**An-Najah National University**

**Faculty of Graduate Studies**

# PANCREATIC IMMUNE CELL ALTERATIONS IN AN ANIMAL MICE MODEL OF LIVER FIBROSIS

**By**
**Diana Abu Arra**

**Supervisor**
**Dr. Johnny Amer**

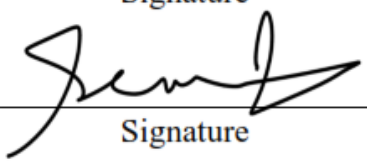
**This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Clinical Biochemistry, Faculty of Graduate Studies, An-Najah National University, Nablus - Palestine.**

**2023**

# PANCREATIC IMMUNE CELL ALTERATIONS IN AN ANIMAL MICE MODEL OF LIVER FIBROSIS

**By**
**Diana Abu Arra**

This Thesis was defended successfully on 19/10/2023 and approved by:

| | |
|---|---|
| **Dr. Johnny Amer**<br>Supervisor |  <br>Signature |
| **Dr. Lina Abu Tair**<br>External Examiner |  <br>Signature |
| **Dr. Samer Abdallah**<br>Internal Examiner |  <br>Signature |

# Dedication

To everyone who helps the seeker of knowledge and spreads hope in him. To all who are passionate about seeking knowledge. To everyone who sought to learn and did not care about his capabilities or circumstances. To every seeker of knowledge who strives with his knowledge to benefit the nation with all that God has given him of knowledge.

To everyone who left me with hope that made me look at life in a strange way. To everyone who taught me a letter and walked on the path of knowledge and sincere intentions. To the fountain of selfless giving, to my dear mother, may God protect her.

To the one who did not sting on me in anything, to the one who sought my comfort and happiness, my dear father. To my brothers and sisters who are the source of optimism and support for me. To everyone who helped me and had a role from near or far in completing this research.

# Acknowledgment

All praise and thanks to Allah who makes blessings last, the Lord of the Worlds, who taught me of His knowledge in His All-Knowing Name, and instilled in me the ability and will, and His kindness and planning accompanied me in all stages of my life and for guiding me all throughout this journey.

Those who give, love and sacrifice, my father and mother, have always inspired me and continue to do so. My father, who gave me love, support and respect. My mother, whom I see as a model of a sacrificial mother, thanks you very much for what you have done for my happiness and success. Special thanks to my brothers and sisters who support my decisions and encourage me to advance.

Thanks, and great appreciation to all my teachers for their effort, time, and cooperation. Appreciation and special thanks to my supervisor, Dr. Johnny Amer, who kindly supervises the preparation and follow-up of this dissertation and provides guidance and direction, you have all the appreciation and praise for what you have provided me. A big thanks to Dr. Ahmad Salhab for his efforts and cooperation with me in conducting the research.

All thanks to the Dr. Lina Abu Tair an external supervisor, and all thanks and appreciation to the Dr. Samer Abdallah an internal examiner.

## Declaration

I, the undersigned, declare that I submitted the thesis entitled:

# PANCREATIC IMMUNE CELL ALTERATIONS IN AN ANIMAL MICE MODEL OF LIVER FIBROSIS

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

**Student's Name:** Diana Abu Arra

**Signature:** 

**Date:** 19/10/2023

# Table of Contents

# List of Figures

# List of Appendices

# PANCREATIC IMMUNE CELL ALTERATIONS IN AN ANIMAL MICE MODEL OF LIVER FIBROSIS

**By**
**Diana Abu Arra**
**Supervisor**
**Dr. Johnny Amer**

## Abstract

**Background:** Liver fibrosis is closely linked to the most common metabolic illnesses and developing acute pancreatitis (AP).

**Aims:** This study aims to investigate the effects of liver fibrosis on modulating changes in pancreatic NK cells and involvement of molecular pathways in a mice model of liver injury.

**Methodology:** Carbon-tetrachloride; $CCl_4$ (i.p injected) of acute (2 weeks) and chronic (4 weeks) models of male C57/BL mice of liver injury was performed. At the end of experiments, mice were anaesthetized, and serum was collected for assessing liver enzymes, pancreatic injury of lipase and amylase. Annexin V examination of the pancreatic tissue for β-islet cell apoptosis, metabolic makers of C-peptide levels as well as for lipid and glucose profiles were performed. The livers were harvested for histological evaluations of inflammatory (H&E staining) and fibrotic (Sirius Red stain) profiles. Moreover, real-time polymerase chain reaction (PCR) was used for □SMA and collagen III. IL-6 levels from pancreatic β-Islet cells were also evaluated. Pancreatic tissue-resident (tr)NK cells were isolated and evaluated for their activity through assessing INF- γ and IL-6 receptor by the ELISA.

**Results:** In the chronic $CCl_4$ induced-model histological characterization of the liver injury was deteriorated as compared to the acute model and the naïve mice. Serum ALT and AST levels, as well as metabolic evaluations of cholesterol, triglyceride, C-peptide, fasting blood sugar, and fibrotic profiles revealed a positive relationship with illness progression and severity of liver fibrosis. Pancreatic enzymes were elevated in liver fibrosis mice model and were associated with β-islet cells apoptosis. An inverse strength association between IL-6 of β-Islet cells and severities of liver fibrosis was achieved. β-Islet cells which exhibit high secretions to IL6 and caused an up-expressions of trNK

IL6R, which in part, affected trNK activity and caused their inability to produce enough quantities of IFN-γ.

**Conclusion:** Liver fibrosis induces pancreatic injury, as evidenced by elevated amylase and lipase levels and increased islet cell apoptosis. Dysregulation of lipid and glucose metabolism might have implications for pancreatic health. Antagonizing IL6 and/or IL6R could improve NK cell activity and delay progression of AP.

**Keywords:** Pancreas; NK cells; liver fibrosis; mice model .

# Chapter One
# Introduction

## 1.1 Background

One of the most significant challenges to global health is liver fibrosis, triggered by viral or metabolic chronic liver disorders (1). fibrosis results from persistent wound-healing and serves as the final pathway response to chronic liver injury. It is characterized by an extensive buildup of extracellular matrix (2), leading to a loss of architecture and subsequent functional failure (3, 4).

Chronic Hepatitis C virus (HCV) and Hepatitis B virus (HBV) infections, toxicity (e.g., alcohol-induced liver disease, lipid overload), non-alcoholic steatohepatitis (NASH), autoimmune disorders such as primary biliary cirrhosis, primary sclerosing cholangitis, and genetic diseases constitute the most common causes (3). These factors lead to hepatocyte injury and immune cell infiltration. In the context of liver fibrosis, the HSCs present in the liver undergo a process known as trans differentiation, when they transform into myofibroblasts. These myofibroblasts play a crucial role in the creation of collagen and contribute to the overall process of tissue healing. In instances of short-term injury, counteracting anti-fibrotic mechanisms balance this process, leading to deactivation or apoptosis of myofibroblasts and subsequent disappearance of scar tissue (1).

In instances of chronic liver illnesses, a discrepancy occurs between processes that promote fibrogenesis and those that inhibit it, leading to the sustained activation of HSCs. The continuous stimulation and multiplication of myofibroblasts disrupt the equilibrium between the deposition and breakdown of the ECM, leading to increased ECM production and the onset of hepatic fibrosis. HSCs and other pro-inflammatory factors are known to induce the production of MMPs and other enzymatic substances that facilitate the degradation of the extracellular matrix, leading to the substitution of normal ECM with a modified matrix (1). MMPs play a crucial role in cleaving fibrillar collagen types I, II, and III into the typical 3/4 and 1/4 fragments (5). The dysregulation of many components has a role in the alteration of matrix stiffness, flexibility, and density during ECM remodeling. The dynamic ECM retains cytokines and growth factors generated by cells, amplifying inflammatory reactions and fibrogenesis (1).

Non-parenchymal cells (NPCs), such as Kupffer cells and other immune cells, exert significant control over the liver's progression, either pushing it towards an uncontrolled fibrosis-promoting stage or guiding it towards an anti-fibrotic scar-dissolving stage. Hepatocyte apoptosis and DAMP release from damaged plasma membranes directly stimulate HSCs and attract and activate lymphocytes and macrophages. The activation sequence described herein facilitates the process of HSC trans-differentiation and myofibroblast activation via triggering the production of pro-inflammatory and pro-fibrogenic cytokine synthesis (1). Additionally, exogenous mito-DAMPs induce fibrogenic activation in HSCs (6).

At the molecular level, a complex network of signaling pathways, initiated by cytokines, orchestrates interactions among pro-fibrogenic cells. HSC activation and fibrosis formation are thought to depend on WNT/-catenin signalling, transforming growth factor beta (TGF-β), platelet-derived growth factor (PDGF), and the inflammasome (NLRP3).Caspase-1 is an enzyme that plays a critical role in the process of programmed, among others (1). HSCs possess a substantial concentration of PDGF receptors, and upon activation, these receptors stimulate HSC proliferation and migration (7).

One of the major contributors to hepatotoxicity, which accelerates the onset of chronic inflammation, oxidative stress, and fibrosis, is the accumulation of lipids in hepatocytes. Among these, triglycerides – considered a non-cytotoxic lipid form – predominantly accumulate in the liver. The buildup of detrimental triglyceride synthesis intermediates, including saturated free fatty acids (FFAs) and their derivatives, free cholesterol, or complex lipids like lysophosphatidylcholine and ceramides, leads to lipotoxicity (1). This process results in the accumulation of noxious lipids, aberrant organelles, cellular damage, and persistent inflammation. The inflammation and fibrosis caused by these FFAs inflict harm on liver cells, intensifying over time and accompanied by a multitude of inflammatory mediators (8).

On the other hand, the buildup of FFAs is one of the contributing components that accelerates the production of ROS. Reactive oxygen species are generated as byproducts of aerobic metabolism, resulting from the reduction of molecular O2 to H2O (9). This group includes superoxide, $H_2O_2$, and hydroxyl radicals, constituting a family of pro-fibrotic mediators. Oxidative phosphorylation and lipid peroxidation in hepatocytes,

HSCs, and macrophages create ROS during normal cellular metabolism (1). Hepatocyte necrosis and apoptosis occur when ROS damage lipids, proteins, and DNA, causing liver cell death. ROS also causes activated Kupffer cells, HSCs, and other inflammatory cells to produce pro-inflammatory and pro-fibrogenic chemicals (1). According to reports, ROS causes several organs to express more TGF-β than usual (10). Activated hepatic stellate cells (HSCs) and myofibroblast contractility cause hepatic sinusoids to constrict in advanced fibrosis. Narrowing blood arteries reduces blood flow and nutrition exchange, causing liver failure (1).

Inflammation of the liver induces the synthesis of inflammasomes, cytokines, chemokines, and their corresponding receptors, in addition to the recruitment of both innate and adaptive immune cells. The presence of liver inflammation leads to the cessation of tissue healing processes. Prolonged inflammation draws in and activates cells that contribute to the formation of ECM, ultimately leading to an excessive accumulation of ECM and the eventual development of liver fibrosis (8).

