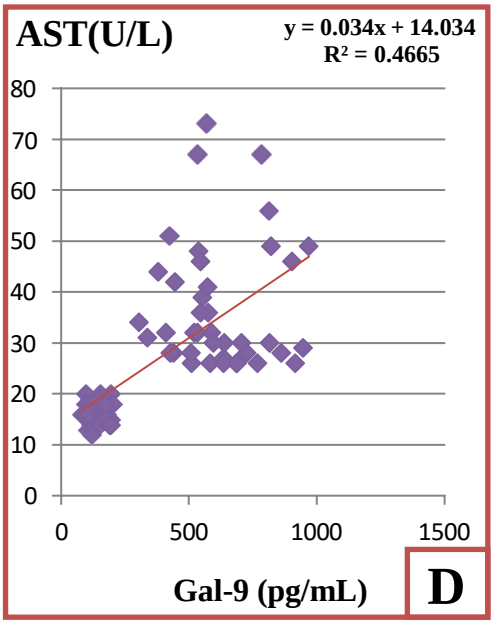


Figure (3-1D): Correlation of Gal-9 with AST

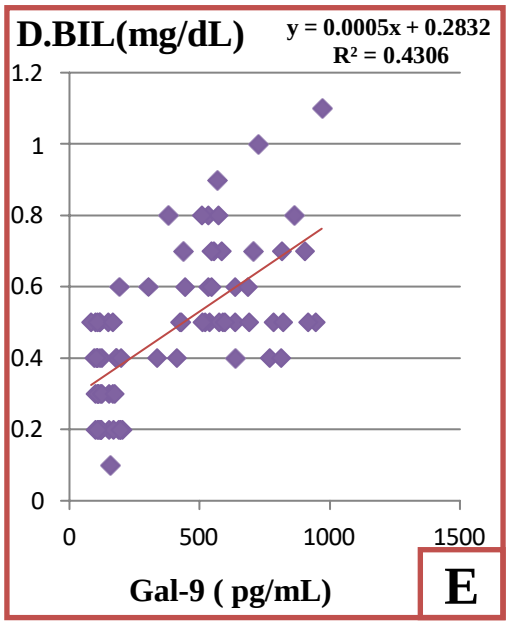


Figure (3-1 E): Correlation of Gal-9 with D.BIL

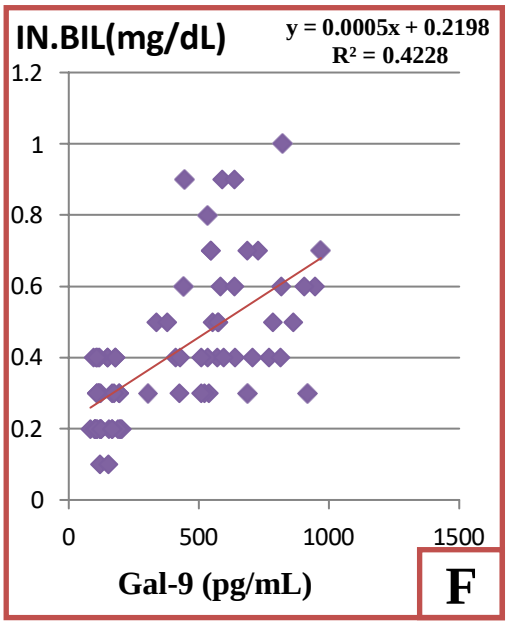


Figure (3-1 F): Correlation of Gal-9 with IN.BIL

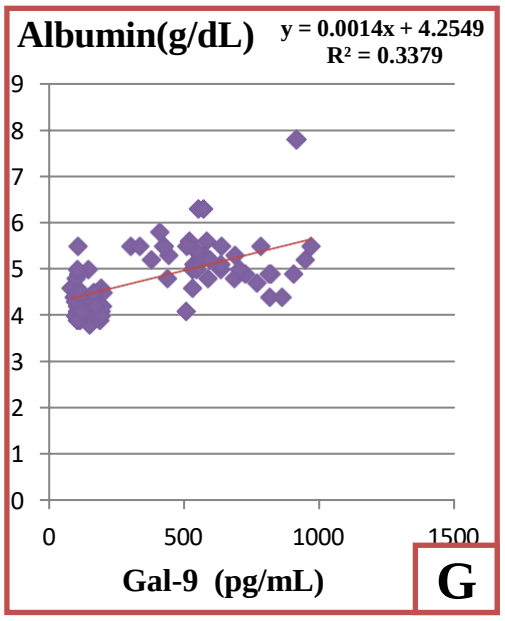
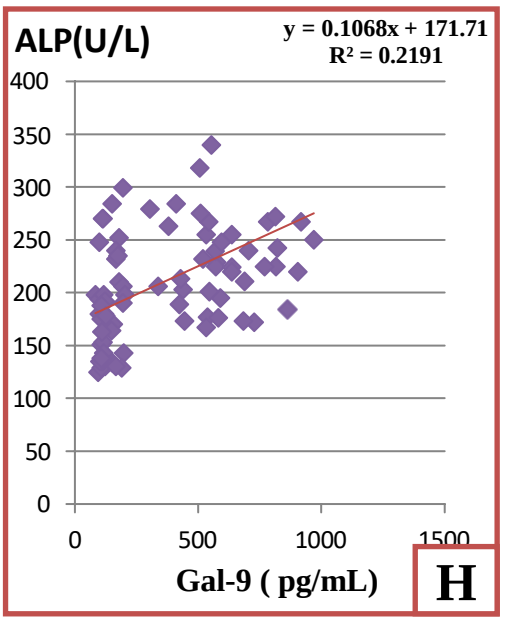


Figure (3-1 G): Correlation of Gal-9 with Albumin



Figure(3-1H):Correlation of Gal-9 with ALP

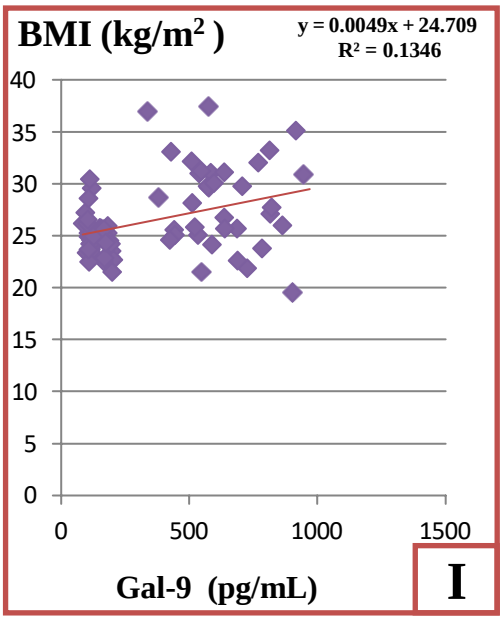


Figure (3-1 I): Correlation of Gal-9 with BMI

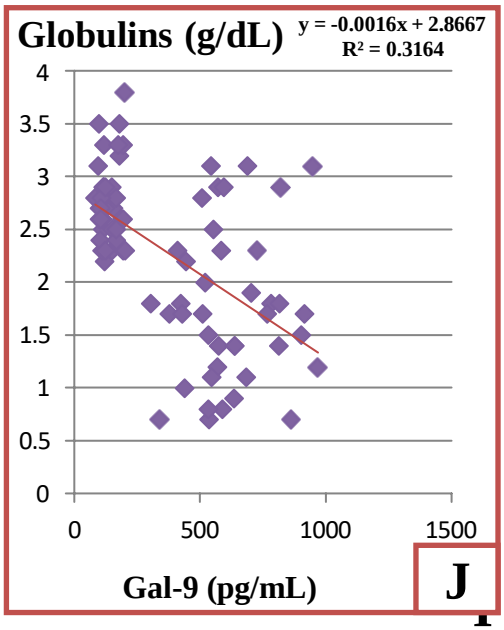


Figure (3-1 J): Correlation of Gal-9 with Globulins

3.5.2 Correlation of Interleukin-33 with Studied Parameters

Analysis serum levels of IL-33 was indicated a very strong positive correlation of IL-33 with GaL-9 (r = 0.919, p< 0.001) as shown in **table (3.9) and figure (3.2 A),** also strong positive correlation of IL-33 with serum levels of T.BIL (r = 0.743, p< 0.001) as shown in **table (3.9)** and **figure (3.2 B)** and ALT (r = 0.729, p< 0.001),as seen in **table (3.9)** and **figure (3.2 C).** A moderately strong positive correlation of IL-33 with serum levels of D.BIL, IN.BIL, AST, Albumin, (r=0.680, P<0.001), (r =0.647, P<0.001) (r =0.644, p<0.001), (r=0.530, P<0.001) as demonstrated in **figures (3.2 D, E, F and G)** respectively, while there is a weak positive correlation between IL-33 with serum levels of ALP (r =0.426, p<0.001) as demonstrated in **table (3.9)** and **figure (3.2 H),** BMI (r=0.254, P= 0.028) as shown in **table (3.9)** and **figure (3.2 I)**.

From **figure (3.2 J)** and **table(3.9)** we noticed that serum levels of IL-33 showed a negative correlation with serum levels of globulins (r=-0.513, P< 0.001) .

Present study of other studied parameter have not shown significant correlation with IL-33 which gave the following results DBP (r=0.182, P=0.119), W/N(r=0.0170, P=0.885), ROP(r=0.103, P=0.379), Age (r=0.048, P=0.675), W/T (r=0.017, P=0.885) ,TSP (r= -0.038, P=0.739), SBP (r= -0.045, P=0.704), W/H (r= -0.081, P=0.492), respectively.

Table (3-9): Correlation of IL-33 with Studied Parameters

IL-33 (ng/mL)	**r**	**p-value**
Gal-9 (pg/mL)	0.919	<0.001
ALT (U/L)	0.729	<0.001
AST (U/L)	0.644	<0.001
ALP (U/L)	0.426	<0.001
T.BIL (mg/dL)	0.743	<0.001
D.BIL (mg/dL)	0.680	<0.001
IN.BIL (mg/dL)	0.647	<0.001
TSP (g/dL)	-0.035	0.755
Albumin (g/dL)	0.530	<0.001
Globulins (g/dL)	-0.513	<0.001
Age Years	0.048	0.675
BMI kg/m2	0.254	0.028
W/H	-0.081	0.492
W/T	0.017	0.885
W/N	0.170	0.885
SBP mmHg	-0.045	0.704
DBP mmHg	0.182	0.119
ROP 1/Min	0.103	0.379

Interleukin-33, a member of the IL-1 cytokine family, is controlled by different immune cells and acts as a warning signal to the immune system after epithelial or endothelial cell damage during cell necrosis, infection, stress, and trauma.[148] A previous study showed that the serum IL-33 and ST2 levels were elevated as serum ALT concentrations increased in CHB patients compared to HBV carriers, healthy control, and CHB patients with low ALT levels. [95] This is consistent with the our findings.

