University of Karbala

College of Applied Medical Sciences

Department of Clinical Laboratories

The Role of Serum Glypican-4 ,Pentraxin-3 and Triglyceride –Glucose index as potential marker in assessments of Non Alcoholic Fatty Liver patient .

A Thesis

Submitted to the Council of the

College of Applied Medical Sciences – University of Kerbala

In Partial of Fulfilment of the Requirements for the Degree of Master in ClinicalLaboratories

Written by

Mariam Salam Abass

B.Sc. Clinical Laboratories\Applied Medical Sciences-University of Kerbala,2021.

Supervised By

Prof. Dr. Ghosoon Ghanem Kaem **Dr .Ahmed Abdul hussein ALhilly**

2025 A.D **1446 A.H**

Supervisor certification

I certify the thesis entitle (The Role of Serum Glypican-4 ,Pentraxin-3 and Triglyceride –Glucose index as potential marker in assessments of Non Alcoholic Fatty Liver patient) was prepared under my supervision at the department of Clinical Laboratories, at the College of Applied Medical Sciences, University of Kerbala, as a partial requirements for the degree of Master in Clinical Laboratories.

Signature

Prof. Dr. Ghosoon Ghanem Kaem

Supervisor

11 /3/ 2025.

Signature

Dr .Ahmed Abdul hussein Alhilly

Supervisor

/ /2025

Head of Department Recommendation

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

Signature

Dr. Riad Hatem Haddawi

Head of Clinical Laboratories Department

College of Applied Medical Sciences/ University of Kerbala

12 /3/ 2025

Discussion Committee Certification

We, the examining committee, certify that we have read the thesis entitled " The Role of Serum Glypican-4 ,Pentraxin-3 and Triglyceride –Glucose index as potential marker in assessments of Non Alcoholic Fatty Liver patient" and have examined the student (**Mariam Salam Abass**) in its content and that in our opinion it is accepted as a thesis for degree of Master of **Clinical Laboratories.**

Signature

Prof. Dr. Narjes Hadi Mansoor

(Chairman)

11 /3 / 2025

Signature

Assist.Prof.Dr.*Ruqya Kareem Mohammed*

(Member)

/ / 2025

Signature

Assist.Prof. Dr.*Halah Abd-alhadi AbdlGhani*

(Member)

/ / 2025

Signature

Prof. Dr. Ghosoon Ghanem Kaem

(Member & Supervisor).

11 / 3 /2025

Signature

Lecture. Dr .Ahmed Abdul hussein ALhilly

(Member & Supervisor)

/ / 2025 .

I have certified upon the discussion of the examining committee .

Signature

Assist. Prof. Dr. Hasan Fisal Alesary

Dean of College of Applied Medical Sciences University of Kerbala

/ / 2025

Approval certification

We Certify that the thesis entitled " The Role of Serum Glypican-4 ,Pentraxin-3 and Triglyceride –Glucose index as potential marker in assessments of Non Alcoholic Fatty Liver patient" fulfills partial requirements of the degree of Master in Clinical Laboratories.

Signature

Head of Clinical Laboratories Department

Dr. Riad Hatem Haddawi

College of Applied Medical Sciences

University of Kerbala

12/ 3 / 2025

Signature

Vice Dean scientific Affairs

Assist. Prof. Dr. **Hasan Fisal Alesary**

College of Applied Medical Sciences

University of Kerbala

/ / 2025

Dedication

To the one who was and still is my support during dark nights, calm my heart with an abundance of reassurance and contentment and be kind to my affairs, the one who is most gracious to me, Imam Aba Al-Fadl (peace be upon him).

To my family, who supported me with everything and worked hard for this achievement and for my name to be raised high and to my life partner , my husband I offer them this in the hope that it will cover part of what they have made.

Mariam

Acknowledgments

First of all, praise be to Allah who enabled me to accomplish this project. From theprinciple that whoever does not thank the creature does not thank then Creator.

So, I express my profound gratitude to my supervisor **Prof. Dr .Ghsoon ghanem and Dr. Ahmed Abdul hussein** for his invaluable assistance, unwavering supervision, and provision of essential project-related information .

We express our sincere gratitude to the University of Karbala, the College of Applied Medical Sciences, and its respected **Dean D. Hassan Faisal Nehme** and Head of Clinical Laboratories. The aid and collaboration of my family members were crucial in facilitating the effective completion of this research. Therefore, I would like to express my sincere gratitude for their unwavering support., persisted encouragement and long patience throughout all the years of study and research ,this work would not have been possible without them, Thank you very much.

Summary

In Western society, fatty liver disease is the most prevalent chronic liver condition. It is brought on by the build-up of fatty acids in liver parenchymal cells as triglycerides rather than by alcohol or the hepatitis virus. One of the most prevalent causes of liver illnesses is non-alcoholic fatty liver disease (NAFLD), and its incidence is rising globally, Non- alcoholic fatty liver disease (NAFLD) involves a spectrum of liver diseases ranging from steatosis, non-alcoholic steatohepatitis(NASH),and fibrosis to cirrhosis. NAFLD is a multisystem disease with extra hepatic disease implications that include type 2 diabetes and cardiovascular disease (CVD). Non-alcoholic fatty liver disease is now known as metabolic dysfunction associated fatty liver disease (MAFLD).

This study aimed to determine the role of Glypican-4, pentraxin-3 Triglyceride –Glucose index a new marker in patient with fatty liver disease, Nonalcoholic fatty liver disease(NAFLD) and Non-alcoholic steatohepatitis(NASH),cirrhosis and in control volunteer ,evaluate the level of lipid profile , insulin resistance and alanine transaminase (ALT),aspartate aminotransferase (AST)in people with and without fatty liver disease. The current study was conducted on ninety patients suffering from fatty liver and thirty healthy. Group1 (30)patient of NAFLD , group2 (30) patient NASH , group3 (30) patient cirrhosis and group 4 (30) healthy as a control. During the period extending between November 2023 to April 2024, Collection of samples carried out from Babylon gastroenterology α hepatology center . The patients' socio demographic data, including their ages, genders, weights, heights .

The human pentraxin-3 (PTX3) serum level and glypican-4 (GPC4) were measured by using the ELISA system, while TyG index was calculated as ln (fasting triglyceride/fasting glucose/2) were measured by DIRUI CS-T180 auto chemistry analyzer ,and other parameter lipid profile (cholesterol and high density lipoprotein(HDL)) and liver enzyme (AST,ALT) were measured by DIRUI CS-T180 auto chemistry analyser.

The result of this study, the age differences between group1(NAFLD), group2 (NASH), group3(Cirrhosis) , and control were statistically significant ($p \leq 0.05$). After comparing the three groups (G1, G2, and G3) to the control, it was discovered that the group 3had a higher mean than the control group. The three groups of body mass index (BMI)(group1,group2, and group3) were determined to be more valuable than the control group, although the highest value was group 1. The BMI differences between the groups are significant.

In TYG and GLY-4, but not in PEN-3, there was a statistically significant difference ($p \leq 0.05$) between the patient groups and the control group. The means of TYG for groups 1 (NAFLD),2(NASH), and 3 (cirrhosis) were higher than the control group but remained quite similar. In GLY-4, the group means (group 1, group 2, group 3) are higher than the control group , with group 3 having the highest mean overall . Group 1 mean was greater than group 2, and group 3 mean was higher than those of the other groups and the control group in PEN-3.

There was a strong positive correlation ($p<0.01$) observed in regard to ALT with both BMI and AST. Additionally, there was a significant positive correlation ($p<0.01$) discovered between LDL and BMI. HDL shows a strong positive correlation with age and a strong negative correlation with BMI, in

contrast to LDL. Triglycerides show a strong positive correlation ($P<0.01$) with ALT and LDL and a negative correlation ($P\leq0.05$) with HDL.

Cholesterol has a negative correlation with HDL and a positive correlation with triglycerides and LDL. It also has a positive correlation ($P<0.01$)with ALT and BMI. VLDL and LDL, TG, and cholesterol have a strong positive association. And unfavorable association with HDL.

Age and glucose are positively correlated, there is a positive correlation ($p<0.01$)between HOMA-IR and glucose.

The statistical analysis revealed a substantial positive correlation ($p<0.01$)between marker PEN-3 and GLY-4. Age, LDL, triglycerides, cholesterol, glucose, and HOMA-IR were found to have a significant positive connection ($p<0.01$), according to TYG.

List Of Content

List of tables

List of figure

List of Abbreviations

Abbreviations	Description
ALD	alcoholic liver disease
AGA	American Gastroenterology Association
AIH	Autoimmune hepatitis
ALT	Alanine aminotransferase
AST	aspartate aminotransferase

AUC	Area under the curve
BMI	Body Mass Index
BP	Blood pressure
CHOL	Cholesterol
CNS	Central nervous system
CR	Caloric restriction
CT	Computed Tomography
CVD	Cardiovascular disease
DNL	*De novo* lipogenesis
EDTA	Ethylene diamin tetra acetic acid
ELISA	Enzyme linked immunosorbent assay
FBG	fasting blood glucose
FDA	Food and drug administration
GPC4,GLY-4	Glypican -4
HbA1c	Hemoglobin A1c
HCC	Hepatocellular carcinoma
HDL	High density lipoprotein

HDL-C	High-density lipoprotein cholesterol
HepG2	Human liver cancer cell line
HOMA-IR	Homeostasis model assessment of insulin resistance
HRP	Horse -radish peroxidase
IR	Insulin resistance
LDL-C	Low-density lipoprotein-cholesterol
MAFLD	Metabolic dysfunction associated fatty liver disease
MASH	Metabolic dysfunction associated steatohepatitis
MetS	Metabolic syndrome
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
Pre-DM	Pre diabetes
PTX3, PEN-3	Pentraxin-3
RBCs	Red blood cells
T2DM	Type 2 diabetes mellitus

TAG	Triacylglycerol
TG	Triglycerides
THR	Thyroid hormone receptor
TMB	Tetramethylbenzidine
TyG	Triglyceride-glucose index
VLDLs	Very low density lipoproteins
WC	Waist circumference
WHR.	Waist –hip ratio

Chapter one

Introduction

1.1INTRODUCTION

In Western society, fatty liver disease is the most prevalent chronic liver condition. It is brought on by the build-up of fatty acids in liver parenchymal cells as triglycerides rather than by alcohol or the hepatitis virus. One of the most prevalent causes of liver illnesses is non-alcoholic fatty liver disease (NAFLD), and its incidence is rising globally. NAFLD is a spectrum of liver diseases that occur in the absence of other known causes, such as excess alcohol use. NAFLD includes hepatic steatosis with more than 5% of liver weight consisting of fat. NAFLD may progress to nonalcoholic steatohepatitis (NASH), a more severe form of NAFLD with steatosis, inflammation, and cellular damage. NASH can progress to fibrosis and cirrhosis. NAFLD is among the leading etiologies for hepatocellular carcinoma (HCC) and liver transplantation (Younossi Z.M *,et al.,* 2020).

NAFLD is a multisystem disease with extrahepatic disease implications that include type 2 diabetes and cardiovascular disease (CVDCompared to people without NAFLD, patients with NAFLD have a twofold increased risk of type 2 diabetes; this risk may be increased by more severe liver disease.A lower income cardio metabolic profile, increased risk of more severe hypertension, atherogenic dyslipidemia, cardiac arrhythmias, and cardiovascular events are all frequently linked to the existence of NAFLD in individuals with type 2 diabetes Non-alcoholic fatty liver disease is now known as metabolic dysfunction associated fatty liver disease (MAFLD)) (Ren Z*,et al., 2023*).

The association of fatty liver disease with obesity, type 2 diabetes mellitus (T2DM), or at least two factors linked to the symptoms of metabolic dysfunction, such as elevated waist circumference (WC), elevated serum C-reactive protein, pre-diabetes (pre-DM), elevated blood pressure, decreased levels of HDL-CH, and elevated triglycerides (TG), is what several experts have defined as MAFLD.

Additionally, they emphasise that the diagnosis of MAFLD no longer needs checking out other causes of chronic liver disease, such as viral hepatitis or alcohol (Fouad Y*,et al.,2022).*

Glypican-4 is an adipokine released from adipose tissue, and related to glycosylphosphatidylinositol- anchored heparin sulfate proteoglycans family. GPC4 is differentially expressed in visceral and subcutaneous adipose tissue, and its expression in human white adipose tissue is highly correlated with Body Mass Index (BMI) and WHR. It was found that GPC4 can interact with, and regulate insulin receptor activation which in turn enhances insulin signaling and adipocyte differentiation. So, it is closely related to metabolic functions (USSAR S*, et al.* ,2012).

Pentraxins are a class of proteins consisting of five monomers arranged in a radial symmetry ring. They are part of a class of receptors that recognize patterns. Endothelial and vascular smooth muscle cells near the site of inflammation are the primary producers of PTX3, a long-chain pentraxin that is thought to be an acute phase marker. Both close to and distant from the inflammation, macrophages, fibroblasts, neutrophils, epithelial cells, dendritic cells, and other cell types also develop it. (Zhang J*, et al.,2012)*

According to Rajkovic *et al.,2016* , Pentraxin -3 has been identified as an independent indicator of inflammation linked to a number of conditions, including atherosclerosis, cancer, respiratory illnesses, and CNS diseases. Elevated levels of PTX3 are connected with an increased risk of developing these conditions or with their progression.

A member of the pentraxin superfamily, pentraxin-3 (PTX-3) is a marker of the acute phase reaction and a traditional mediator of inflammation. According to

studies, PTX-3 is closely linked to the development of conditions like septicemia, atherosclerosis, and type II diabetes . In people with NAFLD, PTX-3 shows a strong positive association with the disease activity index as well as the degree of fatty degeneration and liver fibrosis. (Ristagno G*,et al.,* 2019)

The triglyceride and glucose index is a screening method for insulin resistance that is very simple to use and only requires two laboratory determinations: triglycerides and glucose. The triglyceride-glucose(TyG) index is calculated as LN (fasting triglyceride/fasting glucose/2) ,which can usually be checked in healthy individuals. A recent study reported that the TyG index may be an alternative and reliable measure of IR. Studies have already pointed out that the TyG index is better in predicting the risk level of NAFLD patients compared with homeostasis model assessment-insulin resistance a common diagnostic means for IR clinically(Lee SB, *et al.,*2019).

1.2 AIM OF STUDY

- Evaluate and determined the role of Glypican-4, pentraxin-3and TYG as a marker in Patient with Nonalcoholic fatty liver disease (NAFLD)and Non-alcoholic steatohepatitis (NASH),cirrhosis and control.
- Evaluate the level of lipid profile, insulin resistance and AST,ALT enzymes in patient with fatty liver disease and control.

Chapter Two

Literature Review

2.1 Liver

The liver, located in the right upper quadrant of the body and below the diaphragm, is responsible for several functions, including primary detoxification of various metabolites, synthesizing proteins, and producing digestive enzymes.(Zhang M*, et al .,2020*) The liver is the body's biggest internal organ and performs crucial endocrine, exocrine, and metabolic processes. These include the synthesis of albumin, haptoglobin, and blood homeostasis through the secretion of clotting factors, the synthesis of lipids (lipogenesis), carbohydrates (gluconeogenesis), and the production of bile salt, hemoglobin, iron, vitamins, ammonia, copper, and medications..(Zorn AM.*et al*.,2008)

The liver also plays a significant role in metabolism, regulation of red blood cells (RBCs), and glucose synthesis and storage. In the blood that leaves the gastrointestinal system through portal veins, the liver controls the quantity of chemicals, nutrients, microbial pathogens, and endotoxin components.(Tanwar S, *et al*.,2020) Additionally, it contributes to immune surveillance against infections and counteracts the effects of benign antigens by producing acute phase proteins and cytokine signaling.(Albuquerque-Souza E ,*et al*.,2022)

Liver diseases, including viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), autoimmune hepatitis (AIH), fibrosis, and end-stage liver disease, account for approximately 2 million deaths per year worldwide (*Xiao J,et al .,*2019) Non-alcoholic fatty liver disease (NAFLD) involves a clinicopathological spectrum of liver diseases ranging from steatosis, non-alcoholic steatohepatitis (NASH), and fibrosis to cirrhosis (*Huang .T,et al .,2020)*

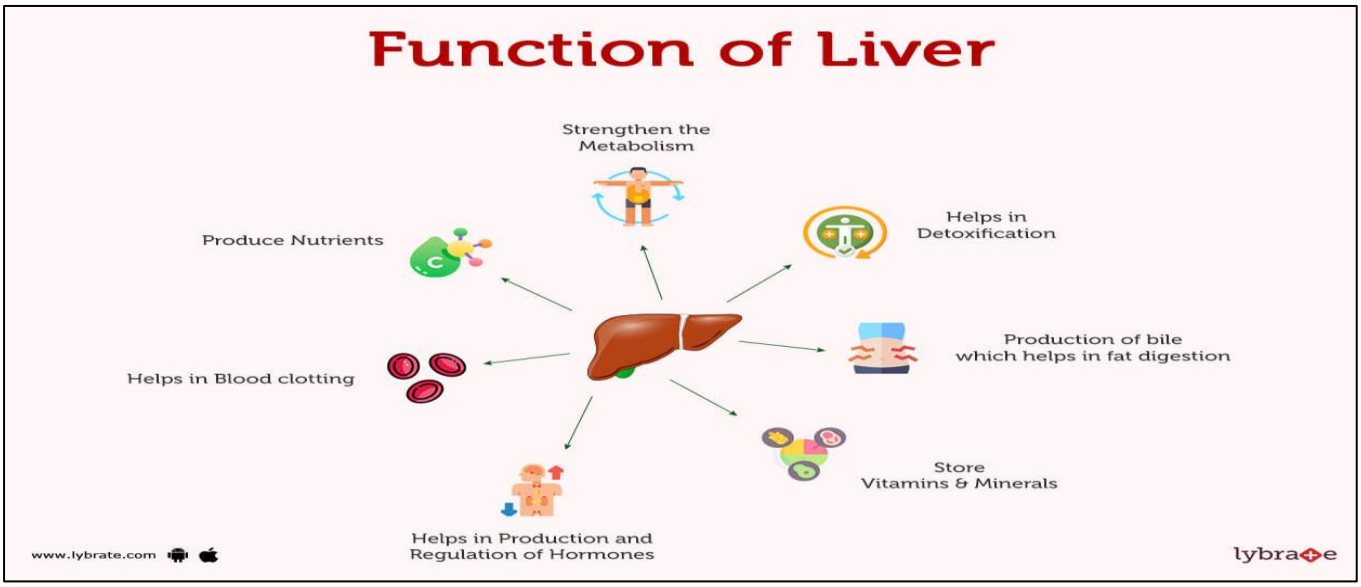


Figure 2-1: function of liver.(Hina Firdous .,et al 2023)

2.2. Liver Disease

2.2.1 Nonalcoholic Fatty Liver Disease(NAFLD)

One of the most prevalent causes of liver illnesses is nonalcoholic fatty liver disease (NAFLD), and its incidence is rising globally (*Stepanova M,et al 2020).* Non-alcoholic fatty liver disease (NAFLD) is defined as a chronic liver disease characterized by excessive fat accumulation in the liver without another obvious cause (no excessive alcohol consumption, hepatotoxic medications, toxins, viral infections, genetic hepatic diseases (Andrea Boccatonda, *et al.,2023*). NAFLD has lately been termed metabolic-associated fatty liver disease (MAFLD) because it is a metabolic illness. According to systematic review, NAFLD affects over 30% of persons globally (Le MH, *et al*.,2023). With a 1% annual increase, NAFLD is becoming more and more common (Henry L. *et al*., 2022). Furthermore, because of the global rise in the frequency of NAFLD, liver steatosis is common in the donor organ pool (Linares I,*et al*.,2019).