Pro-inflammatory mediators are produced due to chronic liver damage; lymphocytes infiltrate the subendothelial region (1). Inflammatory and tissue-regenerative reactions induced by tissue injury are significantly assisted by macrophage (11). Macrophages derived from bone marrow (BM) and/or tissue-resident macrophages are activated and exhibit a pro-inflammatory behavior in response to the local environment. They then swiftly transition into a wound repair phenotype following tissue injury (11). The early inflammatory response often diminishes after tissue injury when recruitment or activation of monocytes or macrophages is prevented. Improper or inadequate macrophage activation can result in incomplete tissue regeneration, potentially delaying wound healing and prolonging exposure to pro-inflammatory stimuli (11).

The liver must find a balance between preventing harm to hepatocytes and other tissues from the antigenic response and immunological activation triggered by antigen exposure. The diminished ability of fibrotic or cirrhotic livers to maintain this equilibrium leads to prolonged immunological activation and eventually, premature immune fatigue (immunosenescence) (12).

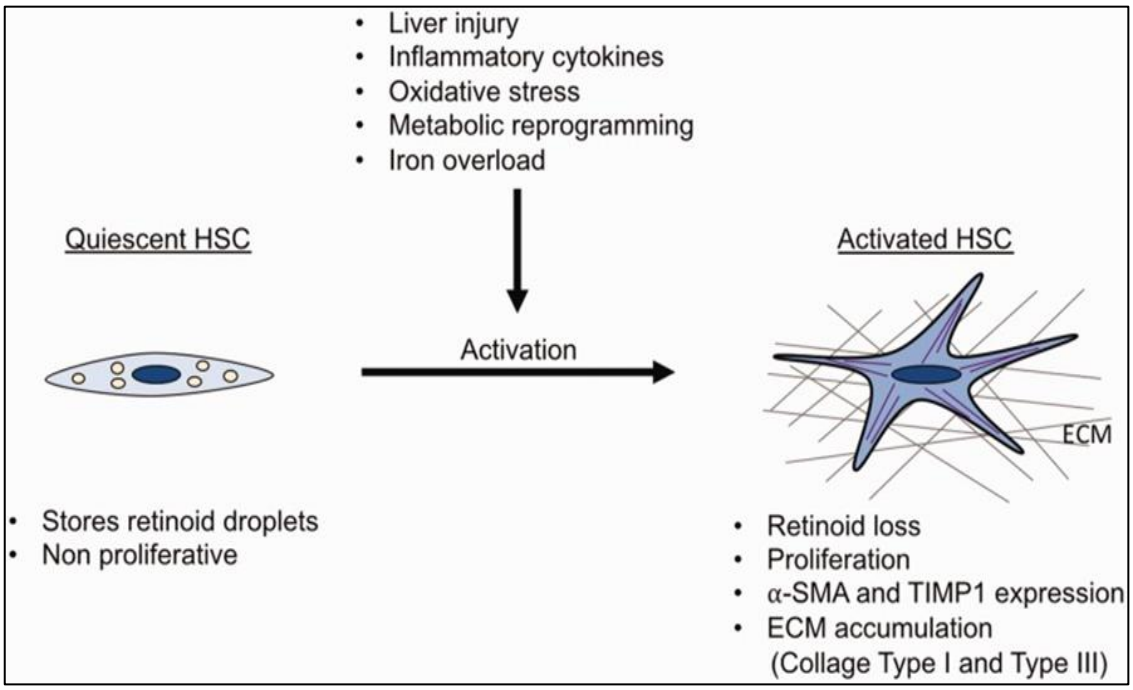
Patients with acute and chronic liver failure experience elevated levels of systemic inflammation and immunodeficiency, increasing their susceptibility to infections and

accelerating the progression of organ failure. Immunodeficiency, in turn, arises from both structural distortion of the liver parenchyma and functional impairment and reprogramming of circulating immune cells due to the metabolic abnormalities associated with liver insufficiency. Circulating monocyte levels are elevated, particularly within the pro-inflammatory and profibrotic non-classical CD14+CD16+ subpopulation. These monocytes exhibit activation indicators, including increased expression of the human leukocyte antigen (HLA-DR) and heightened spontaneous TNF-α production. Neutrophils are depleted and display reduced phagocytic activity and decreased chemotaxis to infection sites due to splenic sequestration. Defective thymopoiesis, heightened splenic pooling, and activation of cell death pathways all contribute to the reduction of circulating CD4+ T-helper cells. Circulating NK cells show diminished cytolytic activity and a subpar response to cytokine stimulation (13).

Immune cells such as innate ILC, Kupffer cells, Th17 cells, and bone marrow-derived monocytes produce cytokines that activate HSCs during hepatocyte and cholangiocyte cell death (10) as illustrated in Figure 1.

**Figure 1**

*Features of quiescent and activated HSCs In case of liver injury*



The increased release of inflammatory cytokines, ROS production, HSCs become activated and transformed to myofibroblast. myofibroblast are characterized by loss of retinoid droplets, increased proliferation, and expression of fibrogenic markers (α-SMA, TIMP1). Moreover, myofibroblast produce ECM proteins such as collagen I and collagen III which are signs of liver fibrosis (10).

By activating STAT3 and NF-κB, the profibrogenic cytokine IL-17 stimulates HSCs to produce increased amounts of collagen type I, α-SMA, and TGF-β. The IL-17 molecule is released by Th17 cells, and in fibrotic livers, neutrophils and mast cells might also contribute significantly to IL-17 production. Monocyte chemotactic protein type I (MCP-1) and CCL5 assist in fibrogenesis. Injured hepatocytes release IL-33, which prompts ILCs to generate IL-13, subsequently stimulating HSCs. Leukocyte chemotaxis and recruitment to the injured liver are governed by small chemotactic cytokines known as chemokines (10). C-C motif chemokine (CCL2), also referred to as MCP-1, is part of the chemokine family released by activated macrophages, fibroblasts, and parenchymal cells in response to injury. In the injured liver, CCL2 promotes the recruitment of monocyte-derived macrophages, which in turn activate myofibroblasts. Kupffer cells are the primary source of TGF-β, a key factor in the production of ECM proteins such as type I and type II collagen. TGF-β also inhibits ECM breakdown by activating Smad2/3. TGF-β promotes HSC activation into myofibroblasts and contributes to liver fibrosis through various mechanisms. Platelets, macrophages, myofibroblasts, and HSCs collectively generate PDGF. This potent HSC mitogen is abundant in fibrotic livers, where it interacts with TGF-β to form a fibrogenic factor (10).

Mice can serve as a valuable model for studying liver disease due to their ability to replicate human conditions, particularly in terms of liver fibrosis. They are practical for drug testing and manipulation. Several approaches are available to induce liver fibrosis in mouse models. One method involves administering a methionine/choline deficient (MCD) diet, while another involves using $CCl_4$, a commonly employed substance for inducing liver fibrosis. In mice, repeated $CCl_4$ dosages plus oxidative stress from a high-fat diet caused fibrosis, inflammation, and apoptosis (14). Liver fibrosis mice model of $CCl_4$ showed association with lipid imbalance (15). Triglycerides accumulated in hepatocytes because of $CCl_4$ stimulation of cytochrome P450 with ß-naphthoflavone or metyrapone. The rate of lipid esterification as well as the production of fatty acids and

triglycerides were both accelerated by $CCl_4$. The synthesis of phospholipids and cholesterol from acetate was also enhanced, whereas the hydrolysis of triglycerides was decreased. Following $CCl_4$ injection, the amount of unsaturated fatty acids in microsomal lipids was about 50% lower while the amount of saturated fatty acids slightly increased (15). Therefore, because of its effect in increasing cholesterol and triglycerides and due to its speed in producing results, we have adapted this model in our research.

Clusters of abdominal obesity, dyslipidemia, hyperglycemia, and hypertension collectively constitute the metabolic syndrome (16). The development of metabolic syndrome and its pathophysiological consequences are linked to obesity and low-grade inflammation (17) as shown in Figure 2 (See appendix D) . The accumulation of body fat in central areas is closely linked to insulin resistance (16). Adipose tissue with compromised metabolism often exhibits fibrosis. The extracellular matrix protein network encompassing adipocytes provides structural support and responds to various signaling cues. This matrix can maintain flexibility while still accommodating healthy adipose tissue growth without disrupting metabolism. However, as obesity progresses, increased interstitial fibrosis within white adipose tissue (WAT) can compromise ECM flexibility and tissue plasticity, eventually leading to adipocyte dysfunction (18). Adipocytes ensconced within a constrictive ECM “shell” lose their proper functions within fat pads and become more prone to necrosis. Both adipocytes and immune cells generate pro-inflammatory cytokines in response to hypoxia, triggering macrophage recruitment and initiating inflammation and dysfunction within adipose tissue (18, 19). Macrophages, neutrophils, lymphocytes, and mast cells are drawn to the vicinity of dysfunctional adipocytes surrounding lipid droplets, initiating a local pro-inflammatory environment. The inflexibility of these fibrotic adipose depots results in both physical and metabolic inflexibility. subsequently, causes the excessive accumulation of lipids in peripheral organs such as the liver and muscle (18).

Insulin resistance (IR) arises from the inflammatory response triggered by chronic infection within fibrosis, elevating the susceptibility to type 2 diabetes mellitus (T2DM) (20). The inflammatory response to infection in the liver severely disrupts insulin signaling, particularly at the level of insulin receptor substrate (IRS) tyrosine phosphorylation and phosphoinositide 3-kinase activation. Furthermore, the accumulation of lipids in the liver due to IR, coupled with the generation of reactive ROS,

may indirectly activate stellate cells, and initiate cellular signaling pathways that contribute to the advancement of hepatic fibrosis (20).

By reducing the production of very low-density lipoprotein (VLDL) and the transfer of FFAs from the liver, hyperinsulinemia, along with consequent insulin resistance, promotes the accumulation of fatty acids (FAs) and triglycerides (TGs) in the liver (20).

Steatogenesis in NAFLD primarily arises from an elevation in hepatic esterification of FFAs, which are derived from dysfunctional or inflamed white adipose tissue and de novo lipogenesis (21). Through the reduction of VLDL production and the transfer of FFAs from the liver, coupled with an increase in IR and hyperinsulinemia, the accumulation of fatty acids (FAs) in the liver is exacerbated, leading to the accumulation of hepatic TGs and the onset of obesity (22).

Furthermore, heightened lipolysis stimulates hepatic insulin resistance by inducing hepatic gluconeogenesis and propelling hepatic lipid synthesis via FFAs esterification. This process furnishes the hepatocyte with an excess of lipogenic substrates (including glucose and non-esterified fatty acids) and hormones, denoted by IR, a prominent characteristic of chronic infection (hyperinsulinemia) (21, 23). Muscle IR enhances de novo lipogenesis by augmenting glucose supply to the liver. Hepatic IR has been explicitly correlated with intrahepatic ectopic lipid storage (21).