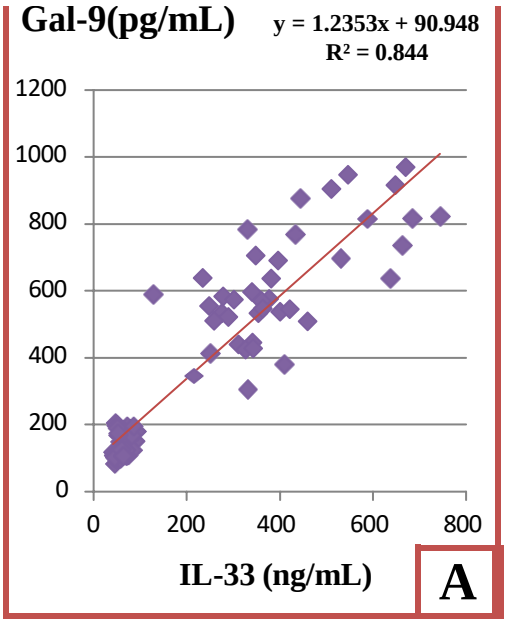


Figure (3-2 A): Correlation of IL-33 with Gal-9

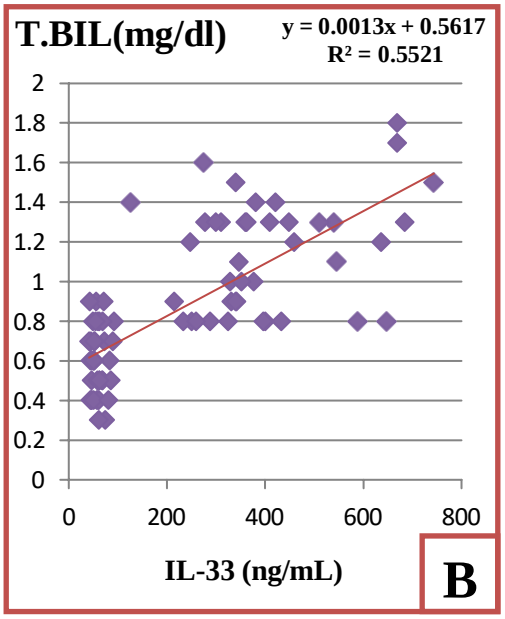


Figure (3-2 B): Correlation of IL-33 with T.BIL

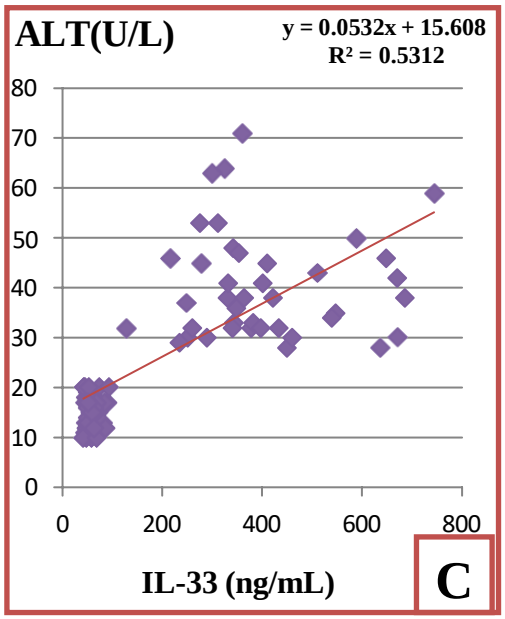


Figure (3-2 C): Correlation of IL-33 with ALT

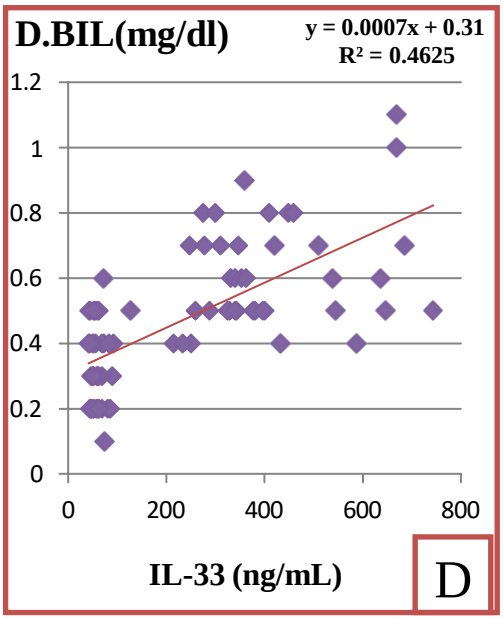


Figure (3-2 D): Correlation of IL-33 with D.BIL

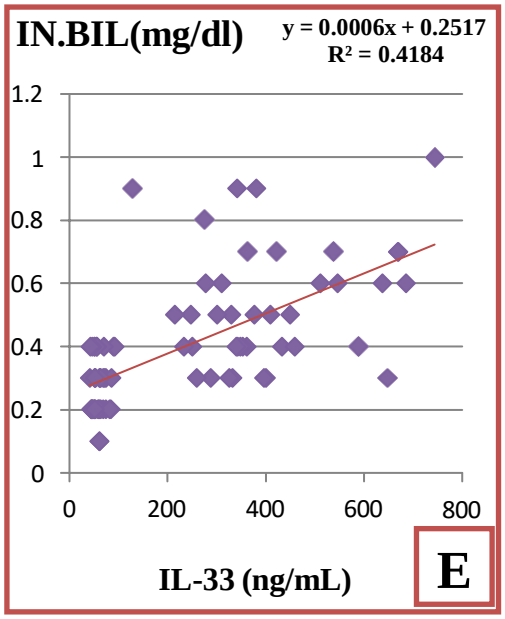
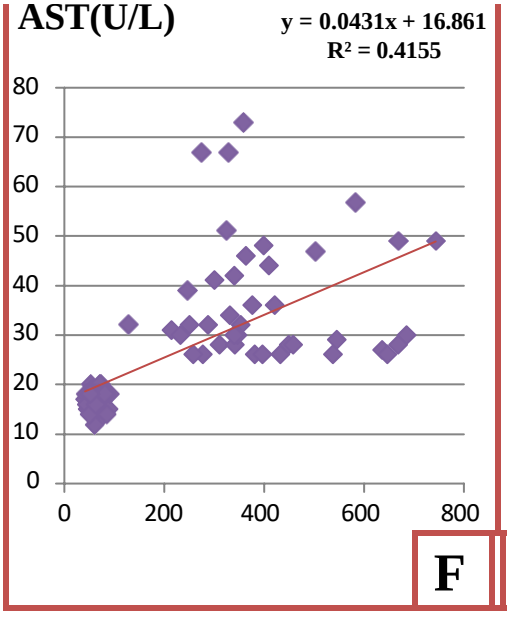


Figure (3-2 E): Correlation of IL-33 with IN.BIL



Figure(3-2 F):Correlation of IL-33 with AST

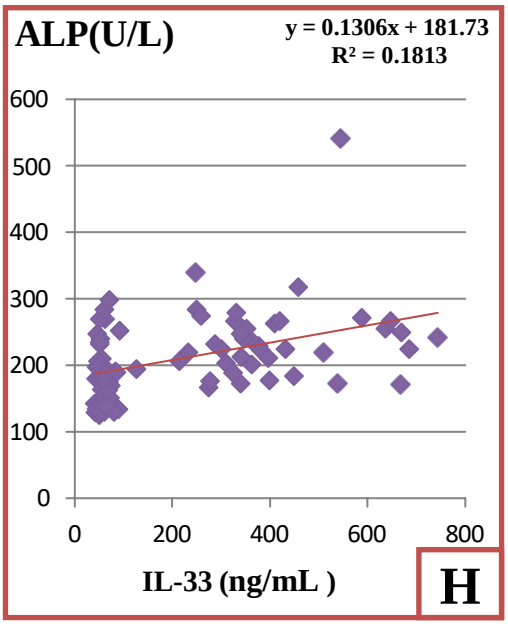
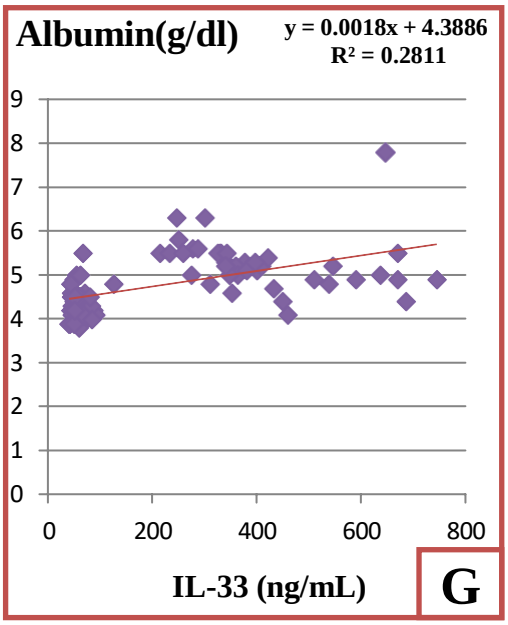


Figure (3-2 H): Correlation of IL-33 with ALP



Figure(3-2 G): Correlation of IL-33 with Albumin

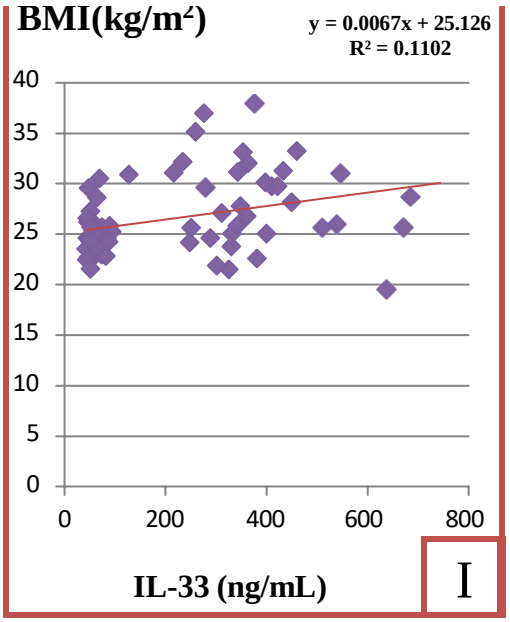


Figure (3-2 I): Correlation of IL-33 with BMI

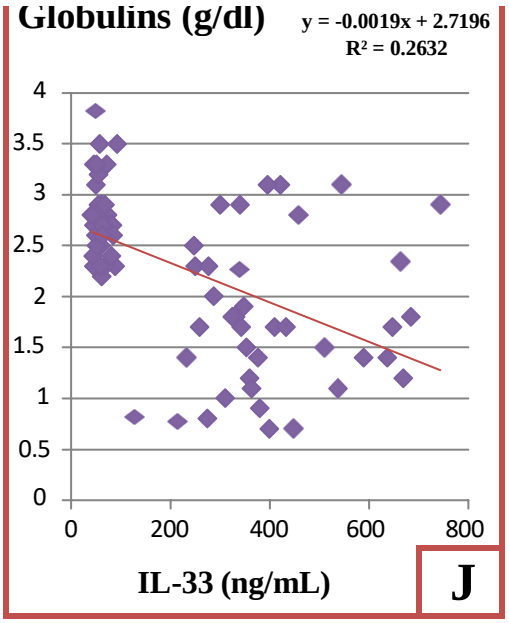


Figure (3-2 J): Correlation of IL-33 with Globulins

3.6 Receiver Operating Characteristic Curve Analysis

The receiver operating characteristic (ROC) curve is a popular graphical method used to investigate ability of continuous variables (markers) to correctly classifed subjects into one of two groups. [149]

The Area under curve (AUC) is an effective measure of sensitivity and specificity because it summarizes the entire ROC curve rather than relying on individual values. [150] It accepts values between [0.5, 1], and the closer to 1 the better the accuracy. [149] The ROC curve evaluation for the outcomes was obtained from the experiments and assessments were carried out within the scope of the current research, the details are provided in **table (3-10)** and **figure (3-3).**