The development of MAFLD is linked to dyslipidemia, obesity, and insulin resistance (IR).These are also the main features of metabolic syndrome (MetS). MetS constitutes a cluster of metabolic abnormalities which stem from IR and chronic low grade inflammation and on the long run increase the risk for cardio-vascular disease (CVD) and T2DM(*Fahed G,et al.,*2022).

Hepatic steatosis, where more than 5% of the liver weight is fat, is a feature of nonalcoholic fatty liver disease (NAFLD). Nonalcoholic steatohepatitis (NASH), a more severe form of NAFLD characterized by steatosis, inflammation, and cellular destruction, can develop from NAFL. NAFLD is one of the main causes of liver transplantation and hepatocellular cancer (HCC)(*Eslam M,et al* 2020). Over the past several years, there has been a noticeable rise in the prevalence of obesity and related metabolic diseases worldwide, including nonalcoholic fatty liver disease (NAFLD), which is now believed to affect 30% of people worldwide (Golabi*,et al.,*2023).

Individuals with NAFLD are generally asymptomatic while few may present with fatigue and right upper quadrant discomfort. History and examination should include risk factor identification for steatosis which is the steppingstone for further investigations for NAFLD (Sheka AC,*et al* .,2020).

Hepatic steatosis, the most apparent clinical characteristic of non-alcoholic fatty liver disease (NAFLD), is brought on by an imbalance between the supply of lipids to the liver cells (dietary lipids, lipid lipolysis, and adipose de novo adipo-genesis) and their use by the liver cells (fatty acid beta-oxidation and VLDL secretion). In the context of other stress signals and pathogenic damage, hepatic steatosis which is caused by insulin resistance, adipose tissue malfunction, and an excess of fructose in the diet predisposes liver cells to damage and cell death (Watt M.J, *et al.,* 2019). Chronic tissue inflammation and liver fibrosis result from im-

munological and interstitial vascular microenvironment remodeling brought on by liver damage (Rui L*, et al .,*2022).

Simple steatosis, a non-alcoholic fatty liver condition NAFL is identified by hepatocellular steatosis, which must be present in excess of 5% of cases. Macrovesicular and microvesicular steatosis are the two categories into which it is divided. About 10% of people with NAFLD may also have microvesicular steatosis, but macrovesicular steatosis is the most common type (Ikejima K ,*et al.,* 2020). Numerous earlier investigations have indicated that NAFL is a benign condition. Several investigations using paired or repeat liver biopsies revealed that NAFL had a far better overall prognosis than NASH, including progression to cirrhosis. But when more information came to light, the idea that NAFL is a benign illness was called into question, and it is now thought to be a progressive one. According to recent studies, fibrosis may occur in approximately 25% of NAFL patients (Mazzolini G, *et al* .,2020).

2.2.1.1 Epidemiology

Epidemiology and disease burden

With prevalence ranging from 13·5% in Africa to 31·8% in the Middle East, nonalcoholic fatty liver disease (NAFLD) is currently the most prevalent cause of chronic liver disease globally. Variations in overall calorie intake, physical activity, body fat distribution, socioeconomic status, and genetic composition are probably the main causes of this variation. NAFLD affects 47·3–63·7% of patients with type 2 diabetes and up to 80% of people with obesity due to its strong correlation with the metabolic syndrome. Nonetheless, some individuals with a healthy body mass index (less than 25 kg/m² for white people and less than 23 kg/m² for Asian people)

might still develop non-obese or lean non-alcoholic fatty liver disease (NAFLD)(Zou B, *et al.,2020*).

Central obesity or other metabolic risk factors are typically present in these patients. Despite the fact that cirrhosis and hepatocellular carcinoma occur in less than 10% of NAFLD patients within 10 to 20 years of diagnosis, the absolute numbers are significant considering the high incidence of the illness. Fatty liver often coexists with other disorders (such as alcohol-related liver disease and viral or autoimmune hepatitis) and may have a synergistic role in liver harm. Crucially, during the next few decades, NAFLD's disease and financial burden are likely to rise (Estes C, *et al.,*2020). Patients with NAFLD, severe fibrosis, type 2 diabetes, and those who need hospitalization have especially high health care utilization and costs.

There is a lack of data on how NAFLD affects patients' day-to-day life, which will be crucial information to gather for upcoming intervention or treatment trials (McSweeney L. *et al*., 2020). In the United States, the prevalence of pediatric obesity, a significant risk factor for non-alcoholic fatty liver disease (NAFLD), has continued to rise, rising from 8·4% in 2011–2012 to 13·9% in 2015–2016. The rise in children's and adolescents' body mass index has accelerated across east and south Asia, despite the fact that it seems to have moderated in many high-income nations. The pooled mean prevalence of non-alcoholic fatty liver disease (NAFLD) in children is 34·2% in pediatric obesity clinics and 7·6% in the general population. Compared to people whose condition began in maturity, those whose disease began in infancy are more likely to experience liver-related events and other comorbidities linked to metabolic syndrome throughout their lives (Vittorio J,*et al.,*2020).

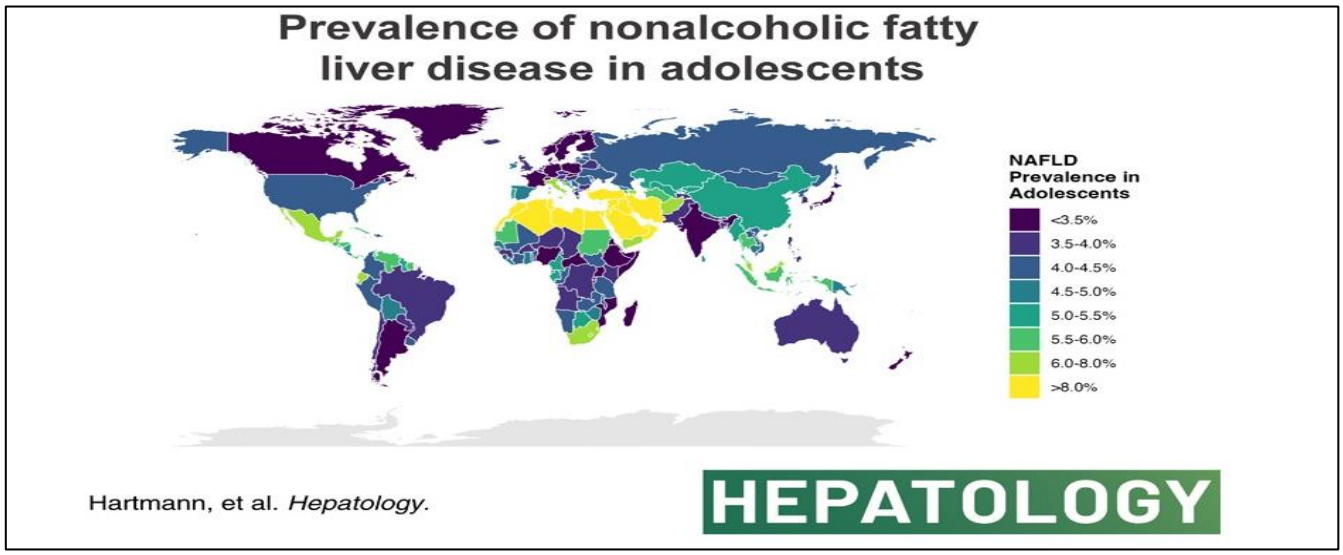


Figure 2-2: prevalence of non -alcoholic fatty liver disease (Hartmann P,*et al* 2023)

2.2.1.2 Etiology

Obesity, diabetes, dyslipidemias, insulin resistance, and metabolic syndrome are known to be associated with the development of non-alcoholic fatty liver disease (NAFLD)(Aguilera-Méndez A. *et a.,* 2019). A temporal association has also been shown between inorganic arsenic exposure and the development of NAFLD reflected by elevated alanine transferees (ALT)(Frediani JK, *et al.,*2019). Due to its close association with metabolic syndrome, NAFLD correlates with cardiovascular risk factors, which also contributes to mortality in these patients in addition to end-stage liver cirrhosis and hepatocellular carcinoma.

2.2.1.3.Pathogenesis

Over nutrition is the main cause of NAFLD since it leads to the growth of adipose depots and the buildup of ectopic fat. In this situation, a pro-inflammatory state that encourages insulin resistance is produced by macrophage invasion of the visceral adipose tissue compartment. Together with increased de-novo lipogenesis, inappropriate lipolysis in the context of insulin resistance causes the liver to receive fatty acids unchecked, beyond its metabolic capacity. An imbalance in lipid

metabolism results in the production of lipotoxic lipids, which in turn trigger cellular stress (such as endoplasmic reticulum and oxidative stress), activate the inflammasome, cause cell death, and subsequently promote inflammation, tissue regeneration, and fibrogenesis (Lefere S, *et al.,*2019). The development of liver fibrosis is linked to inflammatory and profibrogenic macrophages, which may also contribute to long-term inflammatory processes in other tissues (Eslam M,*et al.,*2020).

Many poorly understood metabolic, genetic, and microbiome-related variables influence these pathogenic pathways of non-alcoholic fatty liver disease. There is a heritable component to NAFLD, with individual genetic variations impacting disease risk estimates by 20–70%. In the PNPLA3 gene, a single-nucleotide polymorphism is the most well-characterized genetic variation linked to NAFLD risk.(Romeo S,*et al .,2008)* However, although this percentage varies throughout populations, known genetic variants only make up a modest percentage (10–20%) of total heritability. Several genetic risk variations have a synergistic interaction with obesity, and these genes or genetic variants may affect various features, often with divergent consequences on NAFLD and concomitant illnesses like coronary artery disease(Meffert PJ,*et al .,*2018).

Another factor that may contribute to metabolic dysregulation and inflammation in NAFLD is the liver's interdependence and communication with other organs, especially the gut and adipose tissue.(Aron-Wisnewsky J, *et al .,*2020) Patients with NAFLD exhibit changes in the composition of their gut microbiota, and some evidence points to a fecal-microbiome profile linked to progressive fibrosis (Loomba R, *et al .*,2017). To ascertain the clinical importance of these bacterial signatures and their potential application in future diagnostic procedures, however, confirmation of these signatures in other patient cohorts and geographical locations

while accounting for environmental factors is necessary. Although specific causative effects have not yet been proven, factors generated by bacteria (such as lipopolysaccharide or short-chain fatty acids) or derived from bile acid metabolism may affect liver inflammation and the course of NAFLD(Caussy C,*et al .,*2019).

2.2.1.4.Environmental And Genetic Risk Factors For Disease Progression

The severity of metabolic abnormalities, insulin resistance, and in particular the presence of T2DM, represent the major risk factors for the development of advanced liver disease, and the progression of fibrosis in prospective studies in patients with NAFLD (Dongio- vanni P, *et al* .,2018).

NAFLD-associated genes, genes that facilitate the progression of the disease, including the PNPLA3, TM6SF2, and MBOAT7 genes, which are believed to be linked to the accumulation of fat in the liver and an elevated risk of developing cirrhosis. Genetic predisposition even when environmental variables are not a major risk factor, some people are more likely to have these genes, which increases their risk of the disease progressing to advanced stages.The progression of NAFLD is often influenced by the interaction between genetic predisposition and environmental factors. For instance, individuals with genetic risk factors like the PNPLA3 I148M variant may be more susceptible to developing severe NAFLD if they are also exposed to obesity, a poor diet, and physical inactivity. Conversely, lifestyle modifications like weight loss, exercise, and a healthier diet can potentially mitigate the impact of genetic predisposition on NAFLD progression(Juanola, O, *et al* .,2021).

Consuming industrial fructose has been linked to an increased risk of developing and developing non-alcoholic fatty liver disease (NAFLD), likely because to the stimulation of de novo lipogenesis and an increase in the ratio of dietary saturated

to unsaturated fat intake (Jensen T. *et al.,* 2018).

On the other hand, the role of red meat consumption has not been clearly established Accumulating evidence shows that hepatic fat and NAFLD are strongly inheritable conditions (Trépo E,*et al*.,2020).

2.2.1.5.DIAGNOSIS

Since NAFLD is linked to metabolic syndrome, altering one's lifestyle can help manage it in the short term, but few people can do this over time. For this reason, doctors use pharmaceutical therapy to treat patients as the condition worsens.(Wong, *et al* .,2018) Laboratory tests, physical examinations, and patient histories are currently used to determine the diagnosis. There are several reasons to test for NAFLD, including medical comorbidities like obesity and diabetes, abnormalities in the liver biochemistry like increased aminotransferases, and the identification of advanced liver disease characteristics such a firm liver or dyslipidemia.

- Biochemical diagnostic: According to the American Gastroenterology Association's (AGA) clinical care route, abnormal serum liver biochemistry is the first biochemical indicator that suggests a diagnosis of non-alcoholic fatty liver disease (NAFLD). Liver function tests may show a slight rise (often less than five times the upper limit of normal), despite the fact that most people with NAFLD and NASH are asymptomatic. It is crucial to emphasize that the level of hepatic damage linked to NAFLD/NASH is not reflected in the degree of aminotransferase increase (*Kanwal F,et al .,*2021).

- One significant comorbidity that is commonly observed in NAFLD patients is dyslipidemia, which is defined by hypertriglyceridemia, decreases in high-density lipoprotein cholesterol (HDL-C), and increases in very low-density lipoprotein (VLDL) and low-density lipoprotein cholesterol (LDL-C)(Shahab ,O *et al*.,2018).

According to this research, the severity of NAFLD, the onset of NASH, and liver fibrosis may all be correlated with lipid profile factors (Fan N*,et al .,*2019).

- Biopsy of the liver is the method of choice for diagnosing NAFLD and liver cirrhosis. However, this method is invasive and is not a suitable tool for follow-up (Decharatanachart P*,et al .,*2021).

- FibroScan was used for the measurement of liver stiffness and CAP via transient elastography (Shrestha R, et al .,2021). FibroScan based on ultrasonication is a new technique that allows rapid and noninvasive measurement of steatosis and fibrosis simultaneously, and the median values are used to quantify liver fibrosis and steatosis (Xiaotong Xu,*et al* .,2023)
- Another popular technique for estimating the degree of hepatic steatosis in a patient with an accidental rise of transaminases is abdominal ultrasonography (Rinella, M.E., *et al*.,2023).
- Because of its affordability and ease of use, traditional B-mode ultrasonography is frequently utilized for screening and medical examinations. According to recent data, ultrasound may reliably identify moderate hepatic steatosis, which can occur in as little as 5% of the liver's fat (Ballestri S, *et al*., 2021).
- Liver Elastography Modalities: The fundamental idea behind liver elastography is to send an acoustic impulse through the tissue, causing little tissue displacement and the creation of shear waves in the liver tissue. These waves move more quickly when there is stiffness present, such as in cirrhosis. Elastography can be performed using ultrasound or MRI (Honda, Y, *et al.,*2020).
- Magnetic resonance imaging (MRI), a sensitive imaging test, has been a fundamental technique for assessing NASH in recent years. When deep subcutaneous tissue and visceral adipose tissue in the abdomen were examined by MRI alone, for

instance, longitudinal reduction showed histological improvement of NASH (Shen .W, *et al*., 2022).

- CT (Computerized Tomography):CT scans are less expensive than magnetic resonance imaging (MRI) scans, are widely accessible, and enable quick data capture. On unenhanced CT images, the attenuation difference between the liver and spleen is most frequently used to evaluate hepatic steatosis. By using the spleen as an internal control, the differences in liver parenchyma attenuation measurements between various CT scanners and reconstruction techniques are lessened (Lennartz, S, *et al .,*2021).