Hepatokines or other endocrine mediators discharged by the liver can impinge on insulin action, secretion, and glucose metabolism. Steatotic hepatocytes secrete Fetuin-A, which blocks the insulin receptor and promotes IR. Diminished adiponectin production, ensuing from disruptions in secretory adipocytes due to heightened ECM in fibrosis, exerts a secondary impact on insulin sensitivity (18). This contributes to escalated WAT inflammation and dysfunction, independently associated with the onset of T2DM (21).

Abdominal obesity manifests with persistent low-grade inflammation and immune system activation, which could potentially contribute to the pathophysiology of metabolic disorders associated with obesity. Within the realm of obesity and T2DM, inflammation is evident in the pancreas, liver, muscle, and adipose tissue. Individuals affected by obesity and metabolic syndrome both demonstrate macrophage infiltration within these tissue tissues (19).

During obesity, there is a notable increase in immune cells within the stromovascular portion of adipose tissue. Notably, obesity induces macrophage infiltration in both mouse and human fat tissues. Furthermore, abdominal fat may generate pro-inflammatory molecules in the portal circulation, subsequently causing hepatic inflammation. Saturated fatty acids, which can activate the NLRP3 inflammasome, thereby triggering inflammatory cascades in macrophages and adipocytes, emerge as potential contributors (19).

The inability of adipose tissue to adequately counteract excessive nutritional intake becomes evident through elevated circulating concentrations of non-esterified fatty acids. This connection is linked to the dyslipidemic state characteristic of metabolic syndrome. In scenarios of overload, the liver produces more apo-B-containing particles that transport triacylglycerols to adipose tissue, resulting in the formation of low-density lipoprotein (LDL). This process is particularly pronounced in visceral adipose tissue, which is adept at lipid release as needed. In contrast, due to its typically larger size, subcutaneous adipose tissue possesses a greater capacity for lipid storage. However, the conversion of VLDL or similar particles may experience delays, leading to the development of hypertriglyceridemia when the storage capacity of both sites is exceeded. Furthermore, lipid buildup is also seen in several other tissues, including the liver, muscle, pancreas, and heart. Nevertheless, lipotoxicity may occur due to the organs' limited ability to store lipids without impairing their functional performance (17). Elevated TG/TC ratios impede glucose absorption and stimulate gluconeogenesis in the liver, while hyperlipemia results in a modified insulin signaling pathway (24), eventually culminating in insulin resistance within the pancreas, liver, and muscle (17).

In the context of obesity, inflammation emanates from multiple sources including adipose tissue, the liver, muscles, and pancreas. Within these tissues, a shift towards a pro-inflammatory cell population occurs, marked by the infiltration of macrophages and other immune cells. These cells play a pivotal role in generating pro-inflammatory cytokines, including TNF, within adipose tissue, directly contributing to obesity-induced insulin resistance. Furthermore, IL-6 and IL-1 impede insulin signaling in peripheral tissues, leading to cellular dysfunction and subsequent insulin deficiency through autocrine and paracrine actions (19).

Studies indicate that circulating components such as lipoproteins and SFAs may contribute to islet inflammation, beta cell dysfunction, and β-islet cell hyperplasia (25). Hyperglycemia is often accompanied by high SFAs levels, as these two conditions appear to have cumulative effects on β-islet cell growth. Ceramides may be synthesised from SFAs inside the islet, thus promoting apoptosis of beta cells. This phenomenon plays a role in the development of diabetes (25). Obesity causes T2DM, which is characterised by reduced or inaccurate insulin secretion, insulin sensitivity, dyslipidemia, and liver dysfunction (23).

### 1.2 Pancreas and liver axis

The pancreas and liver share several blood arteries and channels. and, have many blood veins and tubes that connect them (26). The pancreas is a gland with distinct metabolic activities, comprising exocrine and endocrine components. The endocrine cells produce hormones that regulate glucose metabolism and homeostasis, such as glucagon and insulin. In contrast, the exocrine pancreas manufactures and secretes digestive enzymes, which are conveyed to the duodenum through a network of pancreatic ducts. The pancreatic islets, also referred to as islets of Langerhans, consist of hormone-secreting endocrine cells grouped together, while acinar cells in the exocrine compartment are organized into functional units along the ductal network. Many of the endocrine islets – 60% of islets producing insulin and 30% of islets producing glucagon – are located within the pancreatic polypeptide (PP) cells and other cells, constituting the remaining 10% (27).

The liver and pancreatic β-islet cells are key organs for lipid and glucose metabolism. The process of communication between pancreatic β-islet cells and the liver plays a crucial role in the precise control and adaptive responses of the body. It has been observed that the liver's release of proteins and other substances contributes to the control of pancreatic β-islet cell pathophysiology. When metabolic dysregulation is present, the interaction between these two tissues changes. Hepatocytes release proteins known as hepatokines, which manage energy balance and potentially influence the metabolism of distant organs. These proteins act as messages from the liver to the pancreas (28).

Due to IR, β-islet cells are consequently exposed to prolonged high glucose concentrations. When glycolysis becomes saturated, extra glucose is diverted to ROS-generating pathways, causing ROS buildup and oxidative stress. Furthermore, studies

have indicated that excessive fat exposure (hyperlipidemia) elicits oxidative stress responses in β-islet cells, contributing to lipotoxicity (29).

Numerous hepatokines are secreted as the liver's lipid content increases, including hepatokines such as non-esterified fatty acids (NEFA) and fetuin-A (29). The commencement of this process is motivated by heightened energy accessibility, resulting in the buildup of lipids in the liver, the activation of immune cells in the vicinity, and subsequent infiltration. As a result, the liver synthesizes proinflammatory chemicals that have an influence on the neighboring tissues. Certain hepatokines, which play a role in the regulation of bodily metabolism, could infiltrate the pancreatic islets, therefore initiating a series of activities that may either promote the protection or induce harm. Observations suggest that beta cell death is triggered by NEFA and cytokines, with NEFA causing islet inflammation by stimulating local macrophages and activating toll-like receptor 4 (TLR4) (28).

In addition, the process of attracting macrophages to islets is enhanced by the release of chemokines, such as MCP-1 and IL-8. The association between increased plasma fetuin-A levels and insulin resistance is seen specifically in individuals with elevated (NEFA) concentrations. Fetuin-A, derived from hepatic adipose tissue, directly inhibits glucose-stimulated insulin secretion (GSIS) by modulating beta cells without TLR4, while intensifying pancreatic inflammation by inducing a pro-inflammatory response in islet macrophages and monocytes (30). Islet-resident macrophages have the potential to operate as early detectors of metabolic abnormalities within the body. Consequently, these macrophages could undergo shifts in their behavior or localized growth. Conversely, β-islet cells, being capable of sensing excessive fatty acids, have the ability to generate chemokines, thereby attracting Ly6C+ monocytes and macrophages to the islets, positioning them as early responders (31). These circumstances could potentially accelerate the decline of β-islet cells (30).

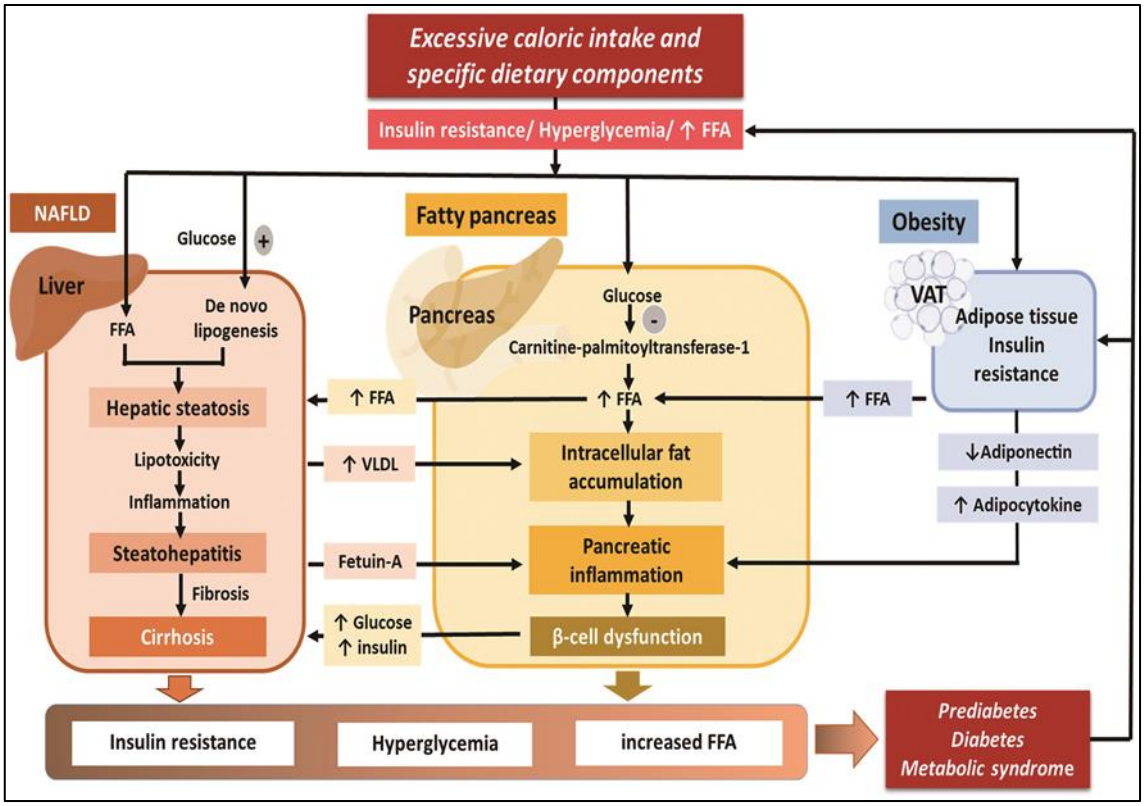
Within minutes of the onset of the illness, immune cells rapidly infiltrate the affected area. Most of these invading cells belong to the innate immune system, including neutrophil granulocytes, monocytes, and macrophages (32). Thus, cytokines or DAMPs resulting from acinar cell necrosis play a pivotal role in activating immune cells. Upon stimulation by CCK, acinar cells have been shown to elicit a cascade of cytokines and

chemokines, including TNF-α, iIL-6, and MCP-1, mediated by activation of NF-κB. Such findings provide insights into the intricate signaling mechanisms underlying acinar cells' physiological and pathological functions. The activated immune cells exacerbate pancreatic damage and contribute to systemic inflammation. Neutrophils combat pathogens by releasing proteases, antimicrobial peptides, and ROS. In pancreatitis, neutrophils are the primary source of ROS, which can lead to oxidative damage to acinar cells and trigger trypsinogen activation. The release of proteases, such as PMN-elastase, facilitates the disintegration of acinar cells and tissue degradation. High levels of TNF-α released by M1 macrophages directly impact pancreatic acinar cells, while invading monocytes releasing TNF-α are identified as causative factors in pancreatic injury and the activation of digestive proteases, according to two distinct studies (32).