Table (3-10): Area Under the ROC Curve for All Analyzed Biomarkers

Parameter	AUC	Std. Error	95% confidence interval	p-value
Age years	0.5138	0.06511	0.3861 to 0.6414	0.8323
BMI kg/m^2	0.7894	0.05407	0.6834 to 0.8954	<0.0001
W/H	0.6294	0.07002	0.4922 to 0.7666	0.0563
W/T	0.7079	0.06204	0.5863 to 0.8295	0.0020
W/N	0.5668	0.06921	0.4311 to 0.7024	0.3207
SBP (mmHg)	0.5232	0.0673	0.3913 to 0.6551	0.7300
DBP (mmHg)	0.6218	0.06522	0.494 to 0.7496	0.0702
POR (1/Min)	0.5614	0.06657	0.4309 to 0.6919	0.3611
ALT (U/L)	1	0	1 to 1	<0.0001
AST (U/L)	1	0	1 to 1	<0.0001
ALP (U/L)	0.7678	0.05365	0.6627 to 0.873	<0.0001
T.BIL (mg/dL)	0.9588	0.01836	0.9228 to 0.9947	<0.0001
D.BIL (mg/dL)	0.9197	0.02853	0.8638 to 0.9756	<0.0001
IN.BIL (mg/dL)	0.9084	0.0304	0.8489 to 0.968	<0.0001
TSP (mg/dL)	0.5644	0.06815	0.4308 to 0.6979	0.3216
Albumine (mg/dL)	0.9222	0.03131	0.8608 to 0.9836	<0.0001
Globuline (mg/dL)	0.8559	0.04692	0.764 to 0.9479	<0.0001

Gal-9 (pg/mL)	1	0	1 to 1	<0.0001
IL-33 (ng/mL)	1	0	1 to 1	<0.0001

All of the parameters tested had some relevance for foretelling CHB, when the test value is greater than the table value (0.5), the speculation AUC end result is significant

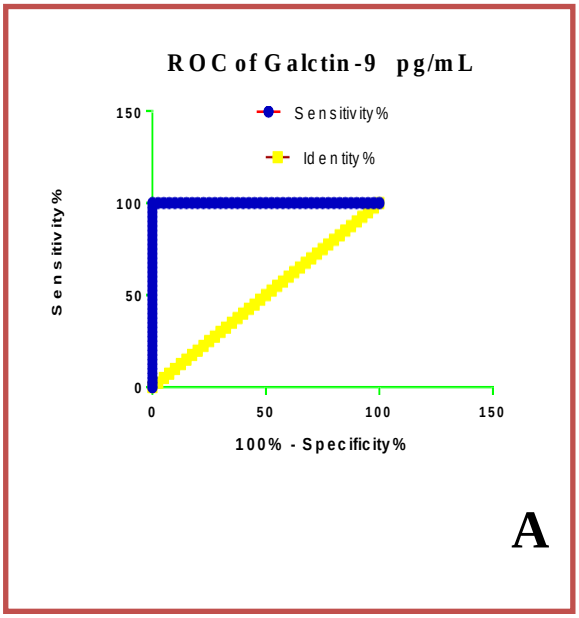
In this regard considering the AUC values collectively with the various parameters listed in **table (3-10)**, it is possible to make the following assessment for parameters, it was divided into five groups in descending order, the first group of which included the values that yielded the result [AUS=1], which represents an ideal value for diagnosing the disease, the ROC curve revealed that the serum Gal-9, IL-33, ALT, AST, of the standards with the highest validity and provides an perfect test with an excellent and fantastic method for discriminating' between healthy control and CHB patients (AUS=1; P <0.0001; 95% confidence interval (CI): 1 to 1 and Std. Error (SE) :0) **(figure 3-3 A, B, C,D)** as a result, it is possible to state that the serum GAL-9, IL-33, ALT, AST, are definitely functional for the diagnosis of CHB disease.

While the parameters in the second group, which gave a higher value of 0.9 , which represents a very strong value in diagnosing the disease were included(T.BIL [AUC = 0.9588; P < 0.0001; 95% CI: 0.9228 to 0.9947 and SE: 0.01836] **(figure 3-3 E),** and also we found Albumin [AUC =0.9222; P < 0.0001; 95% CI: 0.8608 to 0.9836 and SE: 0.03131] (**figure 3-3 F**), as well D.BIL [AUC = 0.9197; P = < 0.0001; 95% CI: 0.8638 to 0.9756 and SE: 0.02853] (**figure 3-3 G**) and IN.BIL [AUC = 0.9084; P = < 0.0001; 95% CI: 0.8489 to 0.968 and SE: 0.0304] (**figure 3-3 H**), respectively.

The third group in which [AUC value from (0.8-0.9) were evaluated and which represent the strong variables in disease diagnosis or prediction which was represented by globulins [AUC = 0.8559; P ≤ 0.0001; 95% CI: 0.764 to 0.9479 and SE: 0.04692] **(figure 3.3 I)**.

The fourth group in which [AUC value from (0.7-0.8) were evaluated and which represent the medium strength in diagnosing and detecting disease, this group includes BMI [AUC =0.7894; P <0.0001; 95% CI: 0.6834 to 0.8954 and SE: 0.06511] (**figure 3-3 J**), moreover, ALP [AUC = 0.7678; P ≤ 0.0001; 95% CI: 0.6627 to 0.873 and SE: 0.05365] (**figure 3.3 K**),and W/T [AUC =0.7079; P 0.0020; 95% CI:0.5863 to 0.8295 and SE: 0.06204] (**figure 3.3 L**).

Finally, Fifth group [AUC value from (0.5-0.7) showed low validity in predicting validity this group includes W/H, DBP, W/N, TSP, ROP, SBP, and age [AUC = 0.6294; P 0.0563; 95% CI:0.4922 to 0.7666 and SE: 0.07002] (**figure 3.3 M**) [AUC =0.6218; P= 0.0702; 95% CI: 0.494 to 0.7496 and SE: 0.06522] (**figure 3.3 N**), [AUC =0.5668; P=3207 ; 95% CI: 0.4311 to 0.7024 and SE: 0.06921] (**figure 3.3 O**), [AUC =0.5644; P= 0.3216; 95% CI: 0.4308 to 0.6979 and SE: 0.06815] (**figure 3.3 P**), [AUC =0.5614; P= 0.3611; 95% CI: 0.4309 to 0.6919 and SE: 0.06657] (**figure 3.3 Q**), [AUC =0.5232; P= 0.7300; 95% CI: 0.3913 to 0.6551 and SE: 0.0673] (**figure 3.3 R**), [AUC =0.5138; P= 0.8323; 95% CI: 0.3861 to 0.6414 and SE: 0.06511] (**figure 3.3 S**) respectively.


ROC of Galctin-9 pg/mL
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
0
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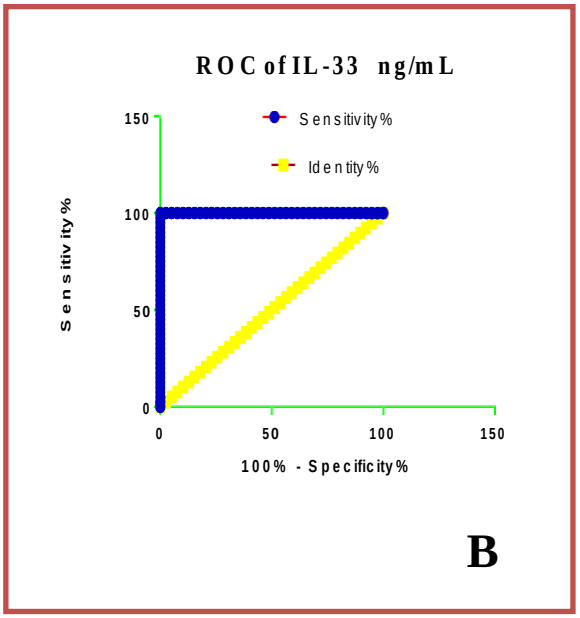
A


ROC of IL-33 ng/mL
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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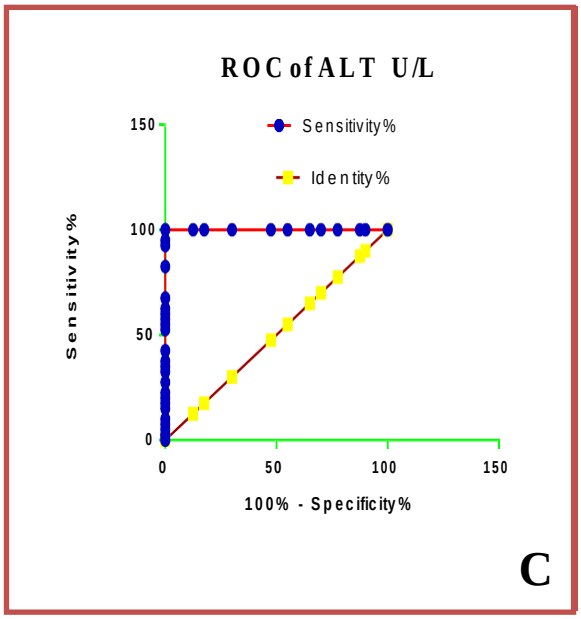
B


ROC of ALT U/L
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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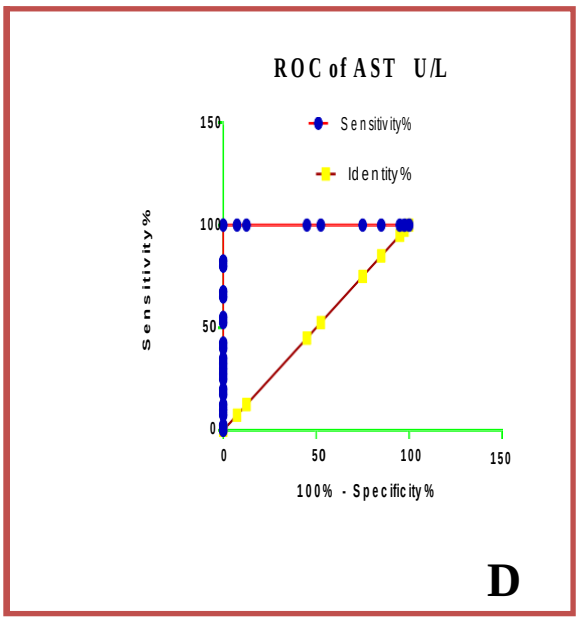
C


ROC of AST U/L
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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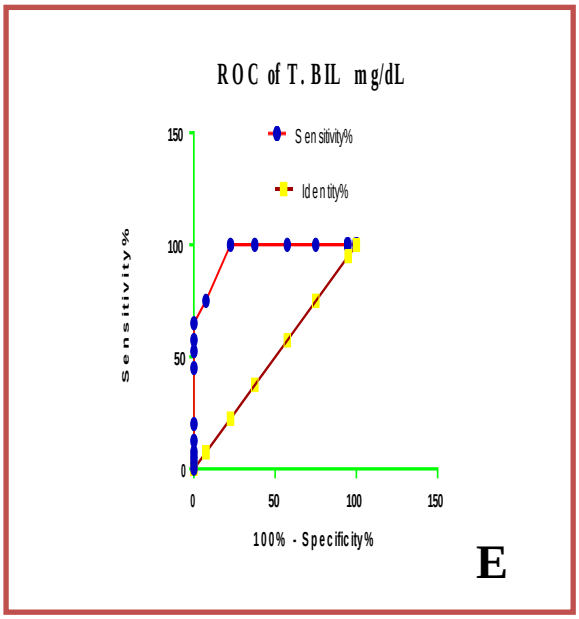
D