2.2.1.6.Treatment

Conservative treatment - lifestyle modifications and weight loss

One of the primary subjects and likely one of the biggest challenges of clinical and experimental research in liver diseases over the past ten years has been the development of efficient treatment strategies to prevent the progression to cirrhosis and its sequelae, thereby reducing liver-related morbidity and mortality in patients with MASLD/MASH. Among the many difficulties have been the following: (1) the requirement to create surrogate endpoints due to the slow clinical progression of liver disease; (2) the absence of verified serological/biochemical biomarkers linked to the progression of liver disease, which necessitated repeated histological evaluation to ascertain treatment response; (3)the inherent limitations and unpredictability of evaluating liver pathology, which have been exacerbated by inconsistent outcomes shown in published randomized clinical studies' placebo arm; (4)the general public's lack of awareness, which has had a detrimental effect on clinical trial enrollment based on repeated histological liver evaluation; and (5) the lack of understanding of disease pathogenesis, which initially caused pharmacolog-

ical approaches to be considered that did not address the primary disease driver, namely hepatic steatosis (Pelusi S,*et al*.,2019).

To date, there is no specific drug treatment for NAFLD, however it is believed that a combination of treatment goals (lifestyle adjustments, increasing physical activity and smoking/ alcohol cessation) can be beneficial (Romero-Gómez M, *et al*.,2017). Dietary changes ,Severely restricting total calories, inducing ketosis, or reducing free sugar intake and limiting carbohydrate consumption can enhance liver protection, thereby making the dietary interventions a promising strategy for the treatment of NAFLD . Caloric restriction (CR) is the most common dietary intervention treatment strategy for NAFLD (Risi R,*et al* .,2021).

Due to this, there was no licensed medication for MASLD/MASH until March 14, 2024, and clinical guidelines mostly addressed weight loss, lifestyle modifications, and managing related metabolic diseases (Francque SM, *et al*., 2021, Pugliese N, *et al*., 2022).

Therefore, the recent FDA conditional approval of resmetirom (previously known as MGL-3196, which will be marketed under the name "Rezdiffra"), an oral liver-targeted thyroid hormone receptor (THR)-β selective medication for the treatment of adults with moderate to advanced fibrosis and non-cirrhotic MASH, should be considered a breakthrough for the MASH community. Results from the phase 3 MAESTRO clinical program, which was intended to assess a variety of safety and effectiveness endpoints, were the primary basis for the FDA's conditional approval of resmetirom (Harrison SA, *et al* 2024).

2.2.2 NON-ALCOHOLIC STEATOHEPATITIS (NASH)

NASH was first described in 1980 and represents a state of chronic liver inflammation. NASH is currently defined as very heterogeneous, especially according to the presence or absence of fibrosis(Ludwig J,*et al.,*1980).

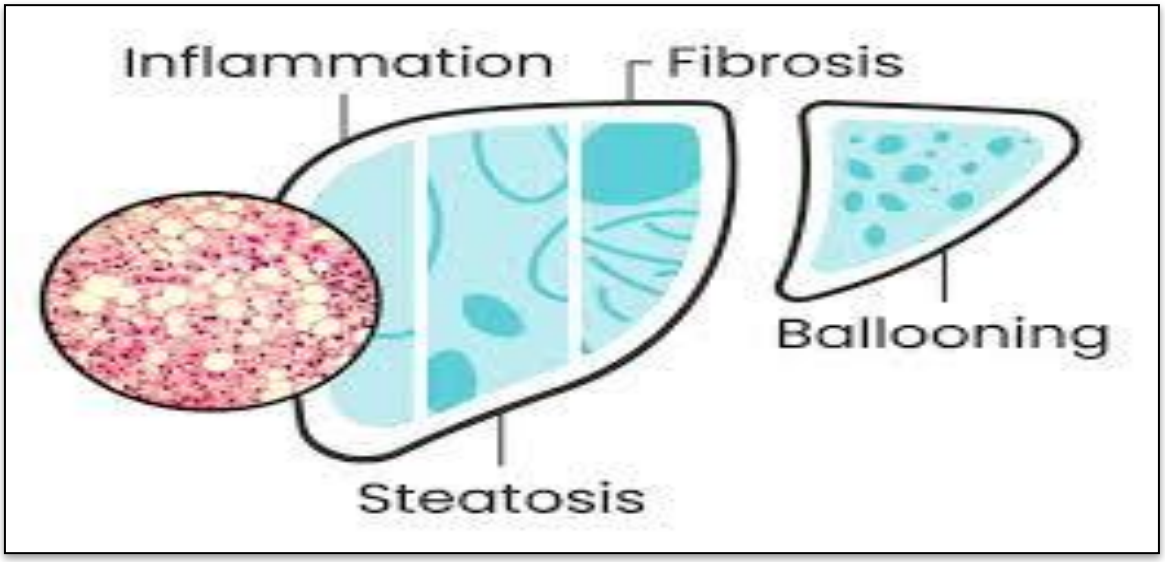
Non-alcoholic steatohepatitis (NASH) is a severe form of non-alcoholic fatty liver disease (NAFLD), characterized by the presence of liver inflammation and hepatocyte injury (ballooning) due to fat accumulation(Chalasani N, *et al*.,2018).

Although it develops typical in the absence of excessive alcohol consumption, NAFLD is related to an unhealthy Diet and a lack of physical activity. The current definition of NASH is highly variable, particularly in terms of whether fibrosis is present or not. A biopsy showing histological evidence of hepatocellular ballooning degeneration, hepatic lobular inflammation, and hepatic steatosis is necessary for a diagnosis of NASH (Sberna AL,*et al* .,2018). NASH is associated with higher levels of aminotransferases, older age (>60 years), Hispanic ethnicity, and underlying metabolic disorders (obesity, diabetes mellitus, or hypertension) (Schuppan D,*et al.,*2018).

There are currently very few alternatives for treating NASH, and the main advice are to lose weight and change one's lifestyle (Dufour JF, *et al.,* 2022). Nonetheless, it is acknowledged that the primary cause of the development of NASH from basic steatosis is necro-inflammation. Knowing the pathophysiological processes that cause liver inflammation in NASH and how inflammatory alterations can lead to fibrotic reactions and hepatic carcinogenesis is therefore important. The adaptive immune system becomes more successful in maintaining chronic hepatic inflammation when liver injury occurs, but the innate immune response is initially

more pronounced. Notably, the main factor causing NASH to develop to liver fibrosis and/or cirrhosis is persistent hepatic inflammation(Sutti S,*et al*.,2020).

Furthermore, oxidative stress, a typical characteristic of NASH pathogenesis, causes hepatocytes and liver endothelial cells to release proinflammatory cytokines and chemokines, which are essential for lymphocyte migration to the hepatic parenchyma. By secreting pro-inflammatory chemicals, T and B lymphocytes that migrate to the liver interact with antigens resulting from oxidative stress and actively contribute to liver damage(Sutti S,et al.,2020).



Figure(2-3) Non-alcoholic steatohepititis (https://somalogic.com/mash-nash/)

2.2.3. liver cirrhosis

Cirrhosis is an important cause of morbidity and mortality among patients with chronic liver disease(Ginès P, *et al .,2021*). Cirrhosis can lead to hepatocellular carcinoma (HCC) and hepatic decompensation, including ascites, hepatic encephalopathy and variceal bleeding and is a leading cause of death worldwide (Tapper EB,*et al .,*2022).

Chronic liver inflammation causes cirrhosis, which is followed by widespread hepatic fibrosis, in which regenerating hepatic nodules replace the normal hepatic architecture and ultimately result in liver failure. Not all patients with chronic liver inflammation develop cirrhosis, but when they do, the rate of advancement varies from weeks in individuals with total biliary blockage to decades in patients with longer-term causes, like viral hepatitis C. A comparatively brief symptomatic phase of months to years may follow the asymptomatic (initial) phase of cirrhosis.Typically referred to as decompensated cirrhosis, the symptomatic phase is linked to a number of problems that lead to frequent hospitalization, a decline in the quality of life for patients and their caregivers, and patient death in the absence of liver transplantation. (*Fabrellas N, et al .,*2020)

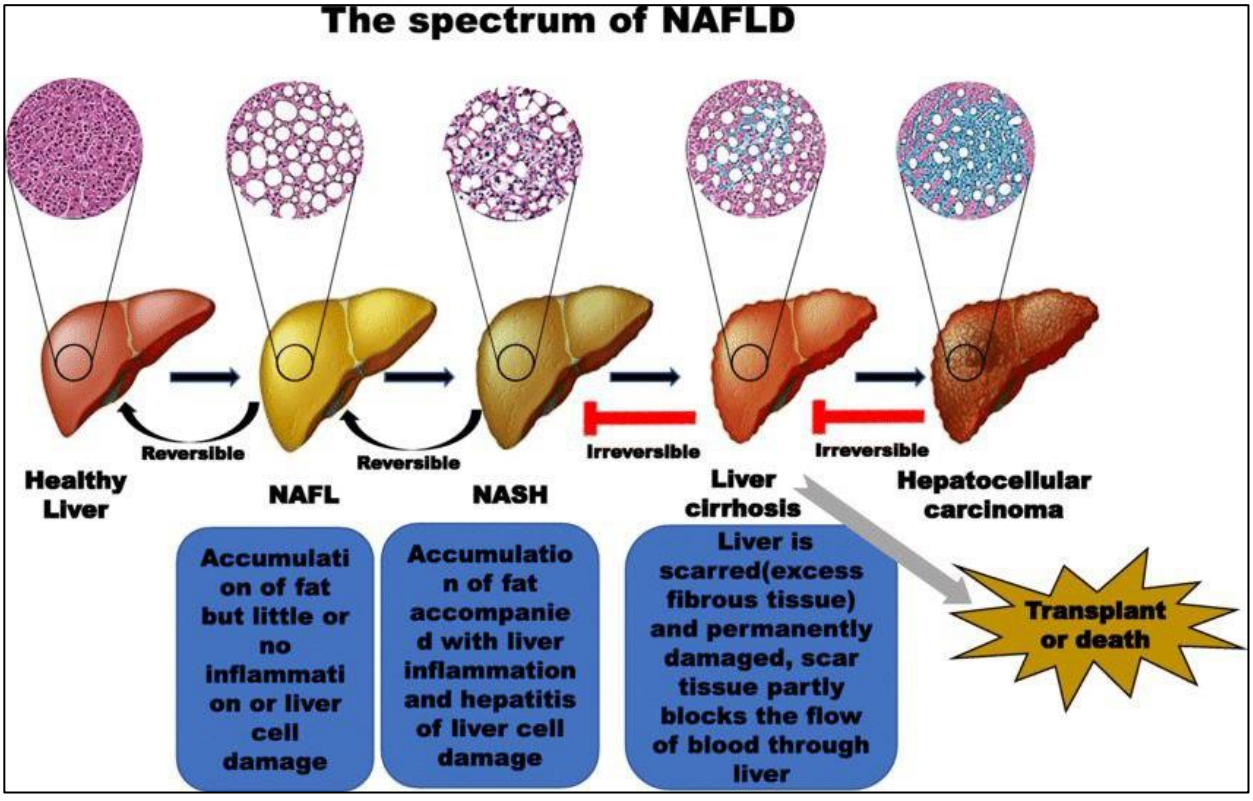


Figure (2-4)Spectrum of NAFLD(Wang,*et al.,2020)*

2.3 Parameter In Non Alcoholic Fatty Liver

2.3.1 Glypican-4

In 1995, Watanaba *et al*. made the initial discovery of Glypican 4 (Gpc4) in the kidneys and brains of mice . In 2012, Ussar et al. verified that Gpc4 is a recently discovered adipokine released by adipose tissue. The glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan family includes Gpc4.

The cell surface proteoglycan known as adipokine glypican-4 (GPC-4) has been linked to metabolic syndrome, obesity, diabetes mellitus, bone formation, and non-alcoholic fatty liver disease (NAFLD) (*Ning, D. P. et al.,2019).* By secreting adipokines, adipose tissue functions as an endocrine organ; obesity can dysregulate this process. GPC-4 enhances insulin receptor signaling in the liver and skeletal muscle cells while simultaneously promoting adipocyte differentiation. Insulin-resistant obese human participants have twice as high levels of GPC-4, an independent indicator of insulin resistance, as compared to their BMI-matched insulin-sensitive controls. Glypican-4 acts directly on the insulin receptor, in contrast to other insulin sensitizers (Tamori, Y.*et al.,2013*).

In Asian women, Yoo *et al*. discovered a strong correlation between blood Gpc4 levels and cardiovascular risk markers such as insulin resistance (IR) and body fat distribution (Yoo HJ, *et al*.,2013).

The body-mass index (BMI) and waist-to-hip ratio (WHR) are correlated with GPC-4, which was more highly expressed in subcutaneous tissue than visceral adipose tissue in lean adults (Altinkaya,S.O, *et al.,*2021). BMI and WHR increased when GPC-4 expression in subcutaneous tissue decreased, while BMI and WHR increased when GPC-4 expression in visceral adipose tissue increased. A new adipokine called Gpc4 might be very important in metabolic diseases. Consequently,

it makes sense for us to question whether Gpc4 and MetS are related (Zhu HJ,*et al*.,2014).

Additionally, serum GPC-4 levels were linked to bone formation and renal insufficiency. They also showed positive correlations with the HOMA-IR score, fasting insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and systolic blood pressure (SBP) (Ning, D. P. *et al.,*2019). When examined within the framework of type 2 diabetes According to research on type 2 diabetes mellitus, patients with impaired glucose tolerance had higher GCP-4 levels, whereas those with a diagnosis of the disease had lower GCP-4 levels (Li, K., *et al*., 2014).

2.3.2 Pentraxin-3

Pentraxins are proteins made up of five monomers arranged in a radial symmetry ring. These receptors belong to a class that recognizes patterns. Pentraxin-3, CRP, and serum amyloid P component are the three primary pentraxins. At the location of inflammation, endothelial and vascular smooth muscle cells primarily produce the long-chain pentraxin known as PTX3, which is regarded as an acute phase marker. Macrophages, fibroblasts, neutrophils, epithelial cells, dendritic cells, and other cell types both close to and distant from the site of inflammation also produce it (Zhang,*et al*.,2012). Also known as tumoral necrosis factor (TNF)-inducible gene 14 protein, PTX3 causes complement activation and acts as a chemo-attractant factor for various inflammatory cells (Bottazzi, B. *et al.,*2016).

The prototypic long pentraxin, pentraxin 3 (PTX3), was identified in the early 1990s in human endothelial cells and fibroblasts as a tumor necrosis factor-alpha of (TNF-a) or interleukin 1ß inducible compound. Despite similarities of action, PTX3 differs from CRP in many basic aspects, such as gene organization, chromo-

somal localization, cellular sources, inducing stimuli, and the recognized ligands (Bottazzi B,*et al* .,2009).

The levels of plasma PTX3 are found higher than normal controls in various inflammatory conditions such as rheumatologic disorders, (Deniz T, *et al.,*2014) asthma (Zhang J, *et al.,*2012), coronary artery diseases (Latini R, *et al .,*2004), vasculitis, and systemic inflammation and sepsis. Because NAFLD is also an ongoing inflammatory condition, we hypothesized that plasma PTX3 levels increase in patients with NAFLD and aimed to investigate the potential relationship of plasma PTX3 levels with the degree of liver damage of NAFLD patients (Fazzini F, *et al .,*2001).

Several innate immune system cells, mainly dendritic cells, macrophages, fibroblasts, and activated endothelial cells, produce PTX3 in response to pro-inflammatory stimuli like lipopolysaccharides (LPS), interleukin-1β (IL-1β), and tumor necrosis factor alpha (TNF-α), which were all thought to be crucial elements in the pathophysiology of NAFLD and NASH (Makhlouf, M,*et al.,*2019).

Increased levels of PTX3, an independent indicator of inflammation linked to a number of conditions such atherosclerosis, cancer, respiratory illnesses, and central nervous system disorders, are linked to the likelihood of developing the disease or its advancement (Rajkovic, *et al.,* 2016). According to earlier research, PTX-3 has great promise as a novel early diagnostic and prognostic biomarker in patients with infectious diseases and septicemia (Liu S, *et al.,* 2014). Other coronary risk variables, such as total cholesterol, high-density lipoprotein (HDL) cholesterol, hemoglobin A1C, smoking status, gender, and obesity, have also been demonstrated to be unrelated to plasma PTX3 level (Inoue K,et al., 2007).

2.3.3 Triglyceride–Glucose (TYG) Index

The newly created triglyceride–glucose (TyG) index is thought to be the best substitute for insulin resistance in the general population. It may be simply computed using fasting blood glucose (FBG) and triglyceride (TG) levels (Hong S. *et al.,* 2020). The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) is another surrogate indicator that is less appropriate for assessing insulin resistance than the TyG index (Britto ADM, *et al.,* 2021). The TyG index was strongly linked to elevated incidence of MS and NAFLD, BMI, total cholesterol (TC), TG, FBG, HbA1c levels, and HOMA-IR (Alizargar J,*et al.,2021)*.

Additionally, earlier research demonstrated a strong correlation between NAFLD and lipid and glucose metabolism. The formation and subsequent progression of nonalcoholic fatty liver disease (NAFLD) are significantly influenced by lipotoxicity and glucotoxicity, which start when hepatocytes are exposed to elevated lipid and glucose levels, respectively (Mota M,*et al*.,2016). As a result, the TyG index has been suggested as a straightforward and trustworthy way to identify those who are at risk for NAFLD (Guo W, *et al.,*2020).

However, there aren't many research comparing the discriminative capacities of the TyG index, lipid parameters, and glycemic parameters for NAFLD risk, particularly in patients with type 2 diabetes, or examining the relationship between the TyG index and NAFLD. The increase in the number of people with non-alcoholic fatty liver disease (NAFLD) is most likely due to impairment of the glucose and lipid metabolic pathways, which has been fueled by the prevalence of obesity and type 2 diabetes (Kitae A,*et al.,2019*).