Circulating monocytes enter and accumulate in inflamed tissue locations during persistent inflammation. The accumulation of immune cells leads to an increase in inflammatory cytokine and chemokine production. Research has shown a heightened presence of myeloid-lineage cells, namely monocytes and macrophages, inside the islets of animal models exhibiting obesity. According to these studies, saturated fatty acids trigger the production of chemokines by β-islet cells, attracting CD11b+ Ly-6C+ M1-type pro-inflammatory monocytes and macrophages to the islets (33). In pancreatic islet cells, there is an enhanced expression and local release of the pro-inflammatory cytokine IL-1β. This local inflammation can hinder insulin production, cause β-islet cell death, and reduce islet mass – all critical steps in the development of T2DM. Furthermore, the existence of amyloid deposits, fibrosis, heightened concentrations of pro-inflammatory cytokines and chemokines, escalated cellular apoptosis, and infiltration of macrophages are further markers of inflammation inside the pancreatic islets (18), as illustrated in Figure 3.

**Figure 2**

*Organ crosstalk in the pathophysiology of fatty pancreas and nonalcoholic fatty liver disease (NAFLD)*




The risk of insulin resistance, metabolic diseases, and fat buildup in the liver, pancreas, and visceral adipose tissue (VAT) is raised by excessive calorie intake and certain dietary components. When there is insulin resistance, the accumulation of fat in the pancreas can be accelerated by hepatic steatosis and elevated hepatic VLDL, ultimately leading to islet cell death. Alterations in adipocytokines, such as decreasing of adiponectin from VAT and pancreatic fat, directly cause β-cell death. Fetuin-A, derived from hepatic adipose tissue, directly inhibits glucose-stimulated insulin secretion (GSIS) by modulating beta cells without TLR4, accelerates β-cell dysfunction, leading to insulin resistance and ectopic fat deposition in other tissues. Insulin resistance is further enhanced by hepatic fat buildup, creating a vicious cycle where insulin increases the creation of FFA, which then floods the pancreas (34).

Several studies have provided evidence suggesting that the presence of metabolic syndrome, which is associated with liver illness, might be regarded as a risk factor for several organs, including the heart, kidney, and pancreas. This association has been

shown to contribute to the development of AP (35). NAFLD might serve as a prognostic factor indicating an increased likelihood of more severe AP (36). NAFLDis characterized by an excessive accumulation of fat within the liver and stands as a significant risk factor for AP, further exacerbating its severity. Previous research has suggested that factors like obesity, Kupffer cells, oxidative stress, and hyperlipemia could collectively contribute to NAFLD's potential role in the development of AP (2). Through logistic regression analysis, it was discerned that NAFLD independently posed a risk for AP (36). However, the precise mechanism through which NAFLD exacerbates pancreatitis remains unknown.

Obesity is frequently observed in NAFLD patients. Patients with obesity have a lengthy history of chronic inflammation, making it susceptible to an increased inflammatory factor response. Furthermore, NAFLD itself is an inflammatory condition that promotes persistent systemic inflammation, potentially contributing significantly to the exacerbation of AP.

Secondly, Kupffer cells, constituting approximately 70% of the liver's total macrophage population, are believed to play a crucial role in AP's pathophysiology by generating an array of inflammatory substances (36). AP is a prevalent condition that often requires hospitalization; it is an acute inflammatory condition of the pancreas that can also impact neighboring tissues or distant organs (37). AP represents pancreatic inflammatory responses triggered by various factors such as cholelithiasis, biliary obstruction, alcohol, hyperlipidemia, autoimmune issues, and other unspecified variables (38). The condition originates from pancreatic acinar cells (PACs) due to an injury leading to intracellular activation of trypsinogen (39, 40), as shown in Figure 4. If AP is not promptly recognized, it can remain untreated, resulting in a systemic inflammatory response and multiorgan failure, posing a life-threatening risk (38).

Pancreatic stellate cells (PSCs) play a pivotal role in the progression of pancreatic fibrosis (40). These cells are triggered by oxidative stress, ethanol, its metabolite acetaldehyde, and cytokines. Their phenotype transforms from quiescent fat-storing cells to activated myofibroblast-like PSCs. These activated PSCs then generate extracellular matrix, adhesion molecules, and various chemokines in response to cytokines and growth factors. Recent research highlights that PSCs also possess a phagocytic function.

PDGF serves as a potent promoter of PSC proliferation. Transforming growth factor beta, activin A, and connective tissue growth factor play both autocrine and paracrine roles in PSC-mediated pancreatic fibrogenesis. Pathophysiological processes within the pancreas, including pancreatic tissue pressure, hyperglycemia, intracellular reactive oxygen species production, activation of protease-activated receptor 2, induction of cyclooxygenase 2, and bacterial infections, collectively contribute to sustaining pancreatic fibrosis post-pancreatic damage (41).

Tissue fibrosis was initially identified as a contributor to cancer growth and metastasis in the early 1950s (42). The organization of fibrils within the ECM initiates the secretion of pro-fibrotic cytokines and growth factors, resulting in the development of enduring fibrous tissue inside the organ. Moreover, Matrix structural modifications have a substantial influence on cell proliferation and migration since they serve as a regulator of many cellular processes and facilitate cellular communication. This aberrant proliferation of stromal cells, coupled with disrupted ECM dynamics, fosters the emergence of a tumorigenic environment, ultimately facilitating malignant transformation and bolstering the survival and infiltration capabilities of cancer cells.

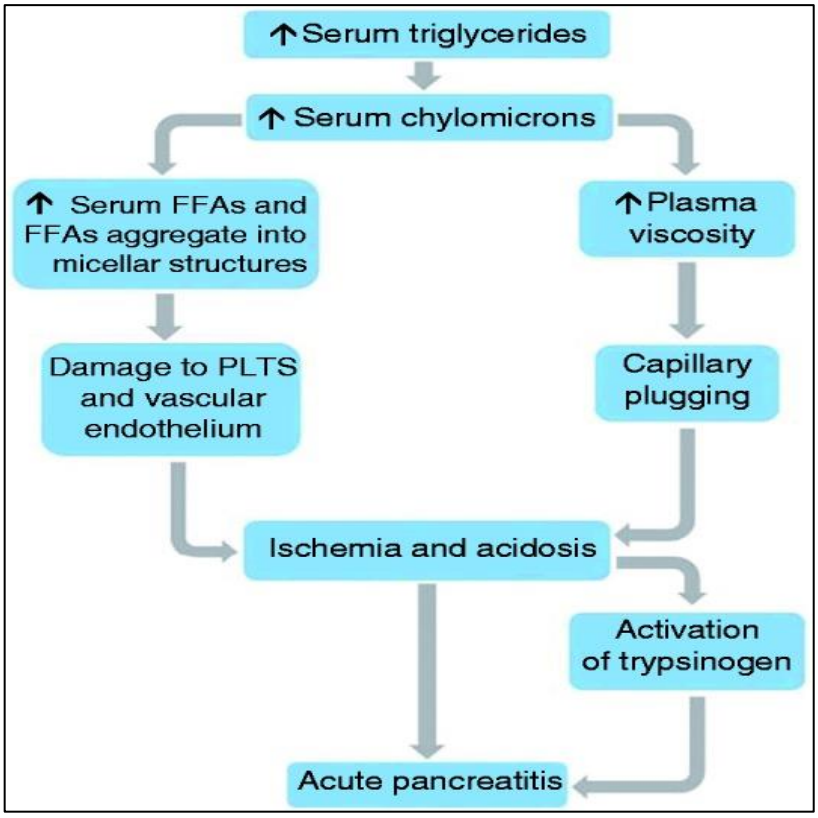
Consequently, carcinogenesis and cancer metastasis are heavily influenced by modified ECM, typically arising from unsuccessful attempts to heal injured tissue. The dysregulation of TGF-β expression in the pancreatic stroma hinders the turnover of ECM and results in the aberrant buildup of matrix proteins and immune cells. Consequently, this creates an environment that promotes the invasion and spread of cancer cells, ultimately leading to metastasis (42). Aoyagi et al. reported an elevation in TGF-β expression, which correlated with increased levels of type I and III collagen. Furthermore, it was observed that there was an increased level of mRNA expression of TGF-β, fibroblast growth factor, PDGF A and C, and epidermal growth factor in the postoperative cancer nodules of individuals diagnosed with pancreatic cancer. These signaling transcription factors combined induce the secretion of inflammatory cells, creating an environment abundant in growth factors, ROS, and inflammatory cells that facilitate the development of tumor cells (42).

The presence of IL-15 has been seen in a rat model of severe AP, where IL-15 functions as a protective factor against organ injury. Research conducted in vitro revealed that

activated NK cells, stimulated by IL-15, have the capacity to selectively target both human PSCs and pancreatic cancer cell lines. This stands in contrast to the behavior of quiescent NK cells. IL-15 is involved in facilitating the proliferation and survival of both NK cells and iNKT cells (40).

**Figure 3**

*Possible mechanisms involved in the pathophysiology of hyper-triglyceridemic pancreatitis (HTGP)*




Note: Hühn MH, Hultcrantz M, Lind K, Ljunggren HG, Malmberg KJ, Flodström-Tullberg M. IFN-gamma production dominates the early human natural killer cell response to Coxsackievirus infection. Cellular microbiology. 2008;10(2):426-36.

### 1.3 Role of immune cells in pancreatic injury

Lymphocytes are pivotal immunoregulatory cells responsible for releasing cytokines that can directly or indirectly modulate immune responses. Pietruczuk *et al.* identified a subset of lymphocytes in patients with AP that displayed significant activation and an enhanced capacity to release Th2-type cytokines to stimulate inflammatory responses (38).

The results suggest that B cells and NK cells could exert a more significant influence on the pathological condition and recovery rate of patients with mild acute pancreatitis (Mild-AP). Among individuals with M-AP, there exists a robust positive correlation

between NK cell frequency and the concentration of serum amylase and lipase activity, which might aid in the precise diagnosis of AP (38). Mouse studies have indicated the critical role of NK cells in ensuring host survival (43).

NK cells constitute the foremost members of the ILC family. Renowned for their capability to eliminate infected or cancerous cells, NK cells also mediate cytotoxicity against various normal immune cells. Vital components of the immune system, NK cells function as sentinels, utilizing molecular detection techniques to identify aberrant cells. Upon activation, NK cells not only induce target cell death via perforin- and granzyme-containing cytotoxic granules but also secrete Interferon gamma (IFN-γ) and tumor necrosis factor (TNF), along with immunoregulatory cytokines such as IL-5, IL-10, IL-13, the growth factor GM-CSF, and chemokines CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), and CXCL8 (IL-8) upon contact with target cells. As a result, they play a crucial role in maintaining immune response regulation and overall homeostasis (44).

Studies on NK cell activation spanning the past decade have illuminated their capacity to respond to inflammatory stimuli arising from tissue injury, thus contributing to both disease development and resolution (45). The function of NK cells in governing cellular immune responses, whether by promoting or suppressing them, as well as their role in maintaining homeostasis through the balance of inhibitory and activating signals involving Killer cell immunoglobulin-like receptors (KIRs), has gained recognition (46).