ROC of T.BIL mg/dL
Sensitivity%
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100% - Specificity%
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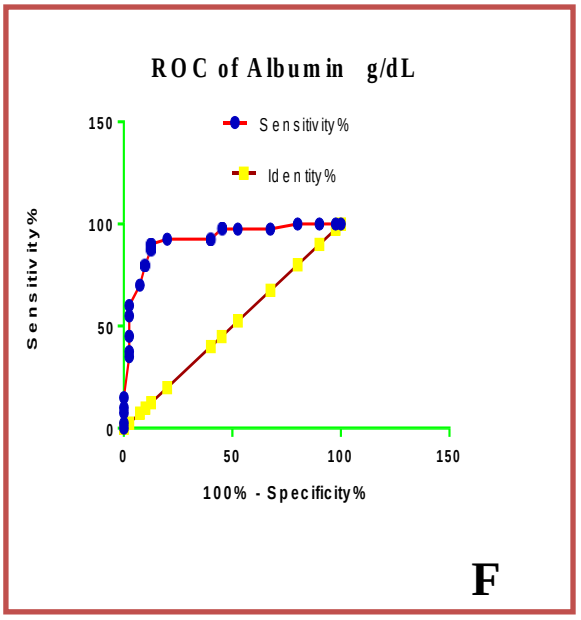
E


ROC of Albumin g/dL
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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100
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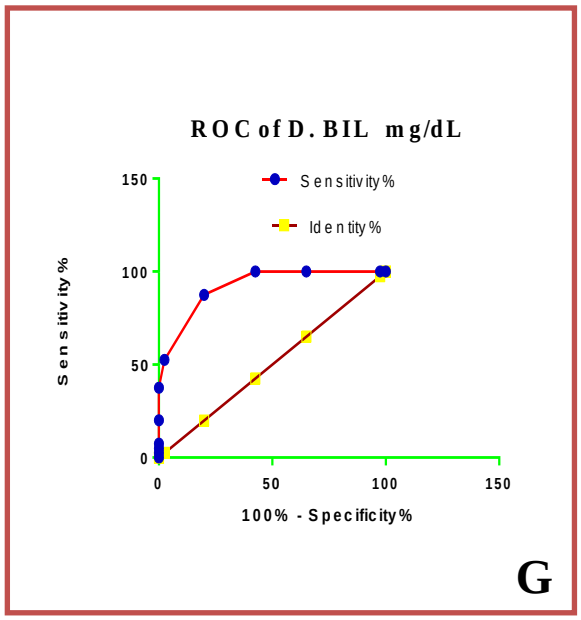
F


ROC of D. BIL mg/dL
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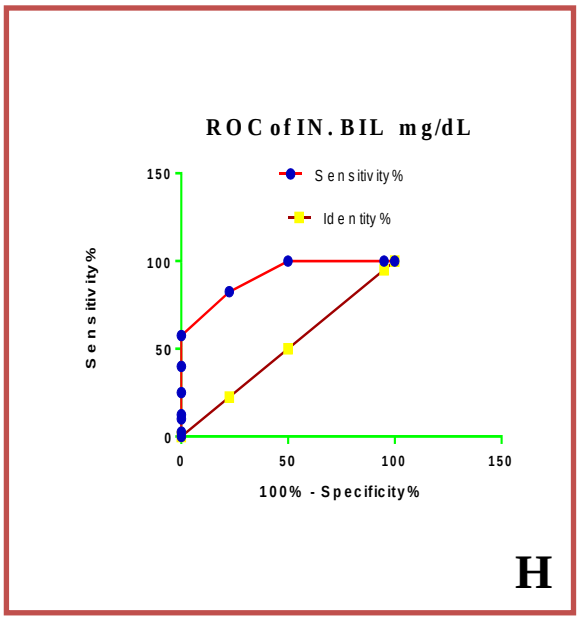
ROC of IN. BIL mg/dL
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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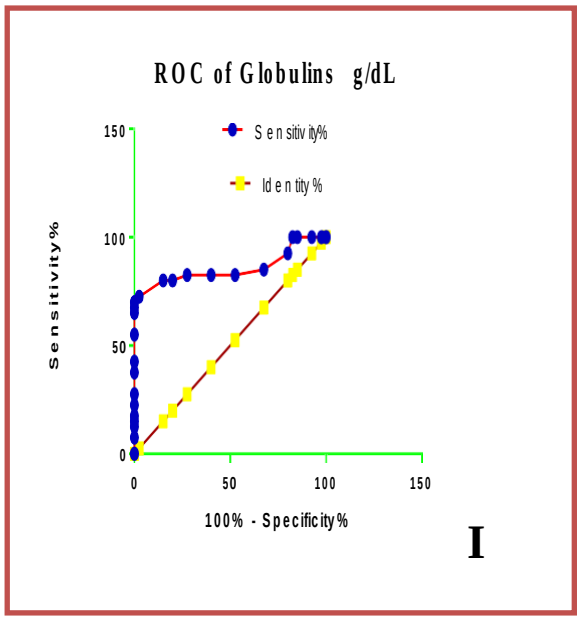
ROC of Globulins g/dL
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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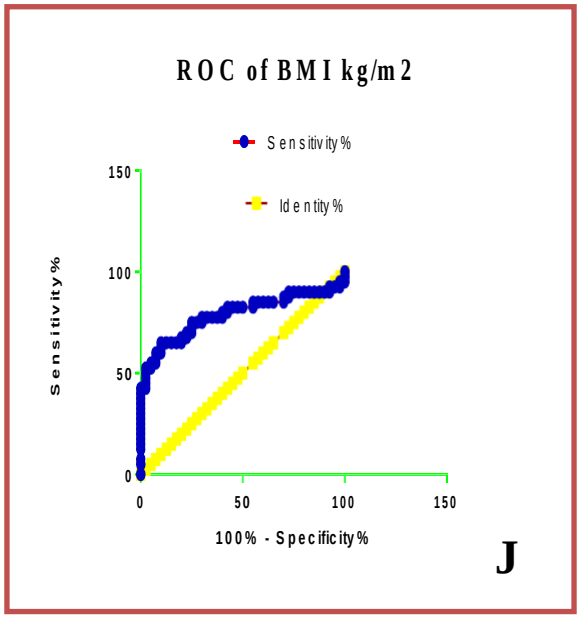
I


ROC of BMI kg/m2
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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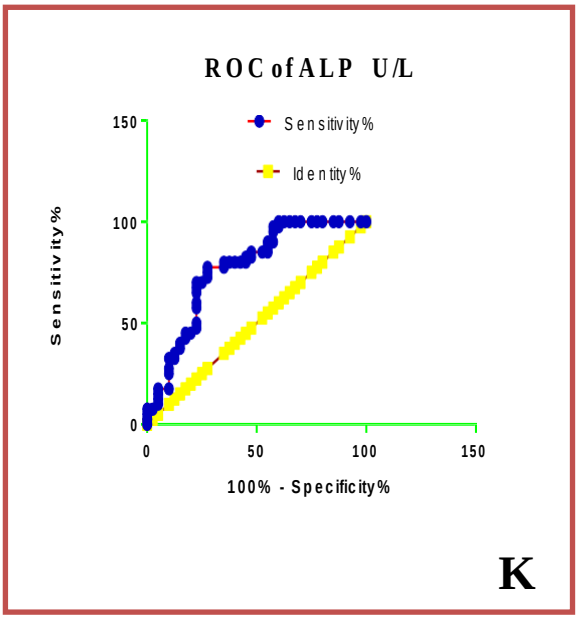
J


ROC of ALP U/L
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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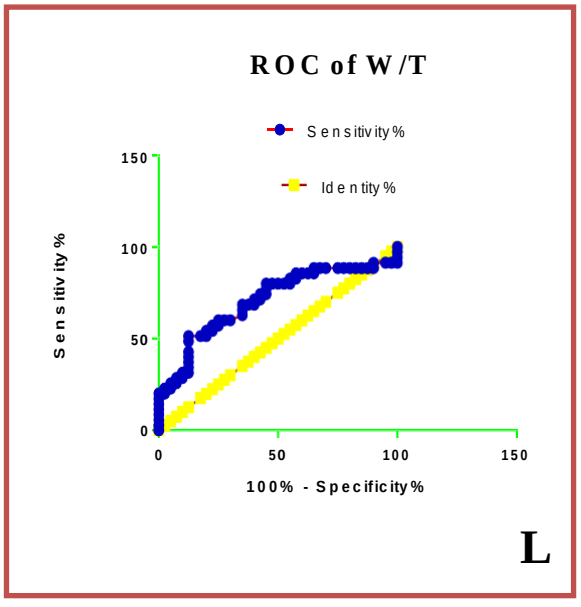
K


ROC of W/T
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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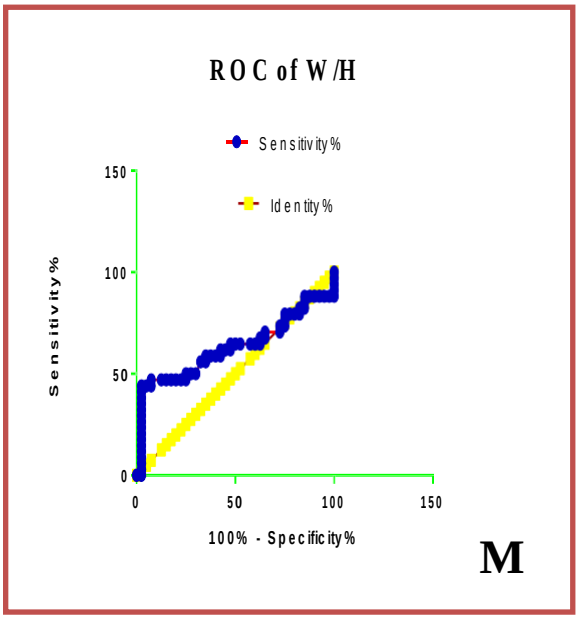
ROC of W/H
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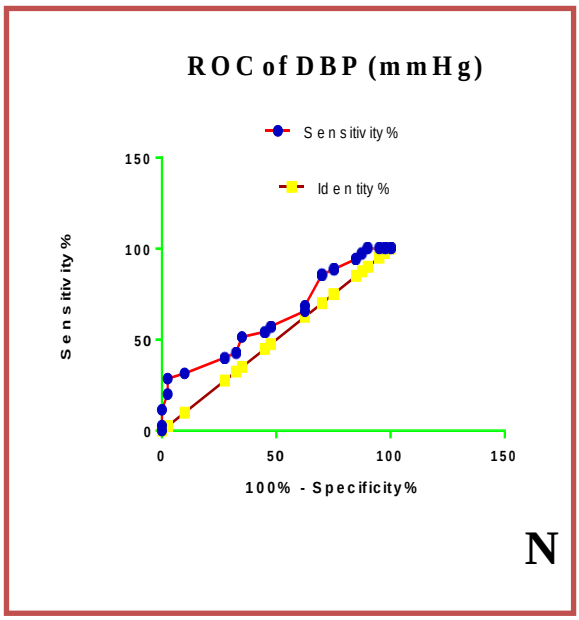
ROC of DBP (mmHg)
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
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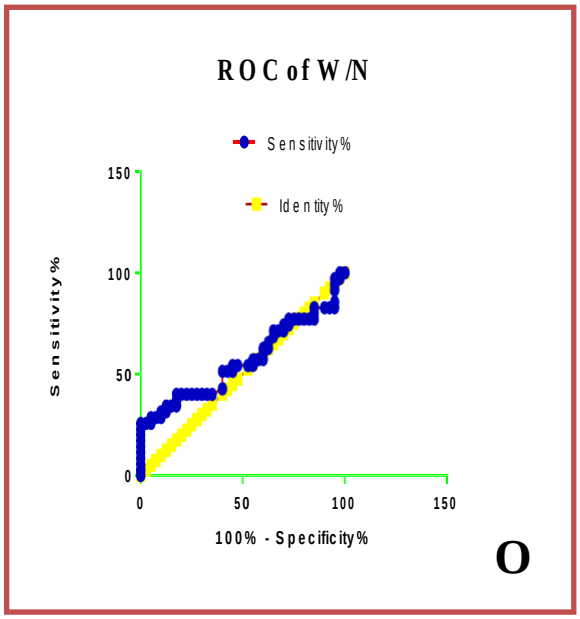
N