2.3.4 Alanine Aminotransaminase (ALT) And Aspartate Aminotransferase (AST).

Alanine aminotransferase (ALT),a commonly used marker to assess hepatocyte damage, is a well-established indicator of non-alcoholic fatty liver disease (NAFLD). It has a strong connection to the prevalence of NAFLD.(Li, *et al*.,2022) Elevated ALT levels are also linked to NAFLD with a more severe histological spectrum, including fibrosis and non-alcoholic steatohepatitis (NASH) (Mansour-Ghanaei, *et al .,*2019).

These enzymes are normally detectable in the serum at low concentrations, typically <30 IU/L (Kalra A, *et al.,*2023). However, any process that leads to loss of hepatocyte membrane integrity or necrosis releases these enzymes into the blood, where the elevated concentrations can be measured (Hustead TR, *et al.,*2017). Several physiological and risk factors may contribute to the serum levels of these enzymes, including age, sex, body mass index, pubertal age, elevated levels of triglycerides, insulin resistance, and blood glucose level (Bussler S,*et al.,*2018)(Chen KW,*et al.,*2018). Since AST can rise as a result of heart or muscular damage, ALT is thought to be a more specific indication of liver illness than AST. In diseases like autoimmune hepatitis (AIH) and alcohol-related liver disease, AST levels may be a more sensitive predictor of liver (Su C.W,*et al*.,2009).

Aspartate aminotransferase AST is another hepatic transaminase that plays a role in diagnosis of steatohepatitis and it seems that this hepatic index is related to metabolic syndrome and BMI (Hsieh MH, *et al.,*2009).

The most frequent anomaly observed in patients with underlying liver illness is a persistent increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are indicators of hepatocyte damage (Agrawal S, *et al* .,2016). Several studies have demonstrated that high ALT levels are correlated with a high-

er risk of NASH.(Ulasoglu *C,et al .,2019*) Some studies have shown that patients with a normal ALT level may also have histological features of NASH and are at risk of disease progression (Ma X, *et al.,*2020). Patients with NAFLD tend to have a ratio less than 0.8, especially early in the disease. However, as NAFLD progresses to NASH with fibrosis, the AST:ALT ratio increases (Newsome PN,*et al.,*2018).

2.3.5 Lipid Profile

It is postulated that deposition of lipids, primarily triacylglycerol (TAG) in the hepatic cells is the main element of the pathogenesis of NAFLD, however, the exact mechanism is still unclear. In obese individuals, dyslipidemia characterized by increased levels of triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-C) levels and decreased levels of high-density lipoprotein cholesterol (HDL-C) is observed. Obesity is the most common cause of secondary dyslipidemia (Iqbal U*, et al.* 2018).

Liver lipid levels are regulated by the interplay between the delivery of lipids to the liver and their hepatic uptake, synthesis, oxidation, and secretion within very lowdensity lipoproteins (VLDLs). Alterations in the equilibrium of one or more of these processes can promote hepatic steatosis (Meex RCR, *et al .,*2017).

2.3.6 INSULIN RESISTANCE

Clinical definitions of insulin resistance are even more specific. It is quantified as insulin's capacity to preserve glucose homeostasis. The hyperinsulinemic euglycemic clamp, which prevents plasma glucose levels from dropping by infusing insulin at a high, constant rate and glucose at a variable rate, is the gold standard for assessing insulin resistance. Insulin sensitivity can be inferred from the amount of glucose needed to maintain normoglycemia. Plasma glucose, plasma insulin, or a combination of these, such as the homeostatic model assessment of insu-

lin resistance (HOMA-IR), are also utilized as surrogate indicators of insulin resistance (Ye FZ, *et al.,* 2020).

Several studies maintain that blood glucose has a role in the onset and progression of non-alcoholic fatty liver disease. More precisely, in individuals with non-alcoholic fatty liver disease (NAFLD), 24-hour glucose concentrations as shown by the area under the curve (AUC) value increase the degree of hepatic de novo lipogenesis (DNL).(Smith GI*, et al.,* 2020) Additionally, the connection between hyperglycemia and NAFLD is confirmed in vitro as well as in vivo. Primary human hepatocytes accumulate lipids when glucose is added (Huggett ZJ, *et al*., 2022). High glucose increases oxidative stress, which causes HepG2 cells to undergo apoptosis (Chandrasekaran K,*et al .,2010*).

Chapter three

Material And Method

3.1. Materials

3.1.1. Apparatus Analysis And Equipment

The apparatus and equipment used in this work are listed in table (3-1)

Table (3-1):The apparatus and equipment

NO	Apparatus And Equipment	*Company*	*Origin*
1.	5ml syringe	Dolphin	Syria
2.	Cotton	Al Salama	Iraq
3.	Tourniquet	Voltaren	China
4.	Gel tube	Xinle	China
5.	EDTA Tube	Vacuum Blood Collection Tube	China
6.	Micropipette	Slamed	Germany
7.	Blue tips	Trust Lab	China
8.	Hitachi CUP	Hitachi	India
9.	Centrifuge Tubes Racks	Trust Lab	China
10.	Micropipettes	Micropipettes	Germany
11.	Centrifuge	Rotofix 32 A (Hettich)	Germany
12.	DIRUI	Auto Chemistry Analyzer (Cs-T180)	China
13.	Elisys uno	Human	Germany
14.	Refrigerator	Kiriazi	Egypt
15.	Abbotte	Architect	Germany

3.1.2 Kits

Kits were used according to the manufacturer and origin, as shown in the following table (3-2).

Table (3-2):The Kits.

No	Kits.	Company	Origin
1.	Human Pentraxin-3 (PTX3) Serum Le Elisa Kit	Pars Biochem	China
2.	Human Glypican-4(GPC4) Serum Le Elisa Kit	Pars Biochem	China
3.	Alanine Aminotransferase(ALT) Kit	DIRUI	China
4.	Aspartate Aminotransferase (AST) Kit	DIRUI	China
5.	Cholesterol Kit	DIRUI	China
6.	Low Density Lipoprotein (LDL)	DIRUI	China
7.	High Density Lipoprotein (HDL)	DIRUI	China
8.	Triglyceride Kit	DIRUI	China
9.	Insulin Kit	Architect	Japan
10.	Glucose Kit	DIRUI	China

3.2 . Methods

3.2.1. Study Design

Case-control research was conducted on 120 people suffering from fatty liver and healthy between November 2023 to April 2024. Samples of patients were taken from Babylon gastroenterology-hepatology center. The patients' socio demographic data, including their ages, genders, weights, heights. The study design demonstrated in figure (3-1)

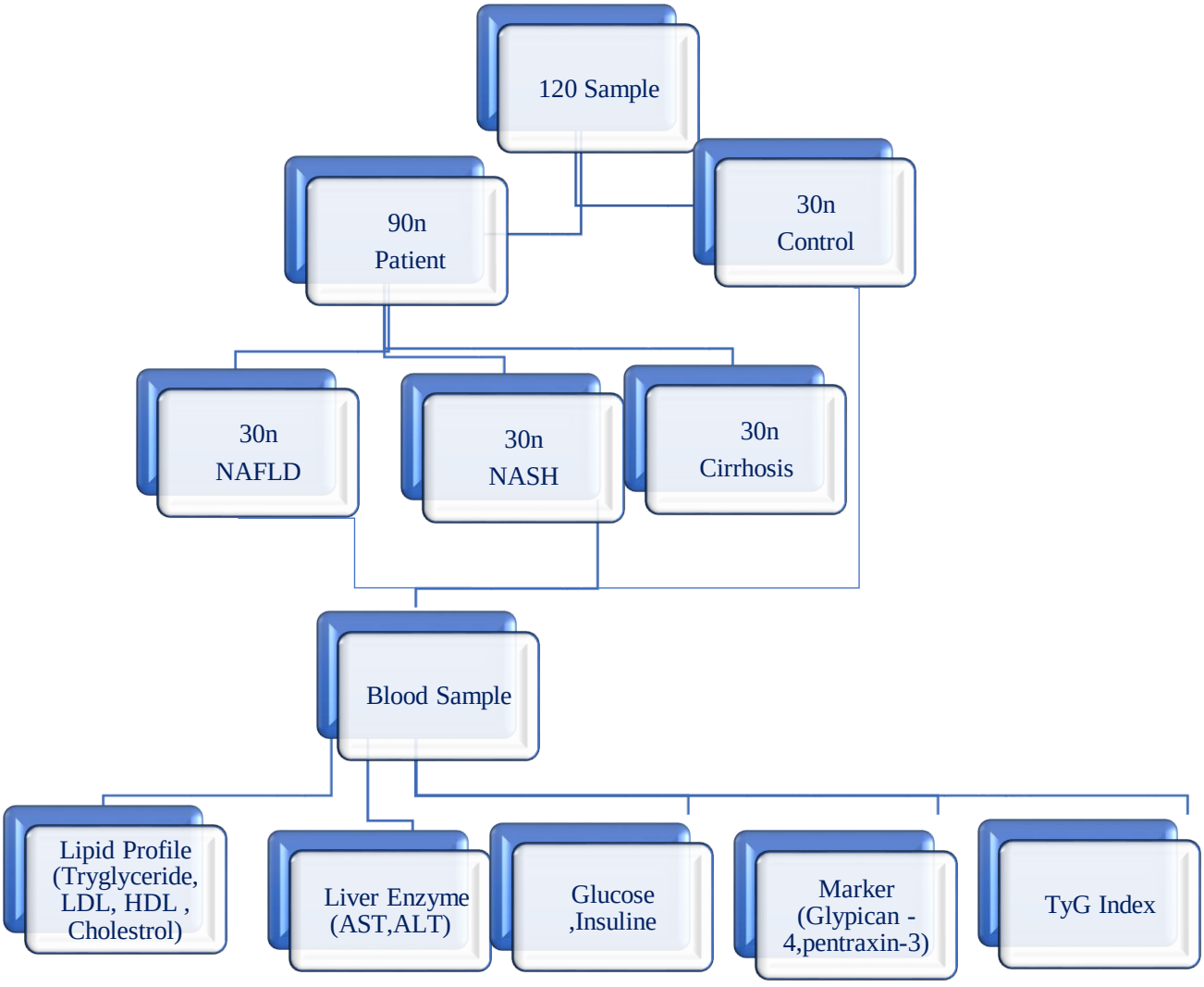


Figure (3-1): The study design

3.2.2. Subjects

The current study included 120 participants, 30 patients suffering from NAFLD,30 patients suffering from NASH ,30 patients suffering from cirrhosis and the control group which included 30 subjects.

3.2.2.1. Collection Of Samples

Using a plastic syringe and the usual safety precautions for venipuncture, five milliliters of venous blood were extracted in an aseptic manner and separated into two portions:

1. For the insulin and glucose tests, one milliliter was put to the EDTA tube.

2. After four milliliters were placed in a gel tube and allowed to coagulate for half an hour, the serum was separated for parameter analysis and centrifugations were performed for 20 minutes at 1500 xg.

3.2.2.2 Exclusion Criteria And Inclusion Criteria

Exclusion Criteria

Patients were excluded if they had

- Current or past consumption of alcohol.
- Patients with malignancy especially Hepatocellular Carcinoma or history of malignancy.
- Autoimmune hepatic disease or celiac disease. chronic hepatic diseases.
- Taking medicines for established diabetes and dyslipidaemias.
- having chronic and acute liver disease including viral hepatitis C, B. Chronic or acute kidney disease.
- Pregnancy.

- Taking medications affecting the liver such as steroids, amiodarone, tamoxifen. Patients with proven hemochromatosis
- missing data on age, sex, blood pressure (BP), body mass index (BMI), laboratory data or ultrasonic examination for liver.
- abnormal copper metabolism or thyroid function tests.

Inclusion Criteria

- people with non- alcoholic fatty liver disease , or NASH or cirrhosis
- Apparently healthy subject, who served as control group.

3.2.3 Ethical Management Of Studies

The College of Applied Medical Science/University of Karbala's Ethical Committee gave its approval to this project. Prior to sample collection, all participants in this study were informed and verbally consented to participate

3.2.4 Measurement Of Body Mass Index

The following equation was used to determine body mass index :

BMI = Weight (kg)/Height (m^2)

The present discourse examined the Body Mass Index (BMI) and its associated concerns, pathological conditions, and clinical relevance. It was vital to comprehend the existing threshold values for each BMI category as established by the World Health Organization (WHO), which provides the following delineations:

- Severely Underweight: < 16 kg/m^2
- Underweight: 16.0 - 18.4 kg/m^2
- Normal weight: 18.5 - 24.9 kg/m^2
- Overweight: 25.0 - 29.9 kg/m^2
- Moderately Obese: 30.0 - 34.9 kg/m^2
- Severely Obese: 35.0 - 39.9 kg/m^2 .

3.2.5 ELISA Kit Of Human Pentraxin-3 (PTX3) Serum Level.

3.2.5.1 Principle Of The Assay

The kit assay Human PTX3 level in the sample ， use Purified Human PTX3 antibody to coat microtiter plate wells, make solid-phase antibody, then add PTX3 to wells, Combined PTX3 antibody which With HRP labeled ， become antibody-antigen- enzyme-antibody complex, after washing Completely, Add TMB substrate solution, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of PTX3 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Table (3.3): ELISA kit of PTX3 serum

Item	Suppliers
User manual	
Closure plate membrane	
Sealed bags	
Microelisa stripplate	
Standard：27pg/ML	

Standard diluent	**(China)**
HRP-Conjugate reagent	
Sample diluent	
Chromogen Solution A	
Chromogen Solution B	
Stop Solution	
wash solution	

3.2.5.2 Assay Procedure

1. Dilute and add sample to Standard: 10 Standard wells were placed on the coated ELISA plates, and 100µl of Standard was added to each well. Next, Standard dilution was added to each well, mixing the two wells; finally, 100µl was removed from the first and second wells and added separately to the third and fourth well. Standard dilution 50µl was then added to the third and fourth wells, mixed; we then removed 50µl from the third and fourth wells, added 50µl to the fifth and sixth wells, and added Standard dilution 50µl to the fifth and sixth wells, mixed, we put 50µl from the fifth and sixth well and added it to the seventh and eighth well, then we added 50µl of the Standard dilution to the seventh and eighth well, mixed, and then we put 50µl from the seventh and eighth well and added it to the ninth and

tenth well, added Standard dilution 50μl to the ninth and tenth well, mixed, and removed 50μl from the ninth and tenth well discard (added Sample 50μl to each well after diluting, density: 18 pg/ML，12 pg/ML ，6 pg/ML，3 pg/ML，1.5 pg/ML)

2. Add a sample. Set the blank wells apart (the HRP-Conjugate reagent and sample are not added to blank comparison wells; all other steps are the same). testing the sample effectively. We add 40 μl of the sample dilution to the testing sample well, followed by 10 μl of the testing sample (the final dilution is five times), add the sample to the wells, avoid touching the well wall as much as possible, and mix gently

3.Incubate: After closing plate with Closure plate membrane , we incubate for 30 min at 37℃.

4.Configurate liquid: 30-fold (or 20-fold)wash solution diluted 30-fold (or 20-fold) with distilled water and reserve.

5.Washing：Uncover Closure plate membrane, discard Liquid, dry by swing, added washing buffer to every well, still for 30s then drain, repeated 5 times, dry by pat.

6.Add enzyme ：Added HRP-Conjugate reagent 50μl to each well, except blank well.

7.Incubate：Operation with 3.

8.Washing：Operation with 5.

9.Color： we add Chromogen Solution A 50 μl and Chromogen Solution B to each well, evaded the light preservation for 15 min at 37℃

10.Stop the reaction： Added Stop Solution50μl to each well, Stop the reaction(the blue color change to yellow color).

11.Assay：taken blank well as zero , Read absorbance at 450nm after Adding Stop Solution and within 15min.

3. 2.5.3 Calculate

Take the standard density as the horizontal, the OD value for the vertical ,draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard density and the OD value ,with the sample OD value in the equation, calculate the sample density, multiplied by the dilution factor, the result is the sample actual density.

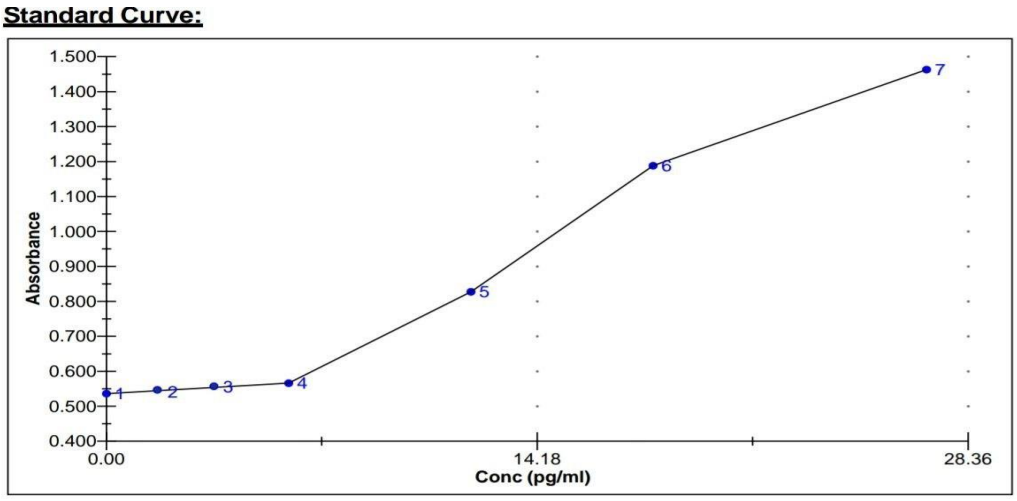


Figure (3-2) Standard Curve Of Human Pentraxin-3

3.2.6 ELISA Kit Of Human Glypican-4(GPC4) Serum Level

3.2.6.1 Principle of the assay

As 3.2.5.1

3.2.6.2Assay procedure

As 3.2.5.2

3. 2.6.3 Calculate

As 3.2.5.3

3.2.7 Measurement of TYG INDEX

TYG index = LN [Fasting triglyceride (mg/dl) × fasting glucose (mg/dl)]/2

3.2.8 Measurement Of Lipid Profile

Lipid Profile (Cholesterol ,Triglyceride, HDL) was measure automatically by DIRUI (Auto Chemistry Analyzer CS_ T180)

3.2.9 Measurement Of insulin resistance

Insulin was measure automatically by Abbotte (Architect), Glucose was measure automatically by DIRUI (Auto Chemistry Analyzer CS_ T180)

The HOMA-IR index was calculated as the product of the fasting plasma insulin level (in millunits per liter) and the fasting plasma glucose level (in millimoles per liter), divided by 22.5.