Natural Killer (NK) cells demonstrate a diverse range of actions, including both their traditional innate involvement in combating tumors and viral infections, as well as regulatory capabilities that impact other immune cells and facilitate tissue formation. One notable aspect that has arisen in comprehending the behavior of NK cells is their metabolic activity. Although the field of NK cell metabolism is still in its early phases of development. Even though, recent research suggests that specific metabolic profiles significantly influence the functional results of NK cells. These profiles may be likened to individual fingerprints that dictate the trajectory of their functionality.

The most robust cytotoxic NK cells stand out due to heightened glucose metabolism facilitated by glycolysis and OxPhos. The predominant energy source for NK cells is glucose, which undergoes processing in both the cytosol and mitochondria through aerobic glycolysis (47). On the contrary, regulatory NK cells exhibit enhanced survival

and functionality under hypoxic conditions characterized by limited glycolytic activity. This observation implies that regulatory processes may be accomplished using restricted amounts of OxPhos and glycolysis. Memory NK cells, which are crucial for long-term survival, possess enhanced mitochondrial functionality, allowing them to adapt and withstand challenges more effectively. This is achieved by removing malfunctioning mitochondria, respiratory capacity, and membrane potential, and decreasing levels of harmful ROS. The available evidence indicates that distinct metabolic profiles, rather than phenotypic characteristics, have a significant impact on distinguishing and directing the many functional outcomes of NK cells (48).

Donnelly et al. examined NK cell responses to TLR3 ligand poly(I:C) activation in vivo after inhibiting glycolysis or mTOR. Using inhibitors from either route substantially lowered the proportion of IFN-γ-+ NK cells in mice 24 hours after poly(I:C) injection. These results suggest that TLR3-induced NK cell IFN-γ relies on glycolysis and mTOR. IFN-γ stability and production rate may be affected by metabolic changes such as protein folding or post-translational modification. Glycosylation is crucial for the folding and stability of proteins, including IFN-γ. IFN-γ may be glycosylated at two locations in humans, improving its secretion and stability (49).

Increasing evidence suggests that poor cellular metabolism is key to the development of malfunctioning NK cells in chronic illnesses like obesity. The metabolic inefficiency highlighted in the statement is linked to PPAR-driven NK cell lipid buildup. This lipid accumulation results in changes in gene expression, namely the downregulation of MYC and mTORC1 signaling. Additionally, it leads to decreased rates of glycolysis and OXPHOS. On the other hand, TGFβ has the ability to directly decrease the metabolic activity of NK cells via many pathways, one of which involves the suppression of mTORC1 signaling (47).

A study was conducted to investigate the dynamic changes in immunity among patients suffering from severe AP and their treatment with traditional Chinese medicine (TCM). The researchers discovered that the TCM group exhibited significantly higher levels of NK cells and B lymphocytes compared to the control group (50).

A study conducted on acute fatty liver pregnancy (AFLP) revealed that the complexity of AFLP increases the risk of acute pancreatitis, although the exact mechanism remains

unknown. Acute fatty liver and pregnancy-associated pancreatitis may be caused by fatty acid metabolites, which damage pancreatic tissue (51). Macrophage migration into islets increases proinflammatory cytokines and chemokines such as TNF-α and IL-1β, which significantly affect insulin sensitivity. Increased expression and release of pro-inflammatory cytokine IL-1β in pancreatic islets regulates inflammation by boosting local synthesis of cytokines and chemokines. Thus, immune cells are recruited to the islets (19, 36).

NK cells, considered innate immune cells, play a crucial role in initiating and sustaining immune responses. These cells are present in peripheral blood and as resident cells in certain tissues. Following cytokine activation, NK cells are among the first immune cells to extravasate into the pancreas upon invading the islets. These entities are well recognized for their ability to generate IFN-γ. On the other hand, NK cells have the inherent potential to directly eradicate target cells through cytotoxic mechanisms (47). The presence of NK cells inside healthy human pancreatic islets indicates their normal migration to the pancreas, which occurs both spontaneously and in response to inflammatory mediators (52). Target cells express inhibitory and activating NK cell receptor ligands that govern NK cell function (43).

Additionally, it has been observed that they accumulate in the pancreas much earlier than T cells, and as the disease progresses, they assume a fatigued and unresponsive state. Activated NK cells release IFN-γ and TNF, two cytokines with immune regulatory functions that activate NK cells and stimulate cytokine secretion. These cytokines are accompanied by cytotoxic granules (53). Recent investigations suggest that exocrine damage and/or inflammation may trigger beta cell neogenesis in the adult pancreas (54). A quick approach for assessing NK cell activity has been recently developed using quantitative sandwich ELISA to quantify released IFN-γ from NK cells exposed to a specific recombinant cytokine (53). An imbalance between the activation and inhibition receptors produced on NK cells may damage beta cells by impairing immunological tolerance. Pancreatic cells are killed due to an inflammatory process initiated by immune system lymphocytes (36).

After pancreatic injury, an increase was observed in the pro-inflammatory phenotype of F4/80+ macrophages, attributed to heightened pancreatic neutrophil levels that released

IFN-γ. Ultimately, the persistence of this innate inflammation accelerated the dedifferentiation of the pancreatic epithelium and hindered pancreatic regeneration (54). In β-islet cells, TNF, IFN-γ, and interleukin-1 (IL-1) induce JNK activation. IL-1-triggered JNK activation reduces GSIS by inhibiting the IRS/PI3K/Akt signaling pathway. FoxO1 phosphorylation decreased, nuclear localization increased, and PDX-1 site DNA binding decreased. Furthermore, it has been asserted that maintaining the complete β-islet cell differentiation state necessitates proper FoxO1 function. Furthermore, Grunnet et al. have shown evidence that the administration of a combination treatment including IL-1, TNF, and IFN-γ may effectively trigger apoptosis in β-islet cells. This apoptotic response is mediated by the induction of mitochondrial stress and the activation of proapoptotic Bcl-2 family members (33).

Damage to insulin-producing β-islet cells causes type 1 diabetes (T1D), an autoimmune disease. NK cells, innate immune cells, may cause and maintain autoimmune responses. NK cells are shown to infiltrate the islets, exhibiting early extravasation into the pancreas. These cells possess cytolytic capabilities that may impact β-islet cells. The migratory ability of natural killer (NK) cells to relocate to the pancreas, both in a spontaneous manner and in reaction to inflammatory agents, is shown by their existence inside the pancreatic islets of healthy individuals. In this context, NK cells were found in the insulitis of T1D patients who underwent immunophenotyping of the condition (52). Although there is considerable interest in comprehending the dynamic alterations occurring in NK cells during AP, the precise function of NK cells in AP remains inadequately described.

The findings of this research, which used experimental models, demonstrated a decrease in NK cells within the peripheral circulation. On the other hand, this drop may be attributable to the active migration of these cells towards the pancreas and draining nodes. These findings strongly suggest the active involvement of these cells in the disease's pathophysiology, as within the islets, they function as cytotoxic agents against β-islet cells, thereby promoting disease progression (52).

Moreover, within this framework, it was shown that pancreatic NK cells had elevated levels of activation markers, a more distinct mature phenotype, and enhanced rates of

proliferation in comparison to other strains of mice. These observations support the notion that these cells play a role in promoting beta cell destruction.

Conflicting findings have been observed in investigations involving human subjects. Several studies suggest that the observed decrease in NK cells over a one-year period of monitoring might potentially be associated with their functional decline. Indeed, a distinct investigation has shown that NK cell activation in persons with T1D takes place roughly one year after the commencement of the illness (52).

### 1.4 Interleukin 6 and metabolic disease

In metabolic disorders, elevated levels of cytokines such as IL-6, TNF-α, and IL-1 are present. This trait causes persistent low-grade inflammation (55). The findings of a performed research on experimental rates with liver damage revealed a significant elevation in oxidative markers, including malondialdehyde, inflammatory markers include TNF-α and IL-6. The liver and pancreas also showed abnormal alterations (28).

The rise of pro-inflammatory cytokines, including IL-1 and tumor necrosis TNF-α, occurs prior to the increase in IL-6 levels and serves as significant inducers. Chronic liver fibrosis, a metabolic syndrome illness, obesity, and T2DM, characterized by ongoing low-level inflammation, there is a significant rise in the systemic concentrations of cytokines (such as IL-1, TNF-α, and IL-6) and acute phase proteins (such as CRP), with an observed increase of 2 to 3 times (55).

The significance of IL-6 in the regulation of glucose homeostasis is extensively acknowledged. Strong evidence indicates that IL-6 regulates adipose tissue and lipid metabolism in a physiological manner (55). Recent research on individuals with rheumatoid arthritis (RA) has unveiled abnormally elevated glucose metabolism, reduced insulin sensitivity, and impaired islet-cell function due to excessive islet apoptosis in these patients. The observed process may be associated with the upregulation of pro-inflammatory cytokines. The RA group exhibited significantly higher levels of IL-6 expression compared to the control group. This irregular glucose metabolism coincided with increased islet apoptosis, resulting in decreased insulin secretion due to a positive correlation between IL-6 and the apoptosis-related enzyme caspase-3.

Furthermore, IL-6 has the potential to exacerbate mitochondrial dysfunction, impair the function of the glucose transporter 2 (GLUT2), and decrease insulin sensitivity by promoting lipolysis in adipocytes and the release of free fatty acids (56). Further Additional studies have showcased the favorable effects of elevated IL-6 levels. It has been uncovered that IL-6 promotes increased GLP-1 synthesis, consequently leading to heightened insulin secretion (55).

The pro-inflammatory cytokine IL-6 showed a robust negative correlation with NK cell activity. A study was carried out on nude mice, revealing a higher percentage of NK cells infiltrating tumors in the peripheral blood and tissues of individuals with esophageal squamous cell carcinomas. The activity and functionality of NK cells are hindered by IL-6 through the STAT3 signaling pathway. STAT3 is a nuclear transcription factor that regulates genes involved in the cell cycle, cell survival, and immune response (57). The activation of STAT3 signaling induced by IL-6 or IL-8 is chiefly accountable for the suppression of NK cells by primary esophageal squamous cell carcinoma (ESCC) cells (58).

IR is commonly observed in NAFLD and is linked with the development of steatosis as well as the progression of the condition to its more severe stages, including steatohepatitis, cirrhosis, and hepatocarcinoma. The nearly universal co-occurrence of IR in studies involving NAFLD patients positions it as an indicative marker for diagnosing NAFLD. IR is assessed using Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) measurements (59). Moreover, NAFLD has an impact on glucose metabolism. As the glucose concentration approaches the diagnostic cutoff for T2DM, the insulin concentration starts to decrease in relation to glucose. Consequently, HOMA-IR no longer solely reflects insulin sensitivity (60).