ROC of W/N
Sensitivity%
Identity%
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100% - Specificity%
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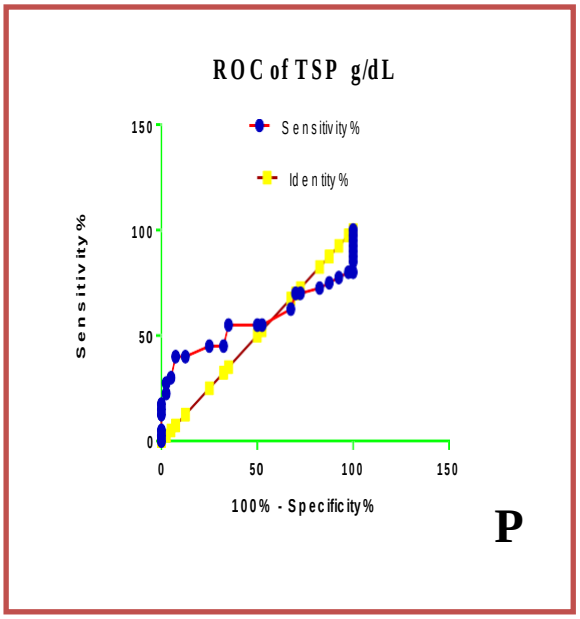
ROC of TSP g/dL
Sensitivity%
Identity%
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100% - Specificity%
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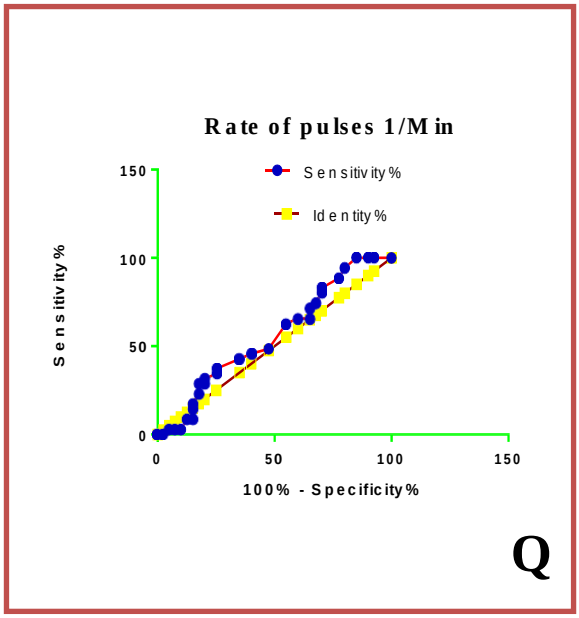
Rate of pulses 1/Min
Sensitivity%
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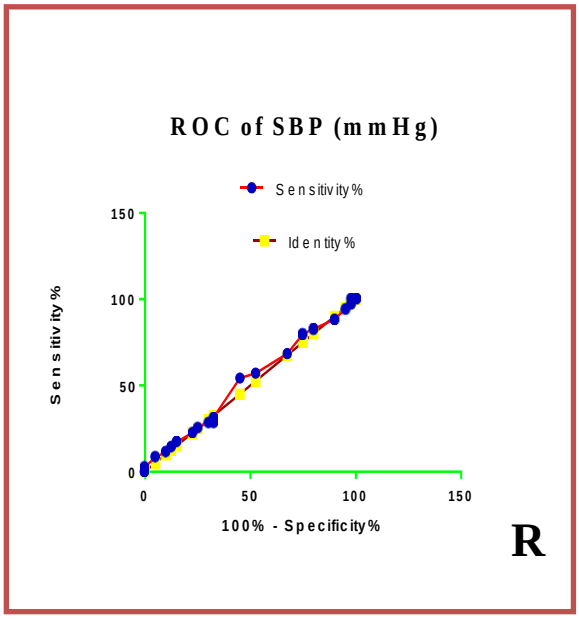
ROC of SBP (mmHg)
Sensitivity%
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Sensitivity%
100% - Specificity%
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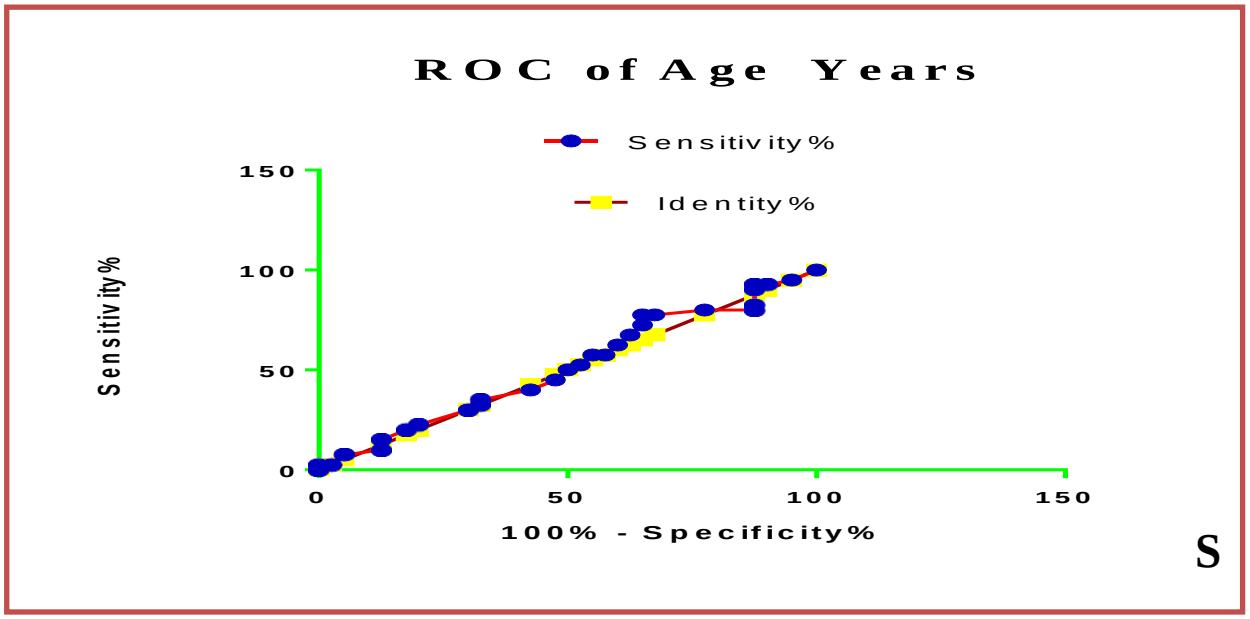

Figure (3.3) (A-S): The Receiver Operating Characteristic Curves Showing AUC between Sensitivity and Specificity for Studied Parameters.

Conclusions

Based on the current findings, this study concludes:

1. There was a very strong positive correlation between serum levels of Gal-9 with IL-33 in CHB patients, therefore, these parameters may be used as biomarkers for risk extrapolation for CHB disease.
2. Serum studied biomarker levels could provide additional information about the risk factors of developing CHB patients.
3. Our data support the postulate that high levels of Gal-9 and IL-33 is concomitant increasing of CHB in Iraqi subjects.
4. Higher serum levels of Gal-9 and IL-33 are great problem in CHB patients. In addition, serum Gal-9 and IL-33 were correlated positively with all parameters with some exceptions, which suggest that are “low-grade inflammation”.
5. Serum levels of Gal-9 and IL-33 may serve as a novel predictor of CHB progress in Iraqi patients.

Recommendations

This study recommends the following based on its current findings:

1- This study recommend to design inhibitors for Gal-9 and IL-33 that show as an encouraging pharmacological goal in the situation of CHB problems, which is a novel category of orally vigorous direct anticytokine medications with potential applications not only in classic inflammatory diseases, such as CHB, but also in common public complications of many other diseases, where the use of biological anticytokine treatments may be quite costly. It remains to be seen whether such compounds can be industrialized, but significant efforts are being made in numerous test centers in this direction.

2- This study recommend examining anthropometric measurements which could be used as free predictive and/or diagnostic indicators in the future, especially if they are assessed and confirmed in papers with a larger sample size.

3- A small sample size of this case control study which may be not successful to explain the vital correlation between Gal-9 and IL-33 levels and additional measured variables in CHB patients, our data suggets studies with larger samples for additional ethnic groups that are required to test the biological roles of serum Gal-9 and IL-33 should be accomplished before these biomarkers are established as confident risk factors in CHB patients.

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Appendix-1

Questionnaires for CHB Patients and Healthy Controls	
File No.	
Sample No.	
Code No.	
Date	**/ / 2020**
Name	
Age	**( ) years**
Gender	Sex ( )
Address	
Weight	**( ) Kg**
High	**( ) cm**
W. of Hip	**( ) cm**
W. of Waist	**( ) cm**
W. of Thoracic	**( ) cm**
W. of Neck	
Smoking:-	**Yes: No:**
Alcohol drinking	**Yes: No:**
Married	**Yes: No: No. of children ( )**
HBsAg	**+ve: -ve:**

Duration of Hepatitis B:-	**(Years) Hepatitis B History**
Age of Getting Hepatitis B:-	(Years)
Treatments:-	A- B-
Blood Pressure	
Systolic blood pressure:-	(mmHg)
Diastolic blood pressure:-	(mmHg)
ECG:-	HR- ( ) BPM
Hepertension drugs	A- B-
Other drugs:-	A- B-

Test	Results	Test	Results
ALT	U/mL	TSP	g/dL
AST	U/mL	Albumin	g/dL
ALP	U/mL	Globulin	g/dL
T.BIL	mg/dL	Gal-9	pg/mL
D.BIL	mg/dL	IL-33	ng/mL
I.BIL	mg/dL		

Appendix II: Clinical and Anthropometric Characteristics of Chronic Hepatitis B Virus Patients and Controls