3.2.10 Measurement Of Liver enzymes

Liver enzymes (AST,ALT) were measured automatically by DIRUI (Auto Chemistry Analyzer CS_ T180).

3.3 Statistical analysis

All data were analyzed using SPSS software (V.28 Inc., Chicago, USA) and Microsoft Excel 2019. Table analysis of variance (ANOVA Table) and the least significant difference [T-test and chi-square] below the significant level of ($P \leq 0.05$)

Chapter Four

Result And Discussion

4.1 Demographic Characteristics Of Study Groups

The current study was conducted on (120)people suffering from fatty liver and healthy. We take 30 sample suffering from NAFLD patient (group1)and 30 sample suffering from NASH (group2),30 sample suffering from cirrhosis(group3) and 30 sample as a control .

Table (4.1):Category of study

Category	Groups	N
Patient	Group 1- NAFLD	30
	Group 2- NASH	30
	Group 3- Cirrhosis	30
Healthy	Control	30

In table (4-2) In this study, the age differences between groups 1, 2, 3, and control were statistically significant ($p \leq 0.05$). After comparing the three groups (G1, G2, and G3) to the control, group 3's mean (53.00±15.31) was greater than the control group's (43.80±15.09).

The results showed that all three groups (groups 1, 2, and 3) were more valuable than the control group; however, group 1 had the highest value, with a mean of (26.13±5.10), higher than the control group's mean of (21.41±2.73). The BMI differences between the groups are significant.

Table (4-2): Demographic characteristics in patient suffering from NAFLD, NASH, cirrhosis compared to healthy volunteer.

Variable	Sample	N	Mean ±S. D	P.V	LSD
Age (19-70) years	Group1	30	41.13 ±12.10	0.02	7.40
	Group2	30	45.80 ±15.72		
	Group3	30	53.00 ± 15.31		
	Control	**30**	**43.80 ±15.09**		
BMI Kg/m^2	Group1	30	26.13 ±5.10	<0.001	2.46
	Group2	30	24.80 ±5.35		
	Group3	30	24.87 ± 5.72		
	Control	**30**	**21.41 ± 2.73**		

N:number ,S.D: standard deviation, P.V: p-value, BMI :body mass index, LSD: Less significance deference

Age is a risk factor for disease-specific mortality in NAFLD (Golabi P,et al.,2021). However, this association may simply reflect longer exposure to hepatic steatosis and, therefore, a higher prevalence of cirrhosis in older patients. In older patients with Non-Alcoholic Fatty Liver Disease (NAFLD), cirrhosis can develop due to prolonged exposure to risk factors like obesity, diabetes, and metabolic syndrome, which accelerate liver damage. As people age, the liver's ability to regenerate declines, and comorbidities like cardiovascular disease can worsen liver function. Additionally, NAFLD can progress to Non-Alcoholic Steatohepatitis (NASH), which leads to inflammation, fibrosis, and eventually cirrhosis. In contrast, among patients without cirrhosis, whether young adults with NAFLD have a more benign disease trajectory than older persons with NAFLD is unknown. Young adults with NAFLD may have a lower burden of metabolic diseases, notably diabetes (Bardugo A, et al.,2021).

Higher amounts of body fat can be seen as predisposing factors for the development of NAFLD and NASH . According to Fan R. *et al*., 2018, obesity is a risk factor for the development of non-alcoholic fatty liver disease (NAFLD), and the prevalence of NAFLD rises in tandem with BMI. However, obesity and NAFLD are not inseparable because many obese people have normal intrahepatic content, whereas many lean people develop NAFLD even when they do not have insulin resistance, type 2 diabetes (T2D), or other metabolic comorbidities (Yilmaz Y, *et al.,*2014).

Bellentani *et al* .,2000, first findings from the Dionysos Study showed that 16% of normal-weight participants had NAFLD. One of the first studies in nonobese Asian populations reported a NAFLD prevalence > 23%, and many of the same characteristics observed in NAFLD patients with obesity, including male sex, higher BMI, older age, hyperuricemia, and elevated metabolic markers, were also common among nonobese (Kim HJ,et al .,2004).

Vos *et al*.,2011 identified "lean-NAFLD," a novel clinical category that refers to the prevalence of NAFLD in nonobese people (BMI < 30 kg/m2). However, because body weight is not a component of the diagnostic criteria for NAFLD, and describing NAFLD itself as lean is imprecise, “NAFLD in lean individuals” has been suggested as a more accurate description of this condition (Albhaisi S, et al., 2019).

4.2 The Relationship Between Biomarker In Patients And Controls.

A statistically significant difference ($p \leq 0.05$) was seen in serum of TYG and GLY-4 level , but not in PEN-3, according to the results presented in Table (4-3).Group1, group2, and group3 had a mean in TYG that is greater than the control (4.66±0.09) but still fairly close.

The group mean (group 1, group 2, group 3) in GLY-4 is higher than the control mean (1.03±0.77), with the highest mean (group 3) being (2.53~±1.75) overall. Group 1 mean was greater than group 2, and group 3 mean was(4.05±7.99) higher than those of the other groups and the control (1.16±2.12) in PEN-3.

Table (4-3) The change in biochemical parameter level of non- alcoholic fatty liver disease compared to control group.

variable	Sample	N	Mean ± S. D	P.V	LSD
TYG	**Group1**	30	5.09 ± 0.26	<0.001	0.10
	Group2	30	5.26 ± 0.21		
	Group3	30	5.26 ± 0.18		
	Control	**30**	**4.66 ± 0.09**		
GLY-4 ng/ml	**Group1**	30	1.26 ± 0.98	<0.001	0.81
	Group2	30	2.46 ± 2.36		
	Group3	30	2.53 ± 1.75		
	Control	**30**	**1.03 ± 0.77**		
PEN-3 pg/ml	**Group1**	30	2.83 ± 3.66	0.13	n.s
	Group2	30	2.37 ± 2.97		
	Group3	30	4.05 ± 7.99		
	Control	**30**	**1.16 ± 2.12**		

N:number ,S.D: standard deviation, P.V: p-value, TYG: triglyceride –glucose index,GLY-4: glypican-4 ,PEN-3:pentraxin-3, LSD: Less significance deference

An alternate technique for assessing insulin resistance is the triglycerides and glucose (TyG) index. Serum triglycerides and fasting plasma glucose are regular and reasonably priced biomarkers that are used to generate the TyG index. In a population comprising patients with type 2 diabetes mellitus (T2DM), the TyG index was initially demonstrated to be correlated with a glucose clamp assessment of insulin sensitivity (Dowman J.K., *et al*., 2011). Since then, it has been applied to a number of other populations, including children and adolescents. TyG index is elevated in NAFLD patients due to insulin resistance, which promotes the accumulation of triglycerides and glucose. This occurs because insulin resistance increases liver fat production (lipogenesis) and impairs fat metabolism. Additionally, metabolic syndrome and chronic low-grade inflammation, which are common in NAFLD, worsen insulin resistance and elevate triglycerides and glucose levels. These factors together contribute to higher TyG values, making it a useful marker for assessing metabolic dysfunction in NAFLD patients (Oka R. *et al.,* 2009).

Remarkably, it has been demonstrated that the TyG index can forecast the incidence and prevalence of NAFLD in the general population (Hossain S,*et al.,*2020). It was previously recommended as being successful in screening a small group of asymptomatic women for NAFLD and NASH (Guo W,*et al.,*2020).

According to other research, the TyG index was a better predictor of NAFLD than the most widely used formula for evaluating insulin resistance in clinical practice, the homeostasis model assessment of insulin resistance (HOMA-IR) (Kim M.K., et al.,2019). In individuals with nonalcoholic fatty liver disease (NAFLD), the TyG index was also strongly correlated with the degree of liver steatosis and the existence of liver fibrosis as assessed by transient elastography (Inavolu P et al., 2018).

This study Agree with Amzolini AM, *et al*., 2022.In reference to the TyG index as a NASH marker, we discovered statistically significant differences ($p \leq 0.005$)

between the TyG index values in individuals who did not have NASH and those who had definite NASH based on histological criteria.

Adipokine glypican-4 (GPC-4) is a cell surface proteoglycan that has been linked in the past to metabolic syndrome, obesity, diabetes mellitus, bone development, and non-alcoholic fatty liver disease (NAFLD) (Oda E *,et al.,*2012). An endocrine organ, adipose tissue secretes adipokines, a process that obesity can dysregulate. In addition to enhancing insulin receptor signaling in the liver and skeletal muscle cells, GPC-4 promotes adipocyte development. Compared to their BMI-matched insulin-sensitive counterparts, insulin-resistant obese human participants had twice as high levels of GPC-4, an independent indicator of insulin resistance. Glypican-4 inhibits the insulin receptor directly, in contrast to other insulin sensitizers. Since then, studies have demonstrated that visceral and subcutaneous adipose tissue also expresses GPC4. Because body mass index (BMI) and waist-to-hip ratio (WHR) are linked to an elevated risk of metabolic and cardiovascular disorders, GPC4 expression is higher in visceral adipose tissue but lower in subcutaneous adipose tissue. Additional research has demonstrated that GPC4 levels directly bind to insulin receptors in the liver, skeletal muscle, and adipose tissue to improve insulin signaling and are associated with human BMI and insulin sensitivity. Elevated Glypican-4 levels in cirrhosis are associated with liver injury, fibrosis, and inflammatory processes. GPC4 contributes to fibrogenesis through its interaction with key signaling pathways like TGF-β and is upregulated in response to tissue damage and remodeling in the liver. These findings make GPC4 a potential biomarker for liver fibrosis and cirrhosis progression (Chen, Z.,*et al*.,2023).

Serum GPC4 levels in Asian non-DM participants were correlated with body fat distribution, IR, arteriosclerosis, and NAFLD. Yoo *et al*., 2013 observed that there

are sex disparities in the population, with men's serum GPC4 levels being considerably higher than women's.

Furthermore, these researchers found a significant correlation between GPC4 in female blood circulation and cardio-metabolic risk factors like arteriosclerosis and IR, as well as a positive correlation between GPC4 and WHR and visceral/subcutaneous fat area ratio. They also proposed that GPC4 is an independent influencing factor of NAFLD.

According to Ning *et al*., 2019, patients with metabolic syndrome have higher serum GPC4 levels. They also showed that GPC4 has a positive correlation with fasting insulin, fasting blood glucose, and the homeostasis model of insulin resistance (HOMA-IR). In conclusion, IR and metabolic disorders are significantly impacted by GPC4.

In this study, Pentraxin 3 (PTX3) is elevated in cirrhosis patients due to its role in chronic inflammation, liver fibrosis, immune system activation, endothelial dysfunction, and tissue regeneration. It serves as a useful biomarker for monitoring disease severity and progression in cirrhosis. According to certain research, people with infectious and inflammatory diseases had far higher serum levels of PTX-3 expression than the healthy population. (Simsek O.*et al.,*2018) Furthermore, PTX-3 plays a crucial role in the diagnosis of liver illness.(Narciso-Schiavon J, *et al.,*2017)

Serum PTX-3 expression significantly rises in adult NASH patients; therefore, PTX-3 can be used as a biomarker for the differential diagnosis of NASH and aid in assessing the degree of liver fibrosis in patients with NASH. Some studies suggest that PTX-3 can be used as an inflammatory marker for the prognosis of patients with hepatic cirrhosis (Hamza R, *et al.,*2016).

This study Agree with Jéssica G,*et al* .,2017.Circulating levels of PTX3 are increased in patients with liver cirrhosis, particularly those with acute decompensation. Serum PTX3 is related to the severity of the disease.

Dis agreement with Feder S, *et al*.,2020 In cirrhosis patients, PTX3 was neither related to ascites volume nor to variceal size. Moreover, PTX3 levels were not induced in patients presenting with ascites or grades III/IV encephalopathy. This excludes PTX3 as a marker of residual hepatic function and common complications of liver cirrhosis. PTX3 levels were not changed in cirrhosis patients with ascites or varices. In summary, PTX3 is not of diagnostic value in cirrhosis and HCC patients.

We discovered that PTX3 plasma levels were noticeably greater in NAFLD patients than in controls. The study by Yoneda *et al*., 2008, which found that PTX3 levels were considerably greater in NAFLD patients than in healthy control subjects, was in agreement with our findings.

Kadir *et al*.,2016 showed that, regardless of metabolic syndrome components, PTX3 levels were higher in NAFLD patients with fibrosis than in NAFLD patients without fibrosis and healthy participants.

In contrast, Maleki *et al*.,2014 reported no discernible change in plasma PTX3 between NAFLD and healthy control patients.

4.3 The Biochemical Parameters Of Patients And Control Group

4.3.1 Parameter Of Lipid Profile

The table (4-4) demonstrates that there is a substantial difference ($p \leq 0.05$) between group1, group2, group3, and control. The results indicate that all three groups (group1, group2, and group3) have more value than the control group. However, the group with the highest mean LDL value, group 2, had a mean of (137.89±41.68), higher than the control group's mean (63.83±24.46).

In contrast to LDL, HDL control more valuable compared to groups(group1,group2,group3)which has a high mean (63.77±9.08) than group1(37.02±5.25)which had a low mean. Compared to the other groups and the control (124.50±20.57), group2 showed a high mean in triglycerides (257.07±70.68). The mean group 2 in cholesterol (227.13±44.72) is higher than the control (152.50~±25.08).

Three patient groups had greater VLDL levels than the control group, while group2 had the highest value (51.41±14.14) compared to the control group (24.90±4.11).

Table (4-4) :The change in lipid profile of non- alcoholic fatty liver disease compared to control group

Variable	Sample	N	Mean S. D	P.V	LSD
LDL mg/dl	Group1	30	136.58 ± 33.96	<0.001	16.49
	Group2	30	137.89 ± 41.68		
	Group3	30	115.98 ± 27.51		
	Control	**30**	**63.83 ± 24.46**		
HDL mg/dl	Group1	30	37.02 ± 5.25	<0.001	6.29
	Group2	30	37.83 ± 9.21		
	Group3	30	46.20 ± 20.58		
	Control	**30**	**63.77 ± 9.08**		
TG mg/dl	Group1	30	222.37 ± 50.27	<0.001	25.68
	Group2	30	257.07 ± 70.68		
	Group3	30	209.10 ± 48.50		
	Control	**30**	**124.50 ± 20.57**		
TC mg/dl	Group1	30	218.07 ± 38.49	<0.001	17.36
	Group2	30	227.13 ± 44.72		
	Group3	30	204.00 ± 24.44		
	Control	**30**	**152.50 ± 25.08**		

VLDL mg/dl	**Group1**	30	44.47 ± 10.05	<0.001	5.14
	Group2	30	51.41 ± 14.14		
	Group3	30	41.82 ± 9.70		
	Control	**30**	**24.90 ± 4.11**		

N:number ,S.D: standard deviation, P.V: p-value, LSD: Less significance deference, LDL: low density lipoprotein ,HDL: high density lipoprotein, TG: triglyceride, TC: cholesterol, VLDL: very low density lipoprotein.

Patients with MASLD frequently have lower levels of high-density lipoprotein cholesterol (HDL-C) and higher amounts of triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) in their blood (Katsiki, N, *et al*., 2016).

The elevated risk of cardiovascular disease seen in MASLD is partly linked to these lipid abnormalities (Targher, G, *et al.,* 2024).The triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio has been proposed as a valuable indirect indicator of type 2 diabetes (T2D) and insulin resistance in recent years. It may also be a highly predictive measure for the diagnosis of metabolic syndrome (Wang, H,*et al.,*2023).

Thus, the TG/HDL-C ratio was found to be closely related to the number of metabolic syndrome components by Cordero et al., and it doubled in patients with metabolic syndrome as compared to healthy subjects(Cordero, A. *et al.,* 2008). A greater TG/HDL-C ratio was also demonstrated by Wang et al. to be a predictor of the incidence of (Wang, H., *et al.*,2023). Furthermore, in both the general population and individuals at high risk of cardiovascular events, a higher TG/HDL-C ratio has been linked to an increased risk for cardiovascular disease (Chen, Y,*et al.,* 2022).

This study also found a connection between cholesterol, LDL, and NASH. Conversely, there was no correlation seen between the degree of liver damage and HDL levels. According to Jiang ZG *et al.,* 2014, VLDL levels in NAFLD patients

can reveal the extent of liver damage. Serum LDL and VLDL subtypes were found to be significantly correlated with inflammation and liver damage.