An observational study included 158 White children aged 10 to 18, classified as overweight or obese (BMI > 85th and 95th percentile according to age- and gender-specific percentiles of BMI). Among them, 80 participants met the criteria for diagnosing NAFLD. HOMA-IR testing unveiled a significant disparity between the NAFLD and control groups (60). In a recent study involving NAFLD patients, the NASH group (17.6%) exhibited elevated HOMA-IR levels and a higher proportion of IR individuals compared to the mild-NAFL group (6.9%). This highlighted a clear association between

IR and the severity of NAFLD. Notably, among patients with NASH, those with higher HOMA-IR levels also displayed more pronounced fibrosis when compared to individuals with lower HOMA-IR levels (61).

The most popular approach in clinical practice, HOMA, leverages fasting blood glucose (FBG) and insulin levels to assess both insulin production and resistance (62). The intensity of AP was exclusively anticipated by an autonomous prognostic determinant, HOMA-IR, which functions as a surrogate for an IR marker (63). Despite its basic representation of cellular activity, the HOMA-B index holds clinical significance (62).

### 1.5 Problem statement

Metabolic syndrome stemming from liver fibrosis constitutes a risk factor for various organs, including the heart, kidney, and pancreas, thereby contributing to the onset of AP (64). NAFLD might serve as a predictive factor for heightened AP severity (36). AP is a prevalent condition that frequently necessitates hospitalization (37). However, the precise mechanism through which NAFLD exacerbates pancreatitis is still unknown (36). NAFLD is characterized by an abnormal accumulation of liver fat, signifying a substantial risk factor for AP that exacerbates its severity (36). A variety of pro- and anti-inflammatory cytokines and chemokines have been produced. The activation of PSCs is initiated by the recruitment of various immune cells such as granulocytes (neutrophils, eosinophils), monocytes, macrophages, and lymphocytes via the release of inflammatory signals. PSCs play a pivotal role in the progression of pancreatic fibrosis (40). Matrix remodeling, a critical repair mechanism, is revealed to be dysregulated during the fibrotic process. An altered ECM, which usually arises due to an unsuccessful attempt to heal wounded tissue, strongly influences tumorigenesis and the spread of cancer. Pancreatic tissue fibrosis was first established as a cause of cancer growth and metastasis in the early 1950s (42).

NK cells accumulate in the pancreas much earlier than T cells, and act as immunoregulatory cells releasing cytokines that can control the immune response directly or indirectly. These cells possess a high capacity for producing IFN-γ and could directly induce cell death in target cells through cytotoxic mechanisms. In addition, NK cells possess the ability to either enhance or suppress T-cell reactions (47).

Pietruczuk *et al.* identified a subset of lymphocytes in patients with AP that exhibited significant activation and an increased capacity to release Th2-type cytokines. These findings suggest that B cells and NK cells might exert a more pronounced influence on the pathological condition and recovery rate of mild AP patients. The NK cells that have been stimulated create two important cytokines, namely IFN-γ and TNF. For instance, these cytokines play a crucial role in regulating the immune system. Additionally, activated NK cells also release cytotoxic granules (53). Additionally, these cells release immunoregulatory cytokines such as IL-5, IL-10, and IL-13 (44).

Moreover, some research has shown a correlation between IFN-γ originating from NK and NKT cells and the improvement of acute pancreatitis AP. To illustrate that indicates a possible involvement of IFN-γ originating from NK and NKT cells in the therapeutic approach for AP (40). NK cells are not only known for their ability to eliminate infected or malignant cells but also for mediating cytotoxicity against various activated immune cells. Hence, they play a crucial physiological role in regulating immune responses and maintaining homeostasis (44). Pancreatic stellate cells (PSCs) play a crucial role in the advancement of pancreatic fibrosis in the setting of pancreatitis. Activated NK cells have shown the capacity to selectively target human PSCs and pancreatic cancer cell lines, exhibiting superior effectiveness in comparison to quiescent NK cells (40).

The close relationship between pancreatic fibrosis and pancreatic cancer (PC) is widely acknowledged. PDAC occurrence and prognosis are distinctly influenced by pancreatic fibrosis (65). Patients under surveillance for Hepatic cell carcinoma due to chronic liver disease were diagnosed with PC in its early stages (66). Cohort research within the general Korean population revealed a strong association between NAFLD and an elevated risk of pancreatic cancer (67). Furthermore, a cross-sectional case-matched analytical and comparative study uncovered significant correlations between histological characteristics of NAFLD and the incidence of PDAC (68).

The measurement of HOMA-IR, a predictive marker for the severity of AP (43), provides valuable insights. Additionally, the clinical utility of the HOMA-B index, despite being a basic indicator of cell activity, offers insights into beta cell activity and pancreatic status (69).

Thus, the significance of NK cells in pancreatitis and the status of pancreatic-islet cells are crucial yet little understood. The exact mechanism through which NAFLD worsens pancreatitis is uncertain (36). While there have been a few studies, the molecular pathway mediated by NK cells that establishes a connection between liver fibrosis and pancreatic damage is not well understood and lacks specific highlight.

This realization has prompted us to propose an approach centered around NK cells to investigate whether these cells are indeed a key factor in the development of pancreatitis and pancreatic damage within the context of a liver fibrosis model.

## 1.6 Study significance

Studying the alterations that occur in pancreatic NK cells and associating these alterations with changes in the metabolic profile in a liver fibrosis model is of great importance. This study, along with a few others conducted (52, 58, 70), could shed light on the crosstalk between liver disease and pancreatic changes. Understanding the molecular pathway mediated by NK cells that could link liver fibrosis and pancreatic damage may guide us in understanding the pathogenesis and subsequently lead us to therapeutic methods for controlling and reducing the damage.

## 1.7 Study objectives

### 1.7.1 General aims

The primary objective of this study is to investigate how liver fibrosis influences the modulation of molecular and immune pathways in the pancreas.

### 1.7.2 Specific aims

Our objectives were to establish potential crosstalk between the liver and pancreas, focusing on the following aspects:

1. Analyzing alterations in the metabolic profile.
2. Evaluating the relationship between the liver fibrosis model and the involvement of the IL-6 cytokine.
3. Investigate pancreas functionality and β-islet cells' activity through the measurement of HOMA-IR.

4. Examining the impact of the IL-6 cytokine (as an immune and inflammatory marker) on pancreatic and β-islet cell activity.
5. Evaluating the correlation between NK cell activity and pancreas functionality using HOMA-IR.
6. Exploring the connection between the IL-6 cytokine and pancreatic NK cell activity in the context of a liver fibrosis model.

## 1.8 Research questions and hypothesis

### 1.8.1 Research questions

1. How does liver fibrosis affect the metabolic profile?
2. How changes in the metabolic profile influence the functionality of the pancreas?
3. What is the relationship between the liver fibrosis model and IL-6 cytokine levels?
4. Is there a connection between levels of the IL-6 cytokine and the functionality of the pancreas in the liver fibrosis model?
5. Does a relationship exist between liver fibrosis and the occurrence of acute pancreatitis?
6. Is there an association between the activity of NK cells and the functionality of the pancreas?
7. What is the relationship between IL-6 cytokine levels and the activity of pancreatic NK cells in the context of a liver fibrosis model?

### 1.8.2 Hypothesis

#### 1.8.2.1 Alternative non-directional hypothesis

1. Relationships exist between changes in the metabolic profile, IL-6 cytokine levels, and pancreas functionality in the liver fibrosis model.
2. A relationship is present between NK cell activity and pancreas functionality in the liver fibrosis model.
3. NK cells play a central role in the molecular pathway linking liver fibrosis and pancreas functionality.

#### 1.8.2.2 Null hypothesis

1. There are no relationships between changes in the metabolic profile, IL-6 cytokine levels, and pancreas functionality in the liver fibrosis model.
2. There is no relationship between NK cell activity and pancreas functionality in the liver fibrosis model.
3. NK cells are not a primary factor in the molecular pathway linking liver fibrosis and pancreas functionality.

## 1.9 Literature review

An examination of 107,754 adult autopsies conducted in Japan revealed that liver disorders, regardless of their origin, might increase the susceptibility of patients to the onset of acute or chronic pancreatitis. Studies have demonstrated that chronic liver disease can hinder exocrine pancreatic function. In rat models, the presence of chronic liver injury exacerbated acute pancreatitis, as detailed in a study (26). A new study was carried out to assess the influence of fatty liver inflammation on the occurrence of cellular dysfunction in patients with T2DM. The findings revealed that an inflamed liver resulting from mild portal endotoxemia was linked to a decrease in pancreatic insulin secretion, implying that this factor might pose a risk for pancreatic cell dysfunction during the development of T2DM (26).

A recent meta-analysis of 14 trials involving 3659 participants infected with HCV genotype 1 revealed a connection between IR and advanced hepatic fibrosis. The results of this study align with previous research that has shown a notable association between IR and HCV genotypes 1 and 4 (21).

Recent research simulated low-grade portal endotoxemia and liver damage in rats with prolonged LPS infusion. Chronic LPS infusion in rats mimicked low-grade portal endotoxemia and liver damage in recent research. This research found increased oxidative and inflammatory markers, such as TNF-α and IL-6, causing pathological alterations in the liver and pancreas of rats (26). Eguchi *et al.* proposed that saturated fatty acids resulting from lipotoxicity and hyperglycemia in liver disease prompted beta cells to release chemokines, which in turn attracted CD11b+ Ly-6C+ M1-type proinflammatory monocytes/macrophages to the islets (25).

Subsequent studies have shown an augmented abundance of macrophages inside the pancreas of C57BL/6J mice and GK rats that were given an HFD. Additionally, there has been an observed elevation in the quantities of monocytes and macrophages within the islets of animal models exhibiting obesity. Consequently, the aforementioned discoveries have shown a correlation between the prevalence of macrophages and heightened amounts of inflammatory cytokines. However, the specific biological sources of these cytokines and their effects on beta cell function remained unclear (25). The research discovered that pro-inflammatory cytokines like IL-1 or TNF-α reduced GSIS and increased beta cell mortality in native islets or beta cell lines. However, the mechanisms through which macrophages disrupt GSIS remained unclear (25).

Another study conducted on 170 cirrhotic patients found that insulin secretion declines in correlation with the degree of liver illness, implying that liver failure has an independent negative impact on pancreatic islets (71). A study carried out in rats showed that there is a connection between chronic subacute hepatic inflammation and impaired insulin secretion caused by mild portal endotoxemia (72). In 2020, a systematic review revealed that changes in NK cells in T2DM patients are still unknown, and more research is needed (73). β-islet senescence can be accelerated by an inflammatory response, hyperglycemia, and IR, according to research conducted on a genetic senescence activation mouse model. Prolonged cell senescence leads to a decline in cellular function, followed by cell depletion and cell death (74).

The authors generated transgenic mice with rat insulin promoter (RIP)-controlled hepatic growth factor (HGF) overexpression in the islets. Dai et al. have perceived that the islets underwent a size reduction, accompanied by a decline in insulin levels and moderate hyperglycemia. Considering that HGF enhances GSIS and cell mass and given the association between obesity and elevated plasma levels of HGF, there is a hypothesis that HGF might act as a mediator between IR and cell hyperplasia (28).