Parameter	Healthy controls			Patients			p-value
	Mean	SD	SEM	Mean	SD	SEM	
ALT (U/L)	15.53	3.154	0.4987	40.35	10.75	1.699	<0.0001
AST (U/L)	16.7	1.937	0.3063	37.03	12.48	1.974	<0.0001
ALP (U/L)	185.5	48.07	7.6	238.6	63.64	10.06	<0.0001
T.BIL (mg/dL)	0.595	0.1709	0.02702	1.143	0.2836	0.04485	<0.0001
D.BIL (mg/dL)	0.3275	0.124	0.01961	0.6125	0.1682	0.0266	<0.0001
IN.BIL (mg/dL)	0.2675	0.08883	0.01405	0.53	0.1911	0.03021	<0.0001
TSP (g/dL)	7.105	0.4646	0.07346	7.03	1.065	0.1684	0.6843
Albumin (g/dL)	4.348	0.3544	0.05604	5.248	0.6135	0.09701	<0.0001
Globulins (g/dL)	2.77	0.3736	0.05907	1.77	0.7297	0.1154	<0.0001
Gal-9 (pg/mL)	136.3	35.48	5.61	618.6	171.7	27.15	<0.0001
IL-33 (ng/mL)	59.84	13.57	2.173	404.3	148	23.4	<0.0001
Age Years	36.7	10.83	1.712	37.4	10.79	1.706	0.7729
BMI kg/m^2	24.94	1.835	0.2901	31.28	9.004	1.424	<0.0001
W/H	0.8928	0.1101	0.01741	0.9159	0.1403	0.02407	0.4298
W/T	0.8881	0.05466	0.00864	0.9276	0.09307	0.01573	0.0260
W/N	2.286	0.2654	0.04196	2.39	0.332	0.05611	0.1365
SBP mmHg	121.3	4.965	0.7851	120.9	4.931	0.8335	0.7703
DBP mmHg	76.63	4.923	0.7785	78.91	5.204	0.8796	0.0543
ROP 1/Min	74.5	10.15	1.605	76.11	8.581	1.45	0.4629

The AUC Area of ROC Curve for Clinical and Anthropometric Characteristics of CHB Virus Patients and Controls

Parameter	AUC	Std. Error	95%confidence interval	P-value
ALT (U/L)	1	0	1 to 1	<0.0001
AST (U/L)	1	0	1 to 1	<0.0001
ALP (U/L)	0.7678	0.05365	0.6627 to 0.873	<0.0001
T.BIL (mg/dL)	0.9588	0.01836	0.9228 to 0.9947	<0.0001
D.BIL (mg/dL)	0.9197	0.02853	0.8638 to 0.9756	<0.0001
IN.BIL (mg/dL)	0.9084	0.0304	0.8489 to 0.968	<0.0001
TSP (g/dL)	0.5644	0.06815	0.4308 to 0.6979	0.3216
Albumin (g/dL)	0.9222	0.03131	0.8608 to 0.9836	<0.0001
Globulin (g/dL)	0.8559	0.04692	0.764 to 0.9479	<0.0001
Gal-9 (pg/mL)	1	0	1 to 1	<0.0001
IL-33 (ng/mL)	1	0	1 to 1	<0.0001
Age Years	0.5138	0.06511	0.3861 to 0.6414	0.8323
BMI kg/m^2	0.7894	0.05407	0.6834 to 0.8954	<0.0001
W/H	0.6294	0.07002	0.4922 to 0.7666	0.0563
W/T	0.7079	0.06204	0.5863 to 0.8295	0.0020
W/N	0.5668	0.06921	0.4311 to 0.7024	0.3207
SBP mmHg	0.5232	0.0673	0.3913 to 0.6551	0.7300
DBP mmHg	0.6218	0.06522	0.494 to 0.7496	0.0702
ROP 1/Min	0.5614	0.06657	0.4309 to 0.6919	0.3611