Additionally, Méndez-Sánchez *et al*., 2020 demonstrated that compared to simple steatosis, steatohepatitis and liver fibrosis are more likely to have elevated VLDL and LDL serum concentrations, there may be a connection between liver inflammation and cholesterol metabolism. The most common form of lipid abnormality in NAFLD is atherogenic dyslipidemia, which is defined as hypertriglyceridemia, low HDL-C levels, and high LDL-C levels.

Prior research showed that lower blood HDL-C levels were linked to the development of NAFLD, which was consistent with NAFLD (Peng K, *et al*.,2017).

Nevertheless, no significant relationship was found in this investigation between HDL-C levels and NAFLD stages. It is becoming more and more clear that NAFLD is a complex illness that is closely linked to metabolic and genetic conditions such as insulin resistance, obesity, dyslipidemia, and cardiovascular diseases (Deprince A, *et al*.,2020).

The frequency of dyslipidemia (increased TG and LDL-C levels and decreased HDL-C levels), which is an important risk factor for NAFLD, is increased in obese patients (Dowla S,*et al.,*2018). In this study, as the TG levels of the patients increased, there was a significant decrease in HDL-C levels (dyslipidemia) and the frequency of NAFLD increased.

Toledo *et al.,*2006, found an increase in serum TG levels and a decrease in HDL-C levels in patients with moderate and severe NAFLD compared to the healthy control group.

Nigam *et al*.,2013, reported that TC ,LDL-C and TG levels were higher and HDL-C levels were lower in patients with NAFLD compared to those without.

Şenyiğit *et al*.,2018, concluded that there was an increase in serum LDL-C and TG levels, and a decrease in HDL-C levels in the group with NAFLD compared to the group without, These findings support the opinion that dyslipidemia is an important risk factor for NAFLD.

4.3.2 Parameter In Liver Enzymes

The table below compares the AST and ALT values of Groups 1, 2, 3, and the control group, and the results show a significant statistical difference ($p \leq 0.05$). The AST mean for group3 is the greatest at (57.80±17.54), higher than the means of group2 (56.13±21.70), G1 (44.40±19.90), and control (25.20±7.17).

The mean of group2 in ALT is 64.43±25.03, which is greater than that of group1 (55.47±26.28), group3 (55.23~±17.22), and control (28.50±7.31).

Table(4-5):The change in liver enzyme of non- alcoholic fatty liver disease compared to control group

Variable	Sample	N	Mean ± S. D	P.V	LSD
AST U/L	**Group1**	30	44.40 ±19.90	<0.001	8.86
	Group2	30	56.13 ±21.70		
	Group3	30	57.80 ±17.54		
	Control	**30**	**25.20 ±7.17**		
ALT U/L	**Group1**	30	55.47 ±26.28	<0.001	10.33
	Group2	30	64.43 ± 25.03		
	Group3	30	55.23 ±17.22		
	Control	**30**	**28.50 ±7.31**		

N:number ,S.D: standard deviation, P.V: p-value, LSD: Less significance deference, AST: aspartate aminotransaminases ,ALT: alanine aminotransaminases.

Although the liver naturally contains some fat, if the liver contains more than 5% to 10% fat, the person is said to have fatty liver (hepatic steatosis). Increased levels of liver enzymes, such as AST and ALT, that are found during regular blood testing, may result from fat buildup in the liver. A liver enzyme called ALT helps liver cells produce energy from proteins. Amino acid metabolism is aided by AST. Blood typically contains low amounts of ALT and AST. An increase in ALT and/or AST could be a sign of illness, injury to the muscles, or damage to the liver. (Sanyal *et al*., 2015)

Hepatocyte cytosol contains aggregates of liver transaminases, primarily alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Suciu A, *et al.,* 2020).

According to Janičko *et al*.,2015, these enzymes are generally found in the serum at low concentrations, usually less than 30 U/L. However, any procedure that results in necrosis or the loss of hepatocyte membrane integrity causes the release of ALT and AST in the serum at larger concentrations. Serum levels of these enzymes may be influenced by a number of physiological and risk factors, such as age, sex, body mass index, high triglyceride levels, insulin resistance, and blood glucose levels (Ipekci SH,*et al.,*2003). Thus, in patients with a variety of liver illnesses, serum levels of transaminases, especially ALT, are crucial indicators that show hepatic inflammation and liver damage (Flores YN, *et al*., 2016).There is a wide range of research on the changes in transaminase levels during MAFLD and MASH. Higher ALT readings, for instance, have been linked in certain studies to an increased chance of developing MAFLD and ultimately MASH (Angulo P,*et al.,* 2007).

On the other hand, some studies have shown that ALT values do not correlate with the severity of histopathological changes in MAFLD (Ipekci SH,*et al.,*2003). variable percentage of MASH patients with normal transaminase values have also

been reported (Fracanzani AL, *et al*., 2008). According to a recent meta-analysis, of the total patients assessed, up to 25% of MAFLD patients and 19% of MASH patients had normal ALT readings (Ma X,*et al.,*2020).

Bayard M ,*et al.,* 2006 demonstrated in his study that laboratory abnormalities often are the only sign of NAFLD. Most commonly elevated liver enzymes are alanine transaminases (ALT) and aspartate transaminases (AST), usually one to four times the upper limits of normal. The ratio of AST/ALT usually is less than 1 (in alcoholic liver disease) but may increase as the severity of the liver damage increases.

Sattar Naved *et al.,*2014 in his study represents most patients with NAFLD are asymptomatic and the disease is typically suspected based on raised alanine aminotransferase (ALT) levels together with other clinical and biochemical features, or an incidental finding during abdominal ultrasonography.

4.3.3GLUCOSE AND HOMA-IR

Table (4-6) displays the glucose data for patients (group1, group2, group3), which indicate a substantial increase ($p < 0.05$) in glucose concentration when compared to the control group. The patient group (group1, group2, group3) had greater glucose than the control group (91.40±7.70), although the group3 had the highest mean (191.83±65.14).In comparison to other groups and the control group (2.40~±0.58), HOMA-IR is greater in group3 (13.21±16.07). The patient groups and control group exhibit significant differences ($P \leq 0.05$).

Table (4-6) The change in HOMA-IR of non- alcoholic fatty liver disease compared to control group.

Variable	Sample	N	Mean ± S. D	P.V	LSD
Fasting glucose mg/dl	**Group1**	30	132.10 ± 57.40	<0.001	24.26
	Group2	30	154.50 ± 39.90		
	Group3	30	191.83 ± 65.14		
	Control	**30**	**91.40 ±7.70**		
HOMA-IR	**Group1**	30	4.06 ± 0.99	<0.001	4.09
	Group2	30	4.71 ± 1.40		
	Group3	30	13.21 ± 16.07		
	Control	**30**	**2.40 ± 0.58**		

N:number ,S.D: standard deviation, P.V: p-value, LSD: Less significance deference, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance

The primary function of the liver is to maintain glucose metabolism through glucose storage and the production of endogenous glucose from the liver's glycogen stores, both of which help to maintain normal blood glucose levels (Roden M,*et al*.,2003).

The development of postprandial hyperglycemia in patients with type 2 diabetes is often caused by increased endogenous glucose synthesis and reduced glucose deposition in skeletal muscle (Krssak M,*et al* ., 2004). Increased insulin resistance and significant postprandial hyperglycemia are characteristics of liver cirrhosis with early-stage hepatogenous diabetes. Liver cirrhosis is identified by declines in both

skeletal muscle and hepatocyte mass as the disease progresses (Imano E, *et al* .,1999).

The group with NAFLD in Tunç's study had noticeably higher insulin levels and HOMAIR than the group without (Tunç S. *et al.,*2021). Furthermore, there was a positive relationship discovered between the severity of NAFLD and the HOMA-IR level.

4.4 Correlation Coefficient Among Biomarkers Parameter

Age and BMI showed a slight correlation, as shown in Table (4-7). There was a strong positive correlation ($p<0.01$) observed in regard to ALT with both BMI and AST.

Furthermore, a strong positive correlation($p<0.01$) was found between BMI and LDL. In contrast to LDL, HDL exhibits a strong negative correlation with BMI and a strong positive correlation with age. Triglycerides exhibit a negative correlation($P>0.05$) with HDL and a strong positive correlation with ALT and LDL. Cholesterol has a negative correlation with HDL and a positive correlation with triglycerides and LDL.

It also has a positive correlation ($P>0.05$) with ALT and BMI . VLDL and LDL, tri, and cholesterol have a strong positive association. And unfavorable association with HDL. Age and glucose have a strong positive association. HOMA-IR and glucose exhibit a strong positive correlation($p<0.01$).

Age, LDL, triglycerides, cholesterol, glucose, and HOMA-IR are all highly positively correlated, according to TYG. There is a strong positive correlation between marker PEN-3 and GLY-4.

TABLE (4-7) Correlation Coefficient Among Biomarkers Parameter

	AGE	BMI	AST U/L	ALT U/L	LDL mg/dl	HDL mg/dl	TG mg/dl	TC mg/dl	VLDL mg/dl	Glucos mg/dl	HOMA -IR	TYG	GLY-4 ng/ml
BMI	.034												
AST U/L	.048	.195											
ALT U/L	-.100	.278**	.848**										
LDL mg/dl	-.024	.334**	.058	.198									
HDL mg/dl	.280**	-.322**	.087	-.088	-.579**								
TG mg/dl	-.038	.076	.110	.210*	.508**	-.210*							
TC mg/dl	.068	.225*	.122	.223*	.903**	-.251*	.726**						
VLDL mg/dl	-.038	.076	.110	.210*	.508**	-.210*	1.000**	.726**					
Gluco mg/dl	.357**	.143	.044	-.047	.114	-.004	-.013	.103	-.013				
HOMA-IR	.102	.116	.146	.062	-.049	-.025	.001	-.056	.001	.315**			
TYG	.283**	.174	.086	.060	.385**	-.114	.571**	.508**	.571**	.794**	.268*		
GLY-4 ng/ml	.084	-.046	-.024	-.068	-.081	-.111	-.112	-.153	-.112	.128	-.003	.044	
PEN-3 pg/ml	-.008	.023	.103	.058	-.028	-.011	-.196	-.093	-.196	-.017	-.062	-.129	.386**

** Correlation is significant at the 0.01level (2-tailed)

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

According to Rui Fan *et al*., 2018, persons with fatty livers had higher BMIs. According to their research, fatty liver risk was substantially correlated with a higher BMI (overweight/obesity).

Presently, there are limited studies that have reported on the association of the ALT/AST ratio with NAFLD risk. Only a few cross-sectional studies have found a positive correlation of NAFLD risk with the ALT/AST ratio (Lu CW, *et al* .,2019). High BMI and dyslipidemia are known to be the most important modifiable risk factors for the onset of NAFLD. A series of studies have elucidated the associations between BMI and dyslipidemia and NAFLD in the past, among which elevated body weight and dyslipidemia were the most important risk factors for the increased risk of NAFLD (Soares ALG, *et al.,*2020).

One of the underlying mechanisms of the positive relationship between liver fat and triglycerides might be that the presence of diabetes in patients with high liver fat leads to a significant increase in hepatic triglyceride production, which may be reversed with diabetes remission (Al-Mrabeh A *,et al.,2020*).

Wu, T,*et al.,*2021 found that the relationship between high serum triglyceride levels and severe intrahepatic lipid accumulation was significant in MAFLD patients except for those with lean/normal weight.

Triglyceride levels were independently associated with the small dense LDL-cholesterol burden along with body mass index. Hirano and colleagues also reported that higher levels of triglyceride were associated with smaller-sized LDL particles (Hirano T,*et al .,*2004*)* small dense LDL-cholesterol.

TyG index and risk of liver fibrosis advancement in NAFLD patients have not been evaluated in many research. This study is the first to assess the relationship between the TyG index and the risk of liver fibrosis progression in northwest Iran. A higher TyG index was observed to be positively connected with both the

deterioration of NAFLD severity and the NAFLD fibrosis score. Additionally, patients with higher TyG index quartiles had greater serum levels of TC, TG, LDL-C, ALT, AST, and HOMA-IR, whereas patients with higher TyG index had lower serum concentrations of HDL-C (Tutunchi, H,*et al*.,2021*).*

However, they did not find any association between glypican-4 levels and fasting plasma glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, or serum adiponectin levels (Ussar S, *et al.,*2012). In contrast, the present study with Asian subjects showed that plasmaglypican-4 levels had no significant correlation with BMI; however, glypican-4 levels showed significant positive associations with VFA/SFA ratio, AST, triglycerides, glucose, and HOMA-IR levels, especially in women(Yoo HJ,*et al.,*2013).

Zhu *et al*., 2014 who found that obese patients with insulin resistance had higher serum levels of GPC4 and that there were positive correlations between serum GPC4 levels and BMI, fasting serum insulin level, HOMA-IR, TC, LDL, ALT, and AST levels.

Additionally, we agreed with Lee *et al*.,2016, who found no significant relationships between GPC4 and any of the following: WHR, TC, TG, LDL, and ALT. Additionally, they noted that GPC4 and AST had a negative connection. However, they concurred with us that there was no relationship between GPC4 and HDL or plasma glucose.

Conclusions And Recommendations

CONCLUSIONS

Current study concludes the following:

1. In this study's cirrhosis patients ,elevated level of Glypican- 4 and pentraxin – 3 compared to NAFLD , NASH and control .
2. In study shows that TyG index was elevated in all patient (NAFLD ,NASH, cirrhosis) than control .
3. According to the current findings ,elevated of liver enzyme in patient in compared to healthy ,where AST is elevated in cirrhosis patient and ALT elevated in NASH patient
4. Insulin resistance was elevated in Cirrhosis patient compared other patient and control .
5. In NASH patients, there are elevated levels of triglycerides (TG), low-density lipoprotein (LDL), cholesterol, and VLDL .
6. Reduced levels of high-density lipoprotein in patient and elevated in healthy .

RECOMMENDATIONS

1. Utilizing in-depth research on the illness due to its significance in terms of its severity and occurrence
2. Add to the research Additional information regarding NAFLD may be obtained from the markers haptoglobin, α-2-macroglobulin, and apolipoprotein a1 (APOA1).
3. A diet that creates a calorie deficit, limits carbohydrates and saturated fats, and promotes fiber and unsaturated fats should be recommended for those with NAFLD.
4. People with NAFLD are advised to follow a customized exercise program that encourages movement and long-term weight loss.).

References

References

- Agrawal, S., Dhiman, R. K., & Limdi, J. K. (2016). Evaluation of abnormal liver function tests. Postgraduate medical journal, 92(1086), 223-234.
- Albuquerque Souza, E., & Sahingur, S. E. (2022). Periodontitis, chronic liver diseases, and the emerging oral gut liver axis. Periodontology 2000, 89(1), 125-141.
- Alizargar, J., Bai, C. H., Hsieh, N. C., & Wu, S. F. V. (2020). Use of the triglyceride-glucose index (TyG) in cardiovascular disease patients. Cardiovascular diabetology, 19(1), 8.
- Al-Mrabeh, A., Zhyzhneuskaya, S. V., Peters, C., Barnes, A. C., Melhem, S., Jesuthasan, A., ... & Taylor, R. (2020). Hepatic lipoprotein export and remission of human type 2 diabetes after weight loss. Cell metabolism, 31(2), 233-249.
- Altinkaya, S. O. (2021). Galanin and glypican-4 levels depending on metabolic and cardiovascular risk factors in patients with polycystic ovary syndrome. Archives of endocrinology and metabolism, 65(4), 479-487.
- Amarapurkar, D. N., Amarapurkar, A. D., Patel, N. D., Agal, S., Baigal, R., Gupte, P., & Pramanik, S. (2006). Nonalcoholic steatohepatitis (NASH) with diabetes: predictors of liver fibrosis. Annals of hepatology, 5(1), 30-33.
- Amzolini, A. M., Forțofoiu, M. C., Alhija, A. B., Vladu, I. M., Clenciu, D., Mitrea, A., ... & Micu, E. S. (2022). Triglyceride and glucose index as a screening tool for nonalcoholic liver disease in patients with metabolic syndrome. Journal of Clinical Medicine, 11(11), 3043.
- Angulo, P., Hui, J. M., Marchesini, G., Bugianesi, E., George, J., Farrell, G. C., ... & Day, C. P. (2007). The NAFLD fibrosis score: a noninvasive

system that identifies liver fibrosis in patients with NAFLD. Hepatology, 45(4), 846-854.

- Azzu, V., Vacca, M., Virtue, S., Allison, M., & Vidal-Puig, A. (2020). Adipose tissue-liver cross talk in the control of whole-body metabolism: implications in nonalcoholic fatty liver disease. Gastroenterology, 158(7), 1899-1912.
- Ballestri, S., Mantovani, A., Byrne, C., Lonardo, A., & Targher, G. (2021). Diagnostic accuracy of ultrasonography for the detection of hepatic steatosis: an updated meta-analysis of observational studies. Metabolism and Target Organ Damage, 1(7).
- Bardugo, A., Bendor, C. D., Zucker, I., Lutski, M., Cukierman-Yaffe, T., Derazne, E., ... & Twig, G. (2021). Adolescent nonalcoholic fatty liver disease and type 2 diabetes in young adulthood. The Journal of Clinical Endocrinology & Metabolism, 106(1), e34-e44.
- Bayard M, Holt J, Boroughs E. Nonalcoholic fatty liver disease. Am Fam Physician 2006 Jun 1;73(11):1961-8.
- Bellentani, S., Saccoccio, G., Masutti, F., Crocè, L. S., Brandi, G., Sasso, F., ... & Tiribelli, C. (2000). Prevalence of and risk factors for hepatic steatosis in Northern Italy. Annals of internal medicine, 132(2), 112-117.
- Bıyıklı, Z. (2009). Non-alcoholic steatohepatitis with normal aminotransferase values.
- Boccatonda, A., Andreetto, L., D'Ardes, D., Cocco, G., Rossi, I., Vicari, S., ... & Guagnano, M. T. (2023). From NAFLD to MAFLD: definition, pathophysiological basis and cardiovascular implications. Biomedicines, 11(3), 883.
- Bottazzi, B., Inforzato, A., Messa, M., Barbagallo, M., Magrini, E., Garlanda, C., & Mantovani, A. (2016). The pentraxins PTX3 and SAP in

innate immunity, regulation of inflammation and tissue remodelling. Journal of hepatology, 64(6), 1416-1427.