According to Weitz et al., they discovered that beta cells under stress produce ATP, which in turn activates macrophages (25). They also found that insulin secretory vehicles can be engulfed by islet macrophages in the context of mouse T1D66. Consistent with their findings, Ying *et al.* observed that obesity significantly increased the number of intact insulin vesicles present in intra-islet macrophages. Furthermore, it has been demonstrated

that macrophages can create open-ended channels referred to as tunneling nanotubes (TNT) for transporting cytoplasmic materials between connected cells. However, the precise processes by which islet macrophages internalize insulin secretory vesicles from beta cells, as well as the impact of obesity on this process, have yet to be fully elucidated (25).

Research using experimental models revealed a reduction in NK cells throughout the peripheral circulation, maybe attributable to their movement towards the pancreas and draining nodes. The results suggest that these cells play a significant part in the pathogenesis of the illness, as they function as cytotoxic agents targeting β-islet cells inside the islets, hence contributing to the advancement of the disease. Furthermore, when compared to other strains of mice subjected to identical settings, it was shown that pancreatic NK cells had elevated levels of activation markers, a more pronounced mature phenotype, and enhanced rates of proliferation. These observations provide support for the notion that these cells contribute to the promotion of β-islet cell destruction (52).

The results obtained from research conducted on nude mice demonstrate an elevated ratio of infiltrating natural killer (NK) cells in the peripheral blood and tissues of individuals diagnosed with esophageal squamous cell carcinomas. The activity and effectiveness of NK cells are hindered by IL-6 through the STAT3 signaling pathway. The primary esophageal squamous cell carcinoma exerts its suppressive effect on NK cells via the stimulation of STAT3 signaling, which may be triggered by either IL-6 or IL-8 (58).

Reportedly, IL-15 treatment has been shown to shield mice from the pathogenesis of cerulean-induced chronic pancreatitis. This protective mechanism involves the development of acinar cell atrophy and the deposition of collagen in the vicinity of blood vessels. In addition, it has been shown that this therapeutic intervention leads to the suppression of profibrotic genes, including TGF-β1, α-SMA, collagen-1, collagen-3, and fibronectin, in murine models with cerulean-induced chronic pancreatitis. The research provides evidence that the activation of iNKT cells, which are sensitive to IFN-γ, by IL-15 has a beneficial effect in preventing pancreatic damage caused by cerulean. This protective mechanism is seen in both the circulation and the tissue.

In recent research conducted in vitro, it was shown that natural killer (NK) cells activated by IL-15 have a greater capacity to eradicate human pluripotent stem cells (PSCs) and pancreatic cancer (PC) cell lines when compared to NK cells in a resting state (40). Additionally, a cross-sectional investigation discovered a linear reduction in NK cell activity as blood glucose levels rise (70).

# Chapter Two

# Methodology

## 2.1 Study design

The experimental model for hepatic fibrosis included the use of male C57BL/6J mice, who were 12 weeks old. The care of these mice adhered to the ethical regulations of AN-Najah National University, and the guidelines set by NIH. All animal protocols were approved by the institutional animal care ethics committee. The mice were accommodated in a barrier facility.

**Liver fibrosis mice model**: The liver fibrosis model was established by administering intraperitoneal injections of 0.5 µl of pure carbon tetrachloride ($CCl_4$; Sigma, C-5331) per gramme of body weight. The $CCl_4$ was diluted in maize oil at a ratio of one to nine. The injections were delivered twice a week, with intervals of two and four weeks, to produce both acute and severe chronic liver disease respectively. During the experimental protocol, the animals were assessed for their weight, thereafter administered anesthesia by inhalation of 5% isoflurane for a duration of 10 seconds, and ultimately underwent cervical dislocation. Each experimental group included six mice.

## 2.2 Study population

The study population comprised mice models, categorized into three groups, each consisting of 6 male mice.

The present study included three groups of mice:

A. Naive mice (mice treated with corn oil -Vehicle).

B. $CCl_4$-treated mice – acute liver injury mode (2-week injections).

C. $CCl_4$-treated mice – chronic liver injury mode (4-week injections).

## 2.3 Study time and setting

The study was conducted in vivo using an animal mice model.

The setting for the in vivo study to induce liver injury required one month.

## 2.4 Study variable

Mice:

1. Mice Age
2. Mice weight
3. Mice gender

Serum analysis parameters:

1. Fasting blood sugar
2. Cholesterol
3. Triglyceride
4. Liver injury enzyme (ALT, AST)
5. C-peptide
6. HOMA-IR
7. Amylase
8. Lipase

Real time PCR:

1. Alpha smooth muscle actin
2. Collagen III

NK cells activity analysis:

1. IL-6R
2. IFN-γ

β -Islet cells expression of IL-6.

## 2.5 Histological assessment

The posterior one-third of the pancreas and liver tissues were fixed in 3% formalin for 24 hours at room temperature and then paraffin-embedded using an automated tissue processor. Tissue sections with a thickness of 7μm were subjected to staining with hematoxylin and eosin (H&E) to evaluate the existence of steatosis, necro-inflammatory regions, and apoptotic bodies. Furthermore, the slices were subjected to staining using a 0.1% solution of Sirius Red F3B in a saturated picric acid stain (Abcam, ab150681) to facilitate the visualization of connective tissue. The tissue sections were deparaffinized by immersing them in xylene and then rehydrated by passing them through a series of graded alcohols, starting with absolute alcohol and ending with distilled water. For the H&E staining, the sections were treated with hematoxylin, a basic dye, and immersed in a hematoxylin solution for a specific period. After staining, the sections were rinsed with running tap water to remove excess stain. Subsequently, the sections were differentiated in an acid alcohol solution to eliminate any excess hematoxylin, followed by rinsing with running tap water. The sections were then counterstained with eosin, an acidic dye, and immersed in an eosin solution for a specific period. Post-counterstaining, the sections were dehydrated by passing them through a series of graded alcohols, starting with distilled water and ending with absolute alcohol. Finally, the sections were cleared using xylene and mounted with a coverslip, utilizing a mounting medium. For Sirius Red F3B staining, the sections were immersed in a Sirius Red solution for a specific duration to stain collagen fibers. After staining, the sections were washed with distilled water to remove any excess stain. Following this, the sections underwent dehydration through a series of graded alcohols, starting with distilled water and concluding with absolute alcohol. Ultimately, the sections were cleared with xylene and mounted with a coverslip, using a mounting medium. The histological results were evaluated and graded by a veterinary pathologist.

For quantify the fibrosis area, stained slides were scanned using a Zeiss microscope equipped with image analysis software (ImageJ). This involved outlining the fibrotic areas present within the tissue section. The calculation of the fibrosis area was performed by dividing the total fibrotic area by the number of fields of view or sections that were analyzed.

### 2.6 RNA isolation, cDNA preparation, and real-time PCR

The liver tissue was subjected to RNA isolation using 2 ml of TRI Reagent (Bio Lab; Cat# 90102331) per cm3 of tissue. The samples were homogenized for 5 minutes at room temperature before adding 0.2 ml of chloroform (Bio Lab; Cat# 03080521). Following a 15-minute incubation period at ambient temperature, the samples were subjected to centrifugation at 1,400 revolutions per minute for a duration of 15 minutes at a temperature of 4 degrees Celsius. The supernatant from each sample was carefully transferred to a fresh micro-centrifuge tube during RNA precipitation. After adding 0.5 ml of isopropanol (Bio Lab; Cat# 16260521), the mixture was incubated at 25°C for 10 minutes. The tubes were then centrifuged (12,000 rpm) for 10 minutes at 4°C, the supernatants were removed, and one ml of 75% ethanol was added to the pellet. Following centrifugation at a speed of 7,500 revolutions per minute (rpm) for a duration of 5 minutes, the resulting pellets were subjected to air-drying at ambient temperature for a period of 15 minutes. After adding 50 µl of DEPC, the samples were thermally treated at 55°C for 10 minutes. The RNeasy Plus Mini Kit (CAT# 74034) was used to analyze NK cell RNA purification according to the manufacturer's recommendations. The R&D High-Capacity cDNA Isolation Kit (1406197) produced cDNA. The αSMA and collagen III gene expressions were quantified using real-time PCR with the TaqMan Fast Advanced Master Mix (Applied Biosystems; Cat# 4371130). The data were normalized using the housekeeping gene expression and analyzed using the QuantStudio™ 5 Real-Time PCR System.

### 2.7 Metabolic profile assessments in serum, pancreatic NK cells and IL-6

On the day of sacrifice, whole blood samples from mice were collected and subsequently centrifuged at 5000 rpm for 30 minutes at 4°C. The following laboratory measurements were obtained: Serum ALT (Abcam; ab285263), AST (Biocompare; MBS2019147), fasting blood sugar (Biocompare; MBS7200879), C-peptide (Biocompare; MBS007738), cholesterol (Abcam; ab285242), triglycerides (Biocompare; MBS726589), amylase (Abcam; ab102523) and lipase (Abcam; ab118969) were detected. In addition, Pancreatic β-islet IL-6, pancreatic NK IL-6R (R&D; D6066) and IFN-γ (R&D; 285-IF) were determinaing of carried out using enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's procedures.

In accordance with standard laboratory protocol, it was ensured that all reagents and samples were equilibrated to room temperature (18–25 °C) prior to their use. A 100 µL volume of each standard and sample was introduced into the respective wells and subjected to incubation for a duration of 2.5 hours at room temperature, with moderate shaking. After the incubation period, the solution was discarded, and wells were washed four times with 1X Wash Solution. It is worth noting that the washing process involved filling each well with Wash Buffer (300 µL), using a multi-channel Pipette or auto washer. Ensuring complete removal of liquid at each step is crucial for optimal performance.

A volume of 100 µL of 1x prepared detection antibody was added to each well for 1 hour at room temperature with gentle shaking. Following this, a volume of 100 µL of prepared streptavidin solution was added to each well for 45 minutes at room temperature with gentle shaking. A volume of 100 µL of TMB One-Step Substrate Reagent (Item H) was added to each well for 30 minutes at room temperature in the dark with gentle shaking. Finally, 50 µL of Stop Solution (Item I) was added to each well. Absorbance was immediately read at 450 nm using an ELISA reader (Tecan M100 Plate Reader).

### 2.8 C-peptide assessment

Participants' blood samples were obtained and collected into suitable tubes or containers. These samples were left undisturbed at room temperature for a designated time to allow clot formation. Subsequently, the clotted samples underwent centrifugation at predetermined speed and duration to separate the serum or plasma from the clot. Careful transfer of the resulting serum or plasma was carried out into new tubes, ensuring no contamination, or mingling with the clot or red blood cells. The transferred serum or plasma samples were then stored at an appropriate temperature, typically at -80°C, until further analysis. An ELISA kit (Biocompare; MBS007738) measured C-peptide levels.