Appendix III: Mean ± SD of Chronic Hepatitis B Virus Patients and Controls

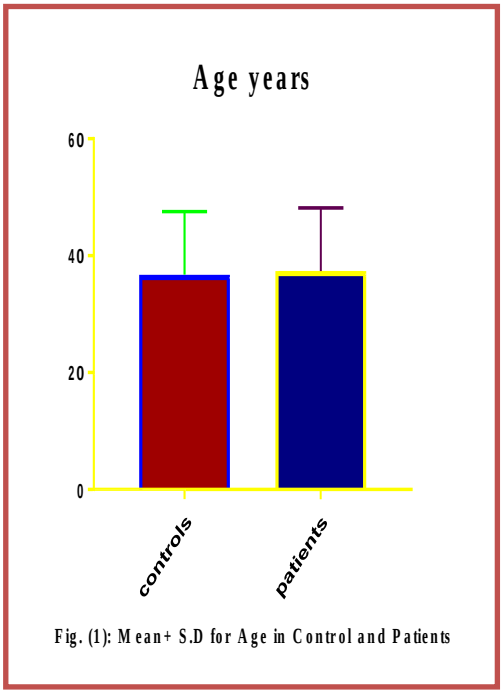

Fig. (1): Mean+ S.D for Age in Control and Patients

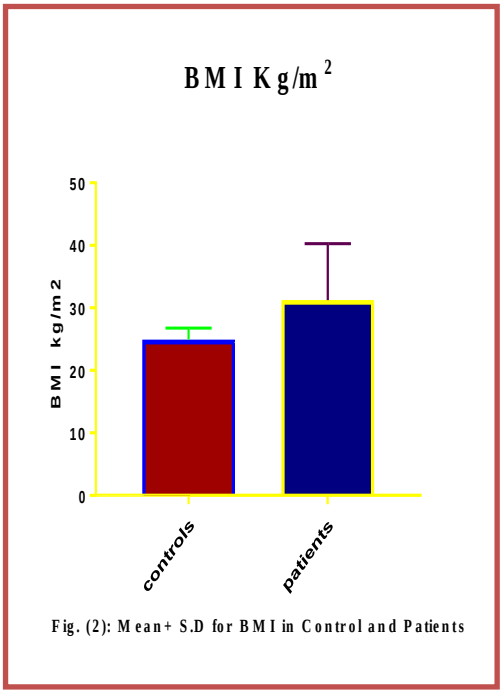

Fig. (2): Mean+ S.D for BMI in Control and Patients

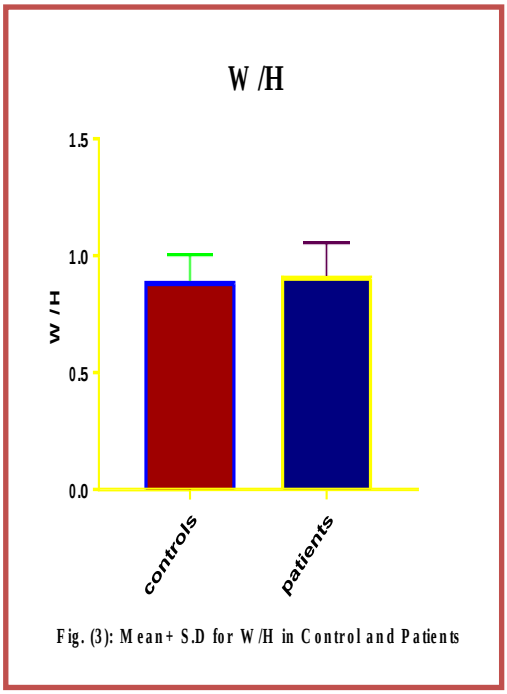

Fig. (3): Mean+ S.D for W/H in Control and Patients

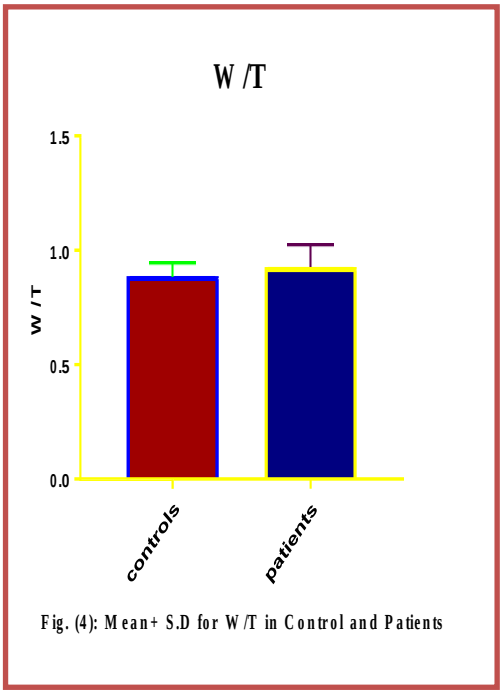

Fig. (4): Mean+ S.D for W/T in Control and Patients

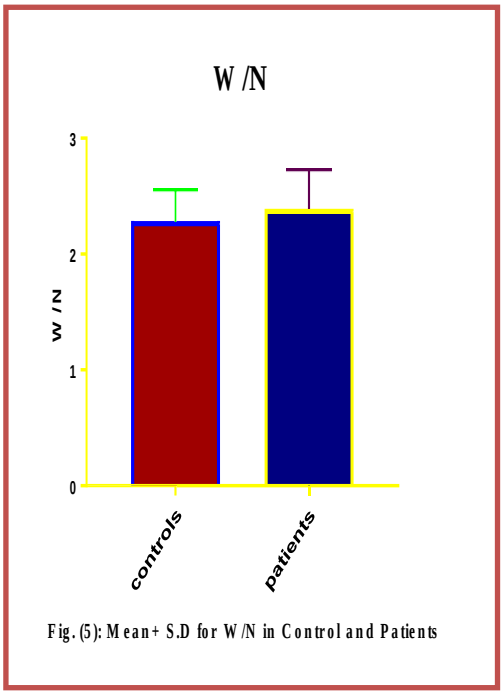

Fig. (5): Mean+ S.D for W /N in Control and Patients

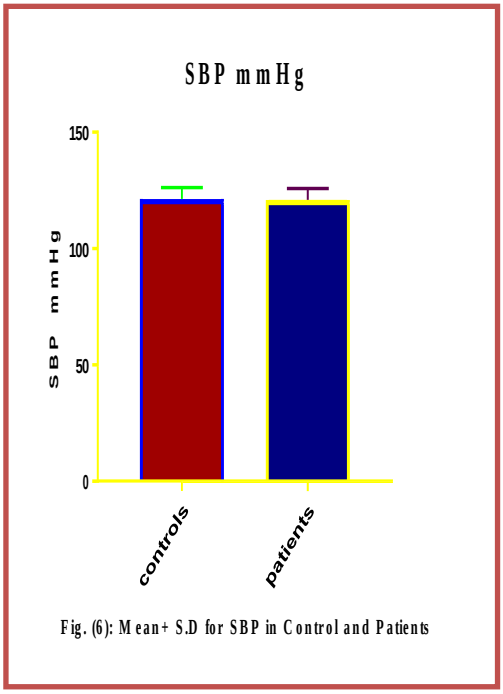

Fig. (6): Mean+ S.D for SBP in Control and Patients

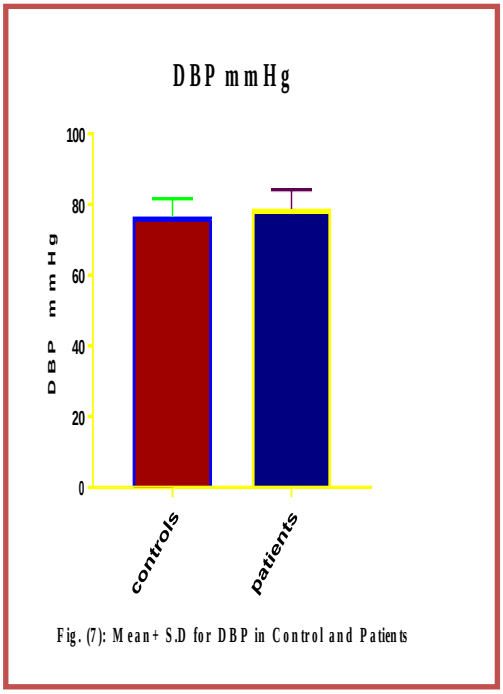

Fig. (7): Mean+ S.D for DBP in Control and Patients

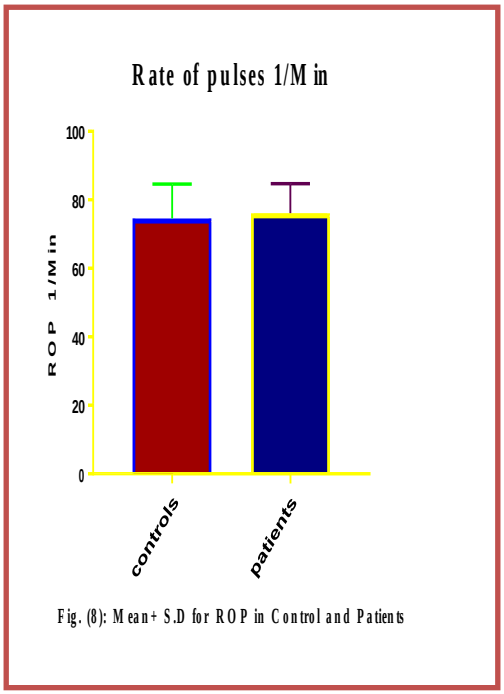

Fig. (8): Mean+ S.D for ROP in Control and Patients

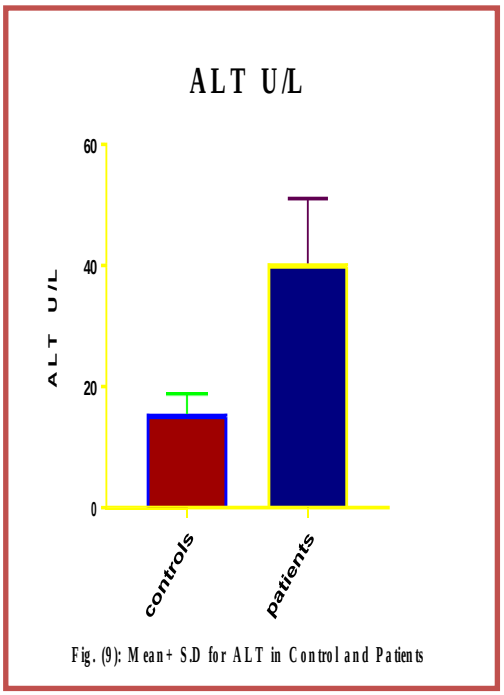

Fig. (9): Mean+ S.D for ALT in Control and Patients

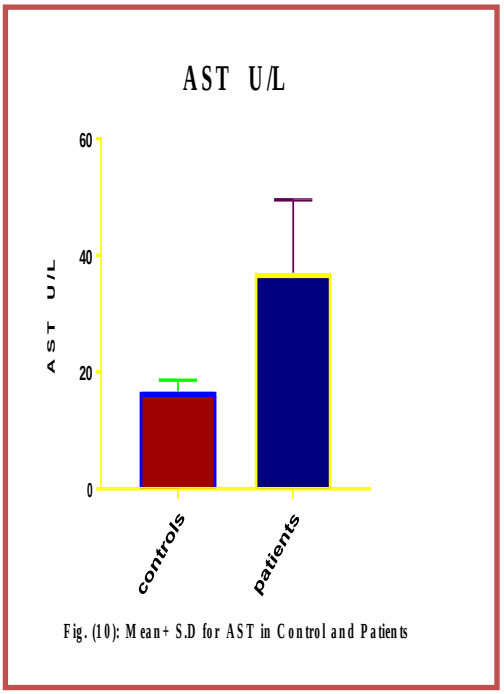

Fig. (10): Mean+ S.D for AST in Control and Patients

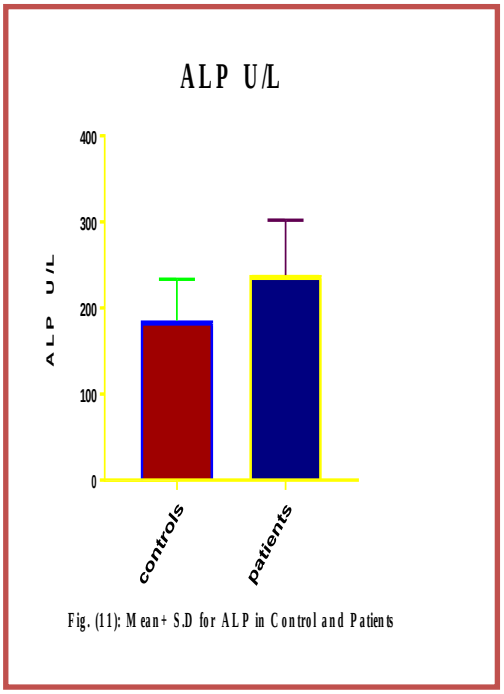

Fig. (11): Mean+ S.D for ALP in Control and Patients

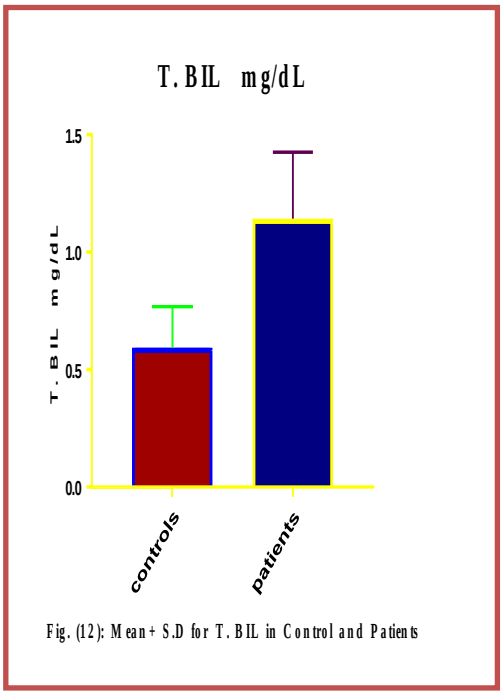

Fig. (12): Mean+ S.D for T.BIL in Control and Patients

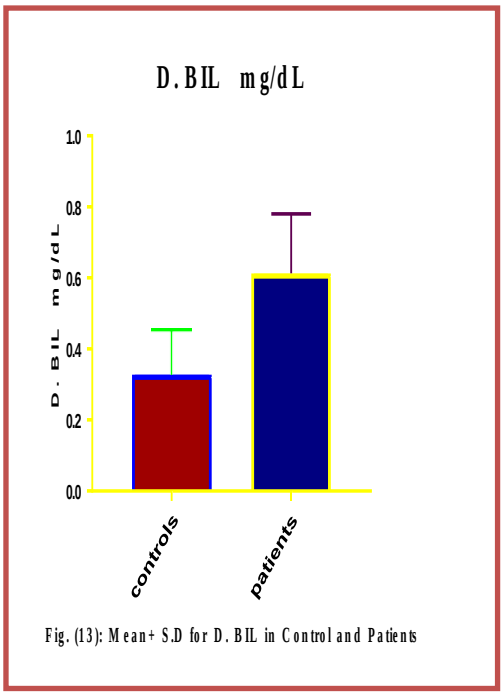

Fig. (13): Mean+ S.D for D. BIL in Control and Patients

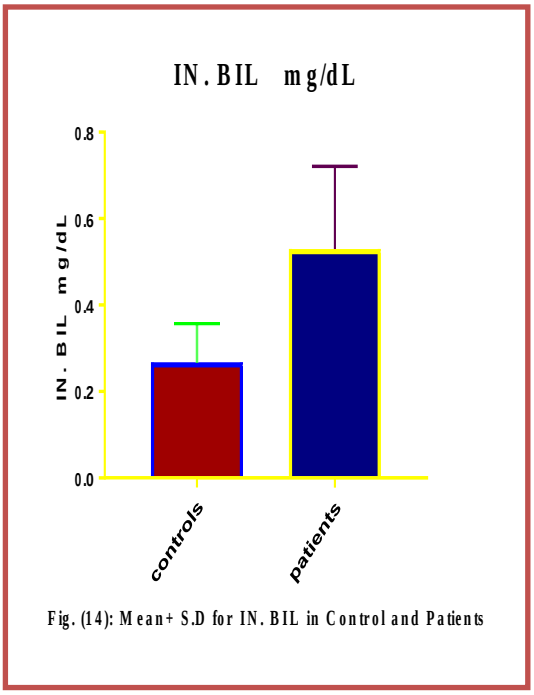

Fig. (14): Mean+ S.D for IN. BIL in Control and Patients

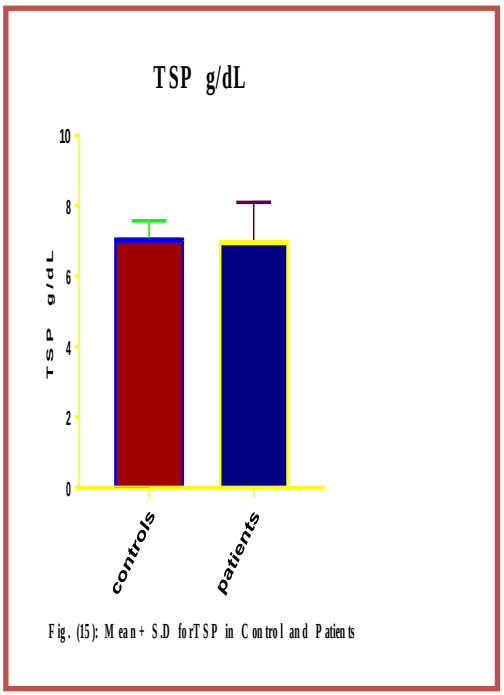

Fig. (15): Mean+ S.D forTSP in Control and Patients

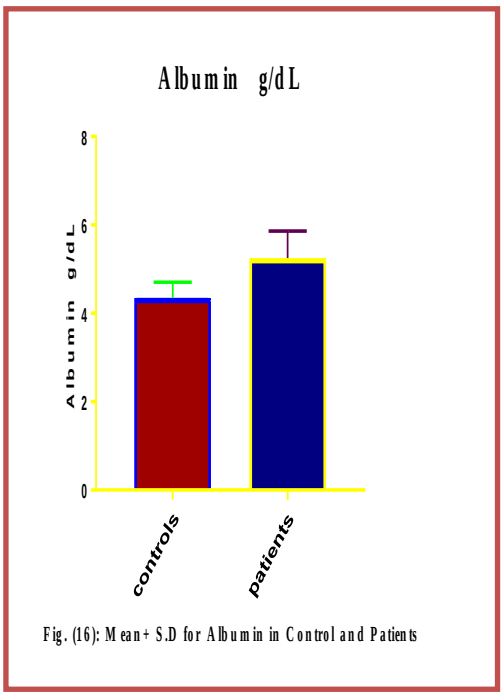

Fig. (16): Mean+ S.D for Albumin in Control and Patients

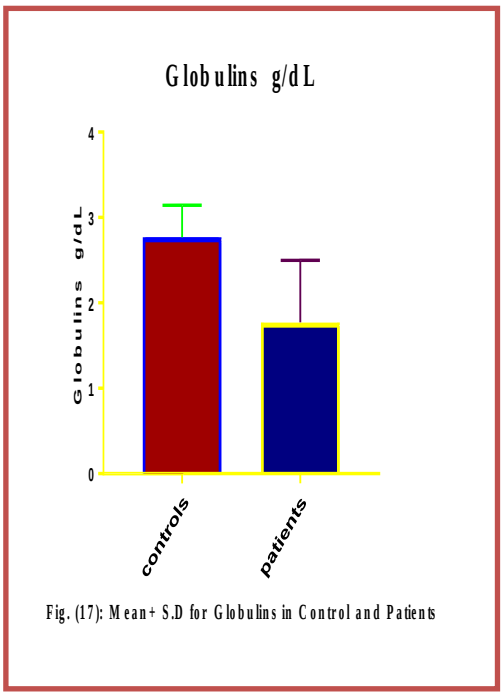

Fig. (17): Mean+ S.D for Globulins in Control and Patients

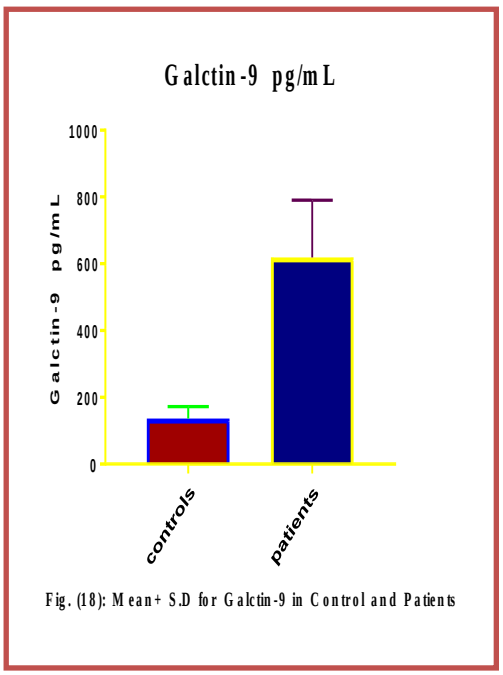

Fig. (18): Mean+ S.D for Galctin-9 in Control and Patients

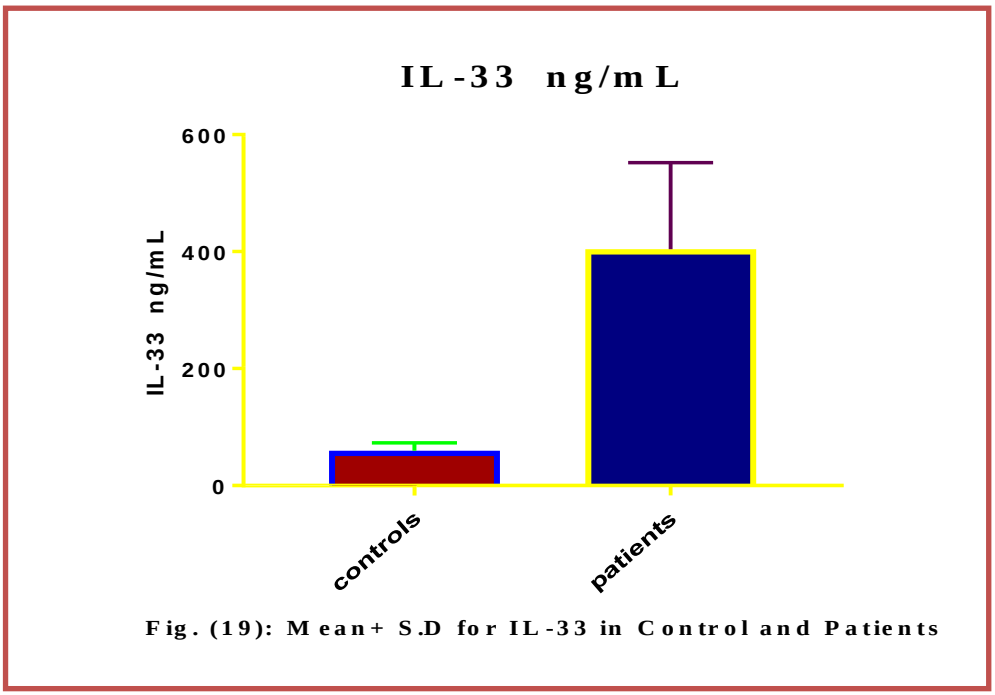

Fig. (19): Mean+ S.D for IL-33 in Control and Patients

الملخص

التهاب الكبد الفيروسي المزمن ب هو من االمراض الشائعة عالميا التي تؤدي الى سرطان الخاليا الكبدية وتليف الكبد .الهدف الرئيسي لهذه الدراسة هو تقيم المستويات المصلية للكالكتين-9 واالنترلوكين-33 واكتشاف العالقة الترابطية الموجودة بينهما في مرضى التهاب الكبد الفيروسي المزمن ب في مدينة الفلوجة.

تضمنت هذه الدراسة 80 عينة وقد تم تقسيمها الى مجموعتين ، هما مجموعة االصحاء و تضمنت 40 عينة)20 ذكور و 20 اناث(ومجموعة المرضى والتي تضمنت 40 عينة)20 ذكور و 20 اناث(العينات تم جمعها من مستشفى الفلوجة التعليمي ومن المرضى الذين يحضرون الى المختبرات الخاصة في مدينة الفلوجة من الفترة الممتدة من ايلول 2020 الى كانون الثاني 2021 وكانت االعمار للعينات تتراوح من)20 -55(سنة .تم تقدير المستويات المصلية للكالكتين-9 واالنترلوكين-33 بواسطة تقنية مقايسة االمتزاز المناعي المرتبط باإلنزيم ، مع ذلك استخدمت الطرق االنزيمية اللونية لتقدير انزيمات الكبد التي تتغير في حالة االصابة بالتهاب الكبد الفيروسي .

اظهرت النتائج مستويات مصلية عالية للكالكتين-9)بيكوغرام\ملتر(مع اختالف معنوي مهم في مجموعة المرضى)P <0.001(مقارنة مع مجموعة أألصحاء. وكان معدل المستويات المصلية لالنترلوكين-33)نانوغرام\ملتر(اعلى مع اهمية معنوية في مرضى التهاب الكبد الفيروسي)P <0.001(مقارنة بمجموعة أألصحاء ، ايضا المستويات المصلية)لإلنزيم الناقل لمجموعة االمين لحامض االنين ، االنزيم الناقل لمجموعة االمين لحامض االسبارتيت ، انزيم الفوسفاتيز القاعدي ، وكذلك البيليروبين واالبومين والتي قد ارتفعت مستوياتها في مجموعة المرضى مقارنة بمجموعة االصحاء ، واظهرت فروقات معنوية)P<0.001(، في حين اظهر معدل المستويات المصلية للغلوبيولين انخفاضا مهما" معنويا" في مجموعة المرضى مقارنة بمجموعة االصحاء)P<0.001(، كما كشفت الدراسة عدم وجود اختالفات معنوية مهمة في المستويات المصلية للبروتين الكلي في المجموعتين المدروسة (P= 0.77).