- Bullon-Vela, V., Abete, I., Tur, J. A., Konieczna, J., Romaguera, D., Pinto, X., ... & Martínez, J. A. (2020). Relationship of visceral adipose tissue with surrogate insulin resistance and liver markers in individuals with metabolic syndrome chronic complications. Therapeutic Advances in Endocrinology and Metabolism, 11, 2042018820958298.
- Chen, C. H., Huang, M. H., Yang, J. C., Nien, C. K., Yang, C. C., Yeh, Y. H., & Yueh, S. K. (2006). Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of Taiwan: metabolic significance of nonalcoholic fatty liver disease in nonobese adults. Journal of clinical gastroenterology, 40(8), 745-752.
- Chen, Z., Zeng, H., Huang, Q., Lin, C., Li, X., Sun, S., & Liu, J. (2023). Serum glypican-4 and clusterin are increased and associated with insulin resistance in patients with polycystic ovary syndrome.
- Decharatanachart, P., Chaiteerakij, R., Tiyarattanachai, T., & Treeprasertsuk, S. (2021). Application of artificial intelligence in non-alcoholic fatty liver disease and liver fibrosis: a systematic review and meta-analysis. Therapeutic Advances in Gastroenterology, 14, 17562848211062807.
- Deniz, T., Kizilgul, M., Uzunlulu, M., Oguz, A., & Isbilen, B. (2014). Levels of pentraxin 3 and relationship with disease activity in patients with ankylosing spondylitis. Acta reumatologica portuguesa, 39(2).
- Deprince, A., Haas, J. T., & Staels, B. (2020). Dysregulated lipid metabolism links NAFLD to cardiovascular disease. Molecular metabolism, 42, 101092.
- Dongiovanni, P., Stender, S., Pietrelli, A., Mancina, R. M., Cespiati, A., Petta, S., ... & Valenti, L. (2018). Causal relationship of hepatic fat with

liver damage and insulin resistance in nonalcoholic fatty liver. Journal of internal medicine, 283(4), 356-370.

- Dowman, J. K., Tomlinson, J. W., & Newsome, P. N. (2011). Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and nonalcoholic steatohepatitis. Alimentary pharmacology & therapeutics, 33(5), 525-540.
- Ekstedt, M., Franzén, L. E., Mathiesen, U. L., Thorelius, L., Holmqvist, M., Bodemar, G., & Kechagias, S. (2006). Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology, 44(4), 865-873.
- Fabrellas, N., Moreira, R., Carol, M., Cervera, M., de Prada, G., Perez, M., ... & Ginès, P. (2020). Psychological burden of hepatic encephalopathy on patients and caregivers. Clinical and translational gastroenterology, 11(4), e00159.
- Fahed, G., Aoun, L., Bou Zerdan, M., Allam, S., Bou Zerdan, M., Bouferraa, Y., & Assi, H. I. (2022). Metabolic syndrome: updates on pathophysiology and management in 2021. International journal of molecular sciences, 23(2), 786.
- Fan, N., Peng, L., Xia, Z., Zhang, L., Song, Z., Wang, Y., & Peng, Y. (2019). Triglycerides to high-density lipoprotein cholesterol ratio as a surrogate for nonalcoholic fatty liver disease: a cross-sectional study. Lipids in Health and Disease, 18, 1-6.
- Feder, S., Haberl, E. M., Spirk, M., Weiss, T. S., Wiest, R., & Buechler, C. (2020). Pentraxin-3 is not related to disease severity in cirrhosis and hepatocellular carcinoma patients. Clinical and experimental medicine, 20, 289-297.
- Fouad, Y., Dufour, J. F., Zheng, M. H., Bollipo, S., Desalegn, H., Grønbaek, H., & Gish, R. G. (2022). The NAFLD-MAFLD debate: Is there

a Consensus-on-Consensus methodology?. Liver International, 42(4), 742-748.

- Francque, S. M., Marchesini, G., Kautz, A., Walmsley, M., Dorner, R., Lazarus, J. V., ... & Lionis, C. (2021). Non-alcoholic fatty liver disease: A patient guideline. JHEP reports, 3(5), 100322.
- Frediani, J. K., Naioti, E. A., Vos, M. B., Figueroa, J., Marsit, C. J., & Welsh, J. A. (2018). Arsenic exposure and risk of nonalcoholic fatty liver disease (NAFLD) among US adolescents and adults: an association modified by race/ethnicity,
- Fujii, H., Imajo, K., Yoneda, M., Nakahara, T., Hyogo, H., Takahashi, H., ... & Japan Study Group of Nonalcoholic Fatty Liver Disease. (2019). HOMA-IR: An independent predictor of advanced liver fibrosis in nondiabetic non-alcoholic fatty liver disease. Journal of gastroenterology and hepatology, 34(8), 1390-1395.
- Fujita, K., Nozaki, Y., Wada, K., Yoneda, M., Fujimoto, Y., Fujitake, M., ... & Nakajima, A. (2009). Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. Hepatology, 50(3), 772-780.
- Ghorpade, D. S., Ozcan, L., Zheng, Z., Nicoloro, S. M., Shen, Y., Chen, E., ... & Tabas, I. (2018). Hepatocyte-secreted DPP4 in obesity promotes adipose inflammation and insulin resistance. Nature, 555(7698), 673-677.
- Godoy-Matos, A. F., Silva Júnior, W. S., & Valerio, C. M. (2020). NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. Diabetology & metabolic syndrome, 12, 1-20.
- Golabi, P., Paik, J. M., Harring, M., Younossi, E., Kabbara, K., & Younossi, Z. M. (2022). Prevalence of high and moderate risk nonalcoholic fatty liver disease among adults in the United States, 1999–2016. Clinical Gastroenterology and Hepatology, 20(12), 2838-2847.

- Guo, W., Lu, J., Qin, P., Li, X., Zhu, W., Wu, J., ... & Zhang, Q. (2020). The triglyceride-glucose index is associated with the severity of hepatic steatosis and the presence of liver fibrosis in non-alcoholic fatty liver disease: a cross-sectional study in Chinese adults. Lipids in Health and Disease, 19, 1-9.
- Hamza, R. T., Elfaramawy, A. A., & Mahmoud, N. H. (2016). Serum pentraxin 3 fragment as a noninvasive marker of nonalcoholic fatty liver disease in obese children and adolescents. Hormone Research in Paediatrics, 86(1), 11-20.
- Harrison, S. A., Ratziu, V., Anstee, Q. M., Noureddin, M., Sanyal, A. J., Schattenberg, J. M., ... & Loomba, R. (2024). Design of the phase 3 MAESTRO clinical program to evaluate resmetirom for the treatment of nonalcoholic steatohepatitis. Alimentary Pharmacology & Therapeutics, 59(1), 51-63.
- Henry, L., Paik, J., & Younossi, Z. M. (2022). the epidemiologic burden of non-alcoholic fatty liver disease across the world. Alimentary pharmacology & therapeutics, 56(6), 942-956.
- Hernandez-Tejero, M., Clemente-Sanchez, A., & Bataller, R. (2023). Spectrum, screening, and diagnosis of alcohol-related liver disease. Journal of Clinical and Experimental Hepatology, 13(1), 75-87.
- Honda, Y., Yoneda, M., Imajo, K., & Nakajima, A. (2020). Elastography techniques for the assessment of liver fibrosis in non-alcoholic fatty liver disease. International journal of molecular sciences, 21(11), 4039.
- Hsieh, M. H., Ho, C. K., Hou, N. J., Hsieh, M. Y., Lin, W. Y., Yang, J. F., ... & Yu, M. L. (2009). Abnormal liver function test results are related to metabolic syndrome and BMI in Taiwanese adults without chronic hepatitis B or C. International journal of obesity, 33(11), 1309-1317.

- Huang, T., Behary, J., & Zekry, A. (2020). Non-alcoholic fatty liver disease: a review of epidemiology, risk factors, diagnosis and management. Internal medicine journal, 50(9), 1038-1047.
- Huggett, Z. J., Smith, A., De Vivo, N., Gomez, D., Jethwa, P., Brameld, J. M., ... & Salter, A. M. (2022). A comparison of primary human hepatocytes and hepatoma cell lines to model the effects of fatty acids, fructose and glucose on liver cell lipid accumulation. Nutrients, 15(1), 40.
- Humphrey, G., Bassirian, S., Singh, S., Faulkner, C., ... & Loomba, R. (2019). A gut microbiome signature for cirrhosis due to nonalcoholic fatty liver disease. Nature communications, 10(1), 1406.
- Ikejima, K., Kon, K., & Yamashina, S. (2020). Nonalcoholic fatty liver disease and alcohol-related liver disease: From clinical aspects to pathophysiological insights. Clinical and molecular hepatology, 26(4), 728.
- Iluz-Freundlich, D., Zhang, M., Uhanova, J., & Minuk, G. Y. (2020). The relative expression of hepatocellular and cholestatic liver enzymes in adult patients with liver disease. Annals of hepatology, 19(2), 204-208.
- Iqbal, U., Perumpail, B. J., John, N., Sallam, S., Shah, N. D., Kwong, W., ... & Ahmed, A. (2018). Judicious use of lipid lowering agents in the management of NAFLD. Diseases, 6(4), 87.
- Janicko, M., Veselíny, E., Orencák, R., Hustak, R., Fedacko, J., Dražilová, S., ... & HepaMeta Study Group. (2015). Redefining the alanine aminotransferase upper limit of normal improves the prediction of metabolic syndrome risk. European Journal of Gastroenterology & Hepatology, 27(4), 405-411.
- Jiang, Z. G., Tapper, E. B., Connelly, M. A., Pimentel, C. F., Feldbrügge, L., Kim, M., ... & Lai, M. (2016). Steatohepatitis and liver fibrosis are predicted by the characteristics of very low density lipoprotein in nonalcoholic fatty liver disease. Liver International, 36(8), 1213-1220.

- Kallai, L., Hahn, A., Roeder, V., & Županić, V. (1964). Correlation between histological findings and serum transaminase values in chronic diseases of the liver. Acta Medica Scandinavica, 175(1), 49-56.
- Kanwal, F., Shubrook, J. H., Adams, L. A., Pfotenhauer, K., Wong, V. W. S., Wright, E., ... & Cusi, K. (2021). Clinical care pathway for the risk stratification and management of patients with nonalcoholic fatty liver disease. Gastroenterology, 161(5), 1657-1669.
- Kim, M. K., Kim, D. M., Lee, S. B., Kang, S., Nam, J. S., Park, J. S., & Ahn, C. W. (2018, May). Relationship between the triglyceride glucose index and the presence and fibrosis of nonalcoholic fatty liver disease in Korean adults. In Endocrine Abstracts (Vol. 56). Bioscientifica.
- Kitae, A., Hashimoto, Y., Hamaguchi, M., Obora, A., Kojima, T., & Fukui, M. (2019). The triglyceride and glucose index is a predictor of incident nonalcoholic fatty liver disease: a population-based cohort study. Canadian Journal of Gastroenterology and Hepatology, 2019(1), 5121574.
- Krssak, M., Brehm, A., Bernroider, E., Anderwald, C., Nowotny, P., Man, C. D., ... & Roden, M. (2004). Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. Diabetes, 53(12), 3048-3056.
- Le, M. H., Le, D. M., Baez, T. C., Wu, Y., Ito, T., Lee, E. Y., ... & Nguyen, M. H. (2023). Global incidence of non-alcoholic fatty liver disease: A systematic review and meta-analysis of 63 studies and 1,201,807 persons. Journal of Hepatology, 79(2), 287-295.
- Lee, S. B., Kim, M. K., Kang, S., Park, K., Kim, J. H., Baik, S. J., ... & Park, J. S. (2019). Triglyceride glucose index is superior to the homeostasis model assessment of insulin resistance for predicting nonalcoholic fatty liver disease in Korean adults. Endocrinology and Metabolism, 34(2), 179-186.

- Lennartz, S., Parakh, A., Cao, J., Zopfs, D., Grosse Hokamp, N., & Kambadakone, A. (2021). Inter-scan and inter-scanner variation of quantitative dual-energy CT: evaluation with three different scanner types. European Radiology, 31, 4438-4451.
- Linares, I., Hamar, M., Selzner, N., & Selzner, M. (2019). Steatosis in liver transplantation: current limitations and future strategies. Transplantation, 103(1), 78-90..
- Ma, X., Liu, S., Zhang, J., Dong, M., Wang, Y., Wang, M., & Xin, Y. (2020). Proportion of NAFLD patients with normal ALT value in overall NAFLD patients: a systematic review and meta-analysis. BMC gastroenterology, 20, 1-8.
- Makhlouf, M., Saleh, S., Rushdy, M., Abdelhakam, S., & Abd-Elghani, E. (2019). Pentraxin-3 in non-alcoholic fatty liver disease and its affection by concomitant chronic hepatitis C infection. Egyptian Liver Journal, 9, 1-8.
- Maleki, I., Rastgar, A., Hosseini, V., Taghvaei, T., Rafiei, A., Barzin, M., ... & Khalilian, A. (2014). High sensitive CRP and pentraxine 3 as noninvasive biomarkers of nonalcoholic fatty liver disease. European Review for Medical & Pharmacological Sciences, 18.
- Mansour-Ghanaei, R., Mansour-Ghanaei, F., Naghipour, M., & Joukar, F. (2019). Biochemical markers and lipid profile in nonalcoholic fatty liver disease patients in the PERSIAN Guilan cohort study (PGCS), Iran. Journal of Family Medicine and Primary Care, 8(3), 923-928.
- Mazzolini, G., Sowa, J. P., Atorrasagasti, C., Kücükoglu, Ö., Syn, W. K., & Canbay, A. (2020). Significance of simple steatosis: an update on the clinical and molecular evidence. Cells, 9(11), 2458.
- McSweeney, L., Breckons, M., Fattakhova, G., Oluboyede, Y., Vale, L., Ternent, L., ... & Anstee, Q. M. (2020). Health-related quality of life and

patient-reported outcome measures in NASH-related cirrhosis. Jhep Reports, 2(3), 100099.

- Meex, R. C., & Watt, M. J. (2017). Hepatokines: linking nonalcoholic fatty liver disease and insulin resistance. Nature Reviews Endocrinology, 13(9), 509-520.
- Meffert, P. J., Repp, K. D., Völzke, H., Weiss, F. U., Homuth, G., Kühn, J. P., ... & Aghdassi, A. A. (2018). The PNPLA3 SNP rs738409: G allele is associated with increased liver disease-associated mortality but reduced overall mortality in a population-based cohort. Journal of Hepatology, 68(4), 858-860.
- Méndez-Sánchez N, Cerda-Reyes E, Higuera-De-La-Tijera F, Salas-García AK, Cabrera-Palma S, Cabrera-Álvarez G, et al. Dyslipidemia as a risk factor for liver fibrosis progression in a multicentric population with non-alcoholic steatohepatitis. F1000Res. (2020) 9:56. doi: 10.12688/f1000research. 21918.1
- Mirr, M., Skrypnik, D., Bogdański, P., & Owecki, M. (2021). Newly proposed insulin resistance indexes called TyG-NC and TyG-NHtR show efficacy in diagnosing the metabolic syndrome. Journal of endocrinological investigation, 44(12), 2831-2843.
- Narro, G. E. C., Díaz, L. A., Ortega, E. K., Garín, M. F. B., Reyes, E. C., Delfin, P. S. M., ... & Bataller, R. (2024). Alcohol-related liver disease: A global perspective. Annals of Hepatology, 29(5), 101499.
- Nigam, P., Bhatt, S. P., Misra, A., Vaidya, M., Dasgupta, J., & Chadha, D. S. (2013). Non-alcoholic fatty liver disease is closely associated with sub-clinical inflammation: a case-control study on Asian Indians in North India. Plos one, 8(1), e49286.
- Ozturk, K., Kurt, O., Dogan, T., Ozen, A., Demirci, H., Yesildal, F., ... & Bagci, S. (2016). Pentraxin 3 is a predictor for fibrosis and arterial stiffness

in patients with nonalcoholic fatty liver disease. Gastroenterology research and practice, 2016(1), 1417962.

- Pelusi, S., & Valenti, L. (2019). Hepatic fat as clinical outcome and therapeutic target for nonalcoholic fatty liver disease. Liver International, 39(2), 250-256.
- Rajkovic, I., Denes, A., Allan, S. M., & Pinteaux, E. (2016). Emerging roles of the acute phase protein pentraxin-3 during central nervous system disorders. Journal of neuroimmunology, 292, 27-33.
- Ren, Z., Simons, P. I., Wesselius, A., Stehouwer, C. D., & Brouwers, M. C. (2023). Relationship between NAFLD and coronary artery disease: A Mendelian randomization study. Hepatology, 77(1), 230-238.
- Rinella, M. E., Neuschwander-Tetri, B. A., Siddiqui, M. S., Abdelmalek, M. F., Caldwell, S., Barb, D., ... & Loomba, R. (2023). AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology, 77(5), 1797-1835.
- Risi, R., Tozzi, R., & Watanabe, M. (2021). Beyond weight loss in nonalcoholic fatty liver disease: the role of carbohydrate restriction. Current Opinion in Clinical Nutrition & Metabolic Care, 24(4), 349-353.
- Ristagno, G., Fumagalli, F., Bottazzi, B., Mantovani, A., Olivari, D., Novelli, D., & Latini, R. (2019). Pentraxin 3 in cardiovascular disease. Frontiers in immunology, 10, 823.
- Roden, M., & Bernroider, E. (2003). Hepatic glucose metabolism in humans—its role in health and disease. Best practice & research Clinical endocrinology & metabolism, 17(3), 365-383.
- Romero-Gómez, M., Zelber-Sagi, S., & Trenell, M. (2017). Treatment of NAFLD with diet, physical activity and exercise. Journal of hepatology, 67(4), 829-846.