ELISA plates were prepared by adding capture antibodies specific to C-peptide to each well, allowing them to incubate before removing the solution and washing the wells to remove any unbound antibodies. Serum or plasma samples were then thawed and diluted based on the assay kit's instructions. These samples were added to the ELISA plate wells, leaving them to incubate, allowing C-peptide to bind to the capture antibodies. A second round of washing was done to remove any unbound components. Detection antibodies,

tailored to C-peptide, were then added to each well and left to incubate. Another round of washing was performed to remove any unbound detection antibodies. A substrate solution was introduced to each well, and the plate was left to incubate, allowing the substrate to react with the bound detection antibodies. The enzymatic reaction was stopped by adding a stop solution. A microplate reader was used to measure the absorbance of each well at a specific wavelength. By comparing the absorbance values to a standard curve created using known concentrations of C-peptide, the C-peptide concentrations within the samples were determined. The acquired C-peptide concentrations were analyzed and interpreted to obtain the necessary data.

### 2.9 HOMA-IR Scoring

The methodology used for evaluating insulin resistance using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was similar to the one described before for measuring liver function and metabolic profiles in the serum analysis. Fasting glucose and C-peptide levels were measured for this purpose.

The obtained glucose and c-peptide values were used to calculate the HOMA-IR index using the following formula: HOMA-IR = (Fasting C-peptide concentration [μU/mL] × Fasting glucose concentration [mmol/L])/22.5.

The calculated HOMA-IR values were recorded for each mouse. Data analysis and interpretation were performed based on the obtained HOMA-IR values. This analysis considered established reference ranges and pertinent clinical or research criteria to derive meaningful insights from the data.

### 2.10 Pancreatic tissue-resident NK (trNK) cells isolation

The pancreas was removed and placed on petri plates with 10 ml of DMEM medium (Biological Industries; Cat# 01–055-1A). The tissues were disseminated using a stainless-steel mesh, and the cells were collected with medium and transported to 50 ml containers with 10 ml of DMEM. After that, the cells were carefully transferred to fresh tubes containing Ficoll (Abcam; Cat# AB18115269) and centrifuged for 20 minutes at 1600 RPM at 20°C. Subsequently, the liquid from each tube was carefully transferred to new tubes and centrifuged for 10 minutes at 1600 RPM at 4 degrees Celsius. To isolate and

purify NK cells, the pellet in each tube was resuspended in 1 ml of DMEM after the second centrifugation (StemCells kit; Cat# 19665).

### 2.11 β −Islet cells isolation

Tissue samples were washed with a sterile solution to eliminate debris and blood. The pancreas was enzymatically digested using collagenase, and the reaction was allowed to proceed for 20 minutes. The digested tissue was then filtered through a mesh to eliminate undigested material. The filtrate was centrifuged at low speed to separate the islets from exocrine tissue. The supernatant containing exocrine tissue was carefully discarded, and the islet pellet was resuspended in a suitable medium. The suspended islets were purified using a density gradient centrifugation method. The purified islet cells were collected and washed with a culture medium to remove impurities. The isolated islet cells were stored in a suitable preservation solution or used immediately for transplantation or further experiments.

### 2.12 β −Islet cells apoptosis

Islet cells were harvested and washed with phosphate-buffered saline (PBS) to eliminate any residual culture media. The cell pellet was resuspended in binding buffer at a concentration of $10^6$ cells per milliliter. Annexin V-AF488 (Alexa Fluor) was introduced to the cell suspension as per the manufacturer's recommended concentration. The cell suspension was gently mixed and incubated in the dark for 30 minutes at room temperature. Following the incubation, propidium iodide (PI) was added to the cell suspension at a final concentration of 5 μg/ml. Gently mixing the cell solution, it was incubated for 20 minutes in the dark at room temperature. Binding buffer was added to the cell suspension after incubation to make 1 ml. The stained cells were then analyzed using the flow cytometry platform LSR-Fortessa II.

### 2.13 Statistical analysis

Statistics were compared using a two-tailed unpaired Student's t-test for two groups or a one-way or two-way ANOVA with a Newman-Keuls post hoc test for multiple groups. The statistical analysis was performed using GraphPad Prism 9.0 software from GraphPad Software in La Jolla, CA. A p-value of $\leq 0.05$ indicates a statistically significant t-test, which measures the difference in means between two variables. The results are

shown as mean ± SD or average means of experimental replicates ± SD. The experiment was repeated three times.

### 2.14 Moral considerations

The study proposal is anticipated to receive approval from the Institutional Review Board (IRB) and the scientific research committee of the public health department, as well as the Scientific Research Board of the Faculty of Graduate Studies at An-Najah National University.

# Chapter Three

# Results

## 3.1 $CCl_4$-induced liver fibrosis presented with propagation in liver histopathological findings

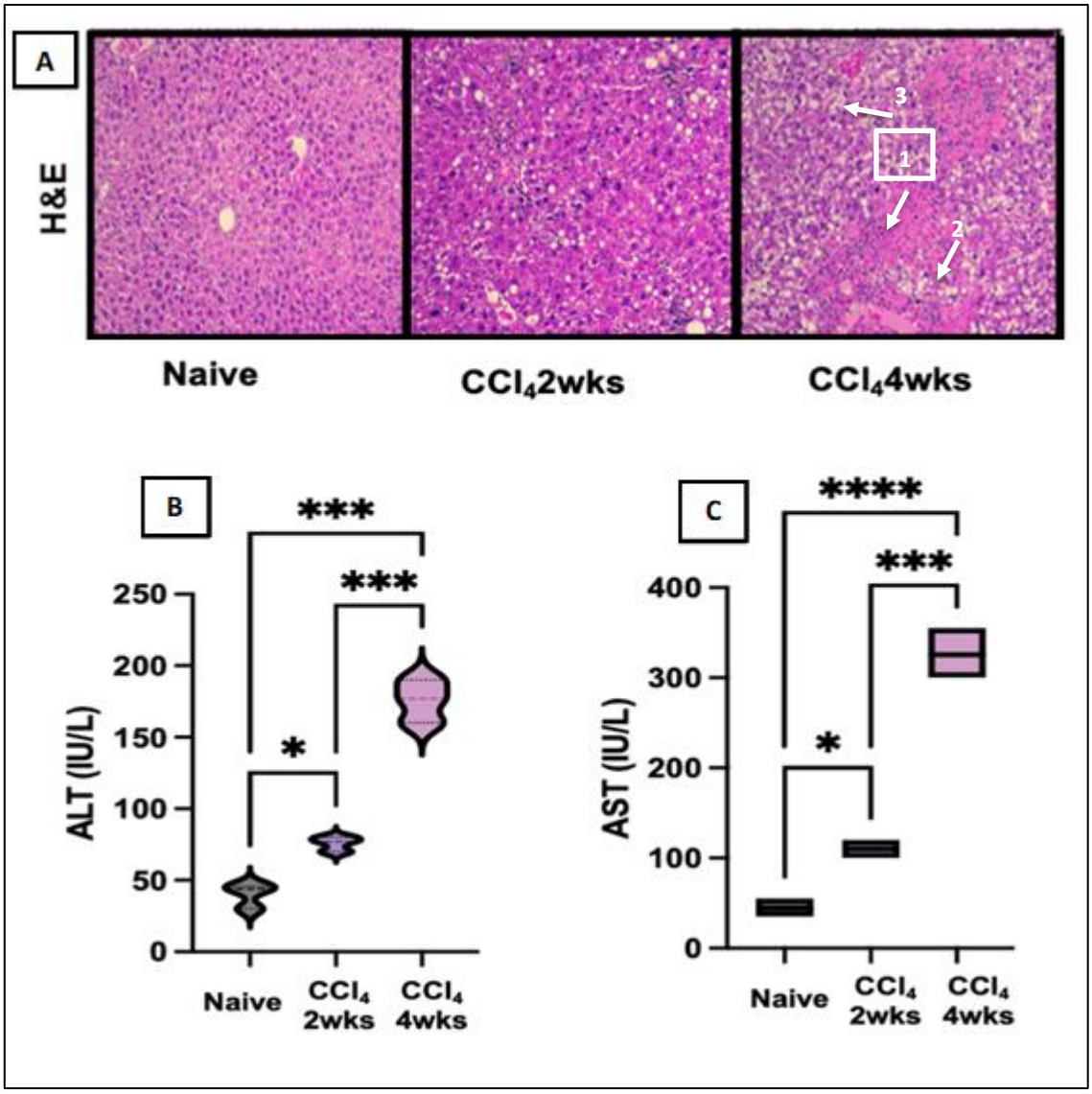
To validate the success of our mice model that received $CCl_4$ injections for inducing liver fibrosis, we conducted H&E staining to evaluate histopathological inflammatory indications. Additionally, we collected serum to assess liver injury enzymes, following the procedure outlined in the methodology.

In Figure 5A, representative H&E-stained liver sections are presented. In $CCl_4$-induced mice, noticeable features included enlarged centrilobular hepatocytes and extensive necrotic regions with pronounced infiltration of inflammatory cells, accompanied by steatosis. The pattern exhibited a greater degree of prominence and clarity in the chronic liver damage model as compared to the acute liver injury model. Histological observations of the acute models indicated lesser instances of micro- and macro-vascular steatosis in comparison to the chronic model. No cell infiltrations were observed in the naive mice. The histopathological scoring was conducted as outlined in Appendix A.

To elaborate on the effects of $CCl_4$ on liver enzymes, serum ALT and AST were assessed. Figure 5B shows the serum ALT levels for acute and chronic liver fibrosis. The data indicates that in the acute and chronic models, ALT levels increased to 80±12.5 and 175±42.3 IU/ml, respectively, in contrast to 46±8.6 IU/ml in the naïve mice. A similar pattern of results was obtained in serum AST levels. Figure 5C shows AST serum level for acute and chronic liver fibrosis. The findings indicate that in the chronic model, there is a statistically significant increase in AST levels that demonstrates a linear correlation with the extent of liver fibrosis. The Pearson correlation value of 0.675 indicates a significant and robust linear association between the variables. In naïve mice, AST was 45±7.8 IU/ml, and 110±17.38 IU/ml in the acute model and 350±43.7 IU/ml in the chronic model. The P-value was ($p<0.05$) in all tested groups.

**Figure 5**

*H&E for assessing histopathological inflammatory findings and serum liver injury enzymes*




The liver slices that were stained with H&E were used for representation. The mice with liver fibrosis exhibited enlarged centrilobular hepatocytes and extensive necrotic regions characterized by a significant infiltration of inflammatory cells (arrow 1), accompanied by steatosis (arrow 2), and chronic liver damage necrosis (arrow 3). (B) serum ALT level for acute and chronic liver fibrosis. (C) AST serum level for acute and chronic liver fibrosis. N=6, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

### 3.2 Histopathological findings revealed increased collagen depositions, as well as elevated gene expression of alpha-smooth muscle actin (α-SMA) and collagen type 3 (Col lll) in liver fibrosis