اظهرت الدراسة الحالية وجود ارتباطا" ايجابيا" قويا" جدا للكالكتين-9 مع االنترلوكين-33)r = 0.919(وكذلك أظهر الكالكتين-9 ارتباطا" ايجابيا" قويا" مع البيليروبين الكلي)r 0.731 =(ومع االنزيم الناقل لمجموعة االمين لحامض االنين)r = 0.725(وارتباطات موجبة مع االنزيم الناقل لمجموعة االمين لحامض االسبارتيت)r = 0.683(، والبيليروبين المباشر

0.656) r = (، والبيليروبين الغير مباشر)0.650 r = (، واالللبومين)0.581 r = (،
وانزيم الفوسفاتيز القاعدي)0.468 r = (وكذلك مع مؤشر كتلة الجسم)0.323 r = (،
واظهر ارتباط سلبي مع الغلوبيولين)0.563 - r = (. اظهرت جميع هذه المتغيرات فروقا" معنوية واضحة الن
قيمة P اقل من 0.05، ولم يالحظ أي ارتباط بين الكالكتين-9 وبين كل
من ضغط الدم االنقباضي ونسبة محيط الخصر الى الرقبة و معدل دقات القلب و نسبة
محيط الخصر الى الصدر و ضغط الدم االنبساطي والعمر ونسبة محيط الخصر الى عرض
الورك وقياس البروتين الكلي .

اما االرتباطات لالنترلوكين-33 مع المتغيرات المدروسة فقد ظهرت كما يلي:
ارتباطات إيجابية قوية للغاية مع الكالكتين-9) r = 0.919) ، بينما لوحظ وجود ارتباط
إيجابي قوي مع االلبومين الكلي (r = 0.743) ، واالنزيم الناقل لمجموعة االنين

(r = 0.729) ، وارتباطات موجبة مع البيليروبين المباشر (r = 0.680)، البيليروبين
الغير مباشر (r = 0.647) ، االنزيم الناقل لمجموعة االمين لحامض االسبارتيت

(r = 0.644) ، أاللبومين)0.530 r = (، انزيم الفوسفاتيز القاعدي (r = 0.426) ،
مؤشر كتلة الجسم)r = 0.254(والعالقة السلبية مع الغلوبيولين)0.513 - r = (مع قيمة
p أقل من 0.05 لجميع هذه المعلمات. ولم يالحظ وجود ارتباط بين كل من االنترلوكين- 33 وكل من ضغط الدم
االنقباضي ونسبة محيط الخصر الى الرقبة ومعدل دقات القلب
والعمر ونسبة محيط الخصر الى الصدر وقياس البروتين الكلي وضغط الدم االنبساطي
ونسبة محيط الخصر الى عرض الورك .

بينت نتائج منحنى)ROC(للمساحة تحت المنحني الترتيب التنازلي الذي يوضح
القدرة التميزية للمتغيرات المدروسة بين مجموعتي المرضى واالصحاء في هذه الدراسة
حيث ان االنترلوكين-9 والكالكتين-33 واالنزيم الناقل لمجموعة االمين لحامض االنين ،
واالنزيم الناقل لمجموعة االمين لحامض االسبارتيت تساوي)1) ومعدل البيليروبين الكلي
)0.9588(، وأاللبومين)0.922(، والبيليروبين مباشر (0.9197)، والبيليروبين الغير
مباشر)0.9084)، والغلوبيولين)0.8559(، ومؤشر كتلة الجسم)0.7894(، وانزيم
الفوسفاتيز القاعدي (0.7678) ، ونسبة محيط الخصر الى عرض الصدر (0.7079)،
ونسبة محيط الصدر الى عرض الورك)0.6294(، وضغط الدم االنقباضي (0.7285)،

و نسبة محيط الصدر الى عرض الرقبة)0.5668(، وقياس البروتين الكلي (0.5644)، ومعدل ضربات القلب (0.5614)، وضغط الدم االنبساطي)0.5232(، والعمر)0.5138(.

تم استنتاج ان المستويات المصلية المنخفضة لكل من الكالكتين-9 واالنترلوكين-33 هي عوامل مرضية ويمكن استخدامها كمؤشرات لتشخيص والتنبؤ بحدوث التهاب الكبد الفيروسي المزمن ب.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي جامعة االنبار
كلية العلوم قسم
الكيمياء

دراسة العالقة الترابطية للكالكتين-9 وآلنترلوكين-33 مع بعض المتغيرات الكيموحيوية لدى مرضى التهاب الكبد الفيروسي المزمن ب في مدينة الفلوجة

رسالة

مقدمة إلى مجلس كلية العلوم- جامعة ألنبار

وهي جزء من متطلبات نيل درجة الماجستير في علوم الكيمياء من قبل

هبه عواد خضير الحديثي

بكالوريوس علوم كيمياء) 2006 (جامعة ألنبار

بإشراف

أ.د. شاكر فارس طليب آلعرجي

1442هـ **2021م**