- Rui, L., & Lin, J. D. (2022). Reprogramming of hepatic metabolism and microenvironment in nonalcoholic steatohepatitis. Annual review of nutrition, 42(1), 91-113.
- Sarin, S. K., Kumar, M., Eslam, M., George, J., Al Mahtab, M., Akbar, S. M. F., ... & Chen, D. S. (2020). Liver diseases in the Asia-Pacific region: a lancet gastroenterology & hepatology commission. The lancet Gastroenterology & hepatology, 5(2), 167-228.
- Sattar, N., Forrest, E., & Preiss, D. (2014). Non-alcoholic fatty liver disease. Bmj, 349.
- Schuppan, D., Surabattula, R., & Wang, X. Y. (2018). Determinants of fibrosis progression and regression in NASH. Journal of hepatology, 68(2), 238-250.
- Seitz, H. K., & Neuman, M. G. (2021). The history of alcoholic liver disease: from an unrecognized disease to one of the most frequent diseases in hepatology. Journal of Clinical Medicine, 10(4), 858.
- Şenyiğit, A., Orhanoğlu, T., & Yaprak, B. (2018). Investigation of the degrees and possible biomarkers of Non-Alcoholic Fatty Liver (NAFL) disease.
- Sharma, S., Hashmi, M. F., & Chakraborty, R. K. (2021). StatPearls [Internet] StatPearls Publishing. Treasure Island (FL): Sep, 18.
- Sheka, A. C., Adeyi, O., Thompson, J., Hameed, B., Crawford, P. A., & Ikramuddin, S. (2020). Nonalcoholic steatohepatitis: a review. Jama, 323(12), 1175-1183
- Shen, W., Middleton, M. S., Cunha, G. M., Delgado, T. I., Wolfson, T., Gamst, A., ... & Lavine, J. E. (2023). Changes in abdominal adipose tissue depots assessed by MRI correlate with hepatic histologic improvement in non-alcoholic steatohepatitis. Journal of hepatology, 78(2), 238-246.

- Simsek, O., Kocael, A., Kocael, P., Orhan, A., Cengiz, M., Balcı, H., ... & Uzun, H. (2018). Inflammatory mediators in the diagnosis and treatment of acute pancreatitis: pentraxin-3, procalcitonin and myeloperoxidase. Archives of medical science, 14(2), 288-296.
- Sinacore, D. R., & Gulve, E. A. (1993). The role of skeletal muscle in glucose transport, glucose homeostasis, and insulin resistance: implications for physical therapy. Physical therapy, 73(12), 878-891.
- Singh, S. P., Nayak, S., Swain, M., Rout, N., Mallik, R. N., Agrawal, O., ... & Rao, M. V. K. (2004). Prevalence of nonalcoholic fatty liver disease in coastal eastern India: a preliminary ultrasonographic survey. Tropical gastroenterology: official journal of the Digestive Diseases Foundation, 25(2), 76-79.
- Soares, A. L. G., Banda, L., Amberbir, A., Jaffar, S., Musicha, C., Price, A. J., ... & Lawlor, D. A. (2020). A comparison of the associations between adiposity and lipids in Malawi and the United Kingdom. BMC medicine, 18, 1-13.
- Souza, M. R. D. A., Diniz, M. D. F. F. D. M., Medeiros-Filho, J. E. M. D., & Araújo, M. S. T. D. (2012). Metabolic syndrome and risk factors for non-alcoholic fatty liver disease. Arquivos de gastroenterologia, 49, 89-96.
- Stender, S., Kozlitina, J., Nordestgaard, B. G., Tybjærg-Hansen, A., Hobbs, H. H., & Cohen, J. C. (2017). Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. Nature genetics, 49(6), 842-847.
- Suciu, A., Abenavoli, L., Pellicano, R., Luzza, F., & Dumitrascu, D. L. (2020). Transaminases: oldies but goldies. A narrative review. Minerva Gastroenterologica e Dietologica, 66(3), 246-251.
- Sutti, S., & Albano, E. (2020). Adaptive immunity: an emerging player in the progression of NAFLD. Nature Reviews Gastroenterology & Hepatology, 17(2), 81-92.

- Tamori, Y., & Kasuga, M. (2013). Glypican-4 is a new comer of adipokines working as insulin sensitizer. Journal of diabetes investigation, 4(3), 250.
- Tapper, E. B., Ufere, N. N., Huang, D. Q., & Loomba, R. (2022). Current and emerging therapies for the management of cirrhosis and its complications. Alimentary Pharmacology & Therapeutics, 55(9), 1099-1115.
- Targher, G., Byrne, C. D., & Tilg, H. (2024). MASLD: a systemic metabolic disorder with cardiovascular and malignant complications. Gut, 73(4), 691-702.
- Toledo, F. G., Sniderman, A. D., & Kelley, D. E. (2006). Influence of hepatic steatosis (fatty liver) on severity and composition of dyslipidemia in type 2 diabetes. Diabetes care, 29(8), 1845-1850.
- Trépo, E., & Valenti, L. (2020). Update on NAFLD genetics: from new variants to the clinic. Journal of hepatology, 72(6), 1196-1209.
- Tunç, S. (2021). Evaluation of Risk Factors Associated with Fatty Liver Disease in Obese Children and Adolescents: A Single Center Experience. Van Medical Journal, 28(2).
- Tutunchi, H., Naeini, F., Mobasseri, M., & Ostadrahimi, A. (2021). Triglyceride glucose (TyG) index and the progression of liver fibrosis: A cross-sectional study. Clinical Nutrition ESPEN, 44, 483-487.
- Van de Velde, F., Bekaert, M., Geerts, A., Hoorens, A., Batens, A. H., Shadid, S., ... & Lapauw, B. (2019). Insulin resistance associates with hepatic lobular inflammation in subjects with obesity. Endocrine connections, 8(9), 1294-1301.
- Verma, S., Jensen, D., Hart, J., & Mohanty, S. R. (2013). Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). Liver International, 33(9), 1398-1405.

- Vittorio, J., & Lavine, J. E. (2020). Recent advances in understanding and managing pediatric nonalcoholic fatty liver disease. F1000Research, 9.
- Vos, B., Moreno, C., Nagy, N., Féry, F., Cnop, M., Vereerstraeten, P., ... & Adler, M. (2011). Lean non-alcoholic fatty liver disease (Lean-NAFLD): a major cause of cryptogenic liver disease. Acta Gastroenterol Belg, 74(3), 389-394.
- Watanabe, K., Yamada, H., & Yamaguchi, Y. (1995). K-glypican: a novel GPI-anchored heparan sulfate proteoglycan that is highly expressed in developing brain and kidney. The Journal of cell biology, 130(5), 1207-1218.
- Watt, M. J., Miotto, P. M., De Nardo, W., & Montgomery, M. K. (2019). The liver as an endocrine organ—linking NAFLD and insulin resistance. Endocrine reviews, 40(5), 1367-1393.
- Wijarnpreecha, K., Thongprayoon, C., & Ungprasert, P. (2017). Coffee consumption and risk of nonalcoholic fatty liver disease: a systematic review and meta-analysis. European journal of gastroenterology & hepatology, 29(2), e8-e12.
- Wong, V. W. S., Adams, L. A., de Lédinghen, V., Wong, G. L. H., & Sookoian, S. (2018). Noninvasive biomarkers in NAFLD and NASH—current progress and future promise. Nature reviews Gastroenterology & hepatology, 15(8), 461-478.
- Wu, T., Ye, J., Shao, C., Li, F., Lin, Y., Ma, Q., ... & Zhong, B. (2021). Varied relationship of lipid and lipoprotein profiles to liver fat content in phenotypes of metabolic associated fatty liver disease. Frontiers in Endocrinology, 12, 691556.
- Xu, X., Jin, J., & Liu, Y. (2023). Performance of FibroScan in grading steatosis and fibrosis in patients with nonalcoholic fatty liver disease: A meta-analysis. Arab Journal of Gastroenterology.

- Ye, Q., Zou, B., Yeo, Y. H., Li, J., Huang, D. Q., Wu, Y., ... & Nguyen, M. H. (2020). Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. The lancet Gastroenterology & hepatology, 5(8), 739-752.
- Ying, X., Qian, Y., Jiang, Y., Jiang, Z., Song, Z., & Zhao, C. (2012). Association of the apolipoprotein B/apolipoprotein AI ratio and low-density lipoprotein cholesterol with insulin resistance in a Chinese population with abdominal obesity. Acta diabetologica, 49, 465-472.
- Yoneda, M., Uchiyama, T., Kato, S., Endo, H., Fujita, K., Yoneda, K., ... & Nakajima, A. (2008). Plasma Pentraxin3 is a novel marker for nonalcoholic steatohepatitis (NASH). BMC gastroenterology, 8, 1-9.
- Yoo, H. J., Hwang, S. Y., Cho, G. J., Hong, H. C., Choi, H. Y., Hwang, T. G., … & Choi, K. M. (2013). Association of glypican-4 with body fat distribution, insulin resistance, and nonalcoholic fatty liver disease. The Journal of Clinical Endocrinology & Metabolism, 98(7), 2897-2901.
- Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., & Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology, 64(1), 73-84.
- Zhang, Q. Q., & Lu, L. G. (2015). Nonalcoholic fatty liver disease: dyslipidemia, risk for cardiovascular complications, and treatment strategy. Journal of clinical and translational hepatology, 3(1), 78.
- Zierle-Ghosh, A., & Jan, A. (2023). Physiology, Body Mass Index. In StatPearls [Internet]. StatPearls Publishing. 2024 Jan PMID: 30571077.

Appendices

APPENDIX.1

Questioner for participants

Name: _____

Age:_____

Gender :_____

Number of telephone:_____

Height:_____

Weight:_____

Chronic disase :_____

Stage of disases :

NAFLD : _____

NASH:_____

Cirrhosis:_____

Calculater of some paramter

Lipid profile :

cholestrol _____

Triglycerides_____

HDL _____

Liver enzyme : AST\ALT _____

APPENDIX.2

MEASUREMENT OF BIO MARKER

The DIRUI device automatically measures the liver enzymes and lipid profile.

I use a centrifuge to separate the blood sample, and then I put the separated serum into the Hitachi cup. I then measure it after placing it in the appropriate location on the DIRUI device.

الخلاصة

في المجتمع الغربي، يعد مرض الكبد الدهني من أكثر الأمراض المزمنة شيوعًا في الكبد. ويحدث بسبب تراكم الأحماض الدهنية في خلايا انسجة الكبد على شكل دهون ثلاثية ، بدلاً من أن يكون بسبب الكحول أو فيروس التهاب الكبد. يعتبر مرض الكبد الدهني غير الكحولي (NAFLD) من أكثر الأسباب شيوعًا لأمراض الكبد، ومعدل انتشاره في تزايد عالميًا. يشمل مرض الكبد الدهني غير الكحولي طيفًا من الأمراض الكبدية تتراوح من الدهون الكبدية والتهاب الكبد الدهني غير الكحولي (NASH) والتليف إلى التشمع. يعتبر مرض الكبد الدهني غير الكحولي مرضًا متعدد الأنظمة وله تداعيات خارج الكبد تشمل مرض السكري من النوع 2 وأمراض القلب والأوعية الدموية (CVD). أصبح مرض الكبد الدهني غير الكحولي يُعرف الآن بمرض الكبد الدهني المرتبط بالخلل الأيضي (MAFLD).

هدفت هذه الدراسة إلى قياس مستوى كليبيكان-4، بنتراكسين-3، ومؤشر الدهون الثلاثية - الجلوكوز (TyG)، في المرضى الذين يعانون من مرض الكبد الدهني، بما في ذلك NAFLD، NASH، التشمع، والمتطوعين الأصحاء. كما هدفت إلى تقييم ملفات الدهون، ومقاومة الأنسولين، ومستويات إنزيمات الكبد (ALT و AST) في الأشخاص الذين يعانون من مرض الكبد الدهني والذين لا يعانون منه. الدراسة اجريت على تسعين مريضًا يعانون من مرض الكبد الدهني وثلاثين شخصًا سليما. المجموعة 1 (30 مريضًا) يعانون من NAFLD، والمجموعة 2 (30 مريضًا) يعانون من NASH، والمجموعة 3 (30 مريضًا) يعانون من التشمع، والمجموعة 4 (30 شخصًا سليما) تمثل المجموعة السيطرة. تم جمع العينات في الفترة الممتدة بين تشرين الثاني 2023 ونيسان 2024 في مركز بابل لأمراض الجهاز الهضمي والكبد. تم تسجيل البيانات الاجتماعية والديموغرافية للمرضى، بما في ذلك أعمارهم، وأجناسهم، وأوزانهم، وطولهم.

تم قياس مستوى بنتراكسين-3 (PTX3) وجليبيكان-4 (GPC4) باستخدام جهاز ELISA، بينما تم حساب مؤشر TyG (ln (fasting triglyceride/fasting glucose/2)) وتم قياسه باستخدام جهاز تحليل الكيمياء الآلي DIRUI CS-T180. قياس ملف الدهون (الكوليسترول والبروتين الدهني عالي الكثافة(HDL) وإنزيمات الكبد (AST و ALT) باستخدام نفس الجهاز.

أظهرت النتائج أن الفروق العمرية بين المجموعة 1 (NAFLD)، المجموعة 2 (NASH)، المجموعة 3 (التشمع)، والمجموعة السيطرة كانت ذات دلالة إحصائية ($p\leq0.05$). عند مقارنة المجموعات الثلاث (G1 و G2 و G3) مع المجموعة السيطرة، تبين أن المجموعة 3 كان لديها

متوسط أعلى من المجموعة السيطرة. كما كان مؤشر كتلة الجسم (BMI) في المجموعات الثلاث (المجموعة 1، المجموعة 2، والمجموعة 3) أعلى من المجموعة السيطرة ، وكان أعلى قيمة في المجموعة 1. كانت الفروق في مؤشر كتلة الجسم بين المجموعات ذات دلالة إحصائية.

كان هناك فرق إحصائي ذو دلالة في مؤشر TyG وكليبيكان-4 بين المجموعات المرضية والمجموعة السيطرة ، ولكن ليس في بنتراكسين-3 ($p\leq0.05$). كانت قيم مؤشر TyG في المجموعات 1 ((NAFLD،(NASH) 2 ، و 3 (التشمع) أعلى من المجموعة السيطرة ولكنها ظلت متشابهة إلى حد كبير. كان مستوى كليبيكان-4 في المجموعات 1 و 2 و 3 أعلى من المجموعة السيطرة ، وكانت المجموعة 3 لديها أعلى قيمة إجمالية. كان متوسط المجموعة 1 أكبر من المجموعة 2، وكان متوسط المجموعة 3 أعلى من المجموعتين 1 و 2 وكذلك من المجموعة السيطرة في بنتراكسين-3.

في هذه الدراسة وجد ارتباط طردي قوي ($p<0.01$) بين ALT وكلاً من مؤشر كتلة الجسم و AST. وارتباط طردي كبير ($p<0.01$) بين LDL ومؤشر كتلة الجسم. أظهر HDL ارتباطًا طرديا قويًا مع العمر وارتباطًا عكسيا قويًا مع مؤشر كتلة الجسم، على عكس LDL. أظهرت الدهون الثلاثية ارتباطًا طرديا قويًا ($p<0.01$) مع ALT و LDL وارتباطًا عكسيا ($p\leq0.05$) مع HDL. وكان للكوليسترول ارتباط عكسي مع HDL وارتباط طردي مع الدهون الثلاثية و LDL. كما له ارتباط طردي ($p<0.01$) مع ALT و مؤشر كتلة الجسم. وارتباط طردي قوي بين VLDL و LDL و TG والكوليسترول وارتباط عكسي مع HDL.

اظهرت الدراسة ارتباط طردي بين العمر والجلوكوز، وارتباط طردي ($p<0.01$) بين HOMA-IR والجلوكوز. أظهرت النتائج الإحصائية ارتباطًا طردي كبيرًا ($p<0.01$) بين ماركرات بنتراكسين-3 وكليبيكان-4. وجد ارتباط طردي كبير ($p<0.01$) بين العمر و LDL والدهون الثلاثية والكوليسترول والجلوكوز و HOMA-IR وفقًا لمؤشر TyG.

بسم الله الرحمن الرحيم

"يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِير۝"

صدق الله العلي العظيم

سوره المجادلة الاية-11

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم التحليلات المرضية

دور الجليبكان-4 والبنتراكسين-3 ومؤشر الدهون السكرية كمؤشرات محتملة في تقييم مرضى الكبد الدهني غير الكحولي.

رسالة مقدمة

إلى مجلس كلية العلوم الطبية التطبيقية – جامعة كربلاء

كجزء من متطلبات نيل درجة الماجستير في علوم التحليلات المرضية

من قبل

مريم سلام عباس

بكالوريوس تحليلات مرضية

العلوم الطبية التطبيقية – جامعة كربلاء، 2021.

تحت إشراف

أ. د. غصون غانم **د. أحمد عبد الحسين الحلي**

2025 م **1446 هـ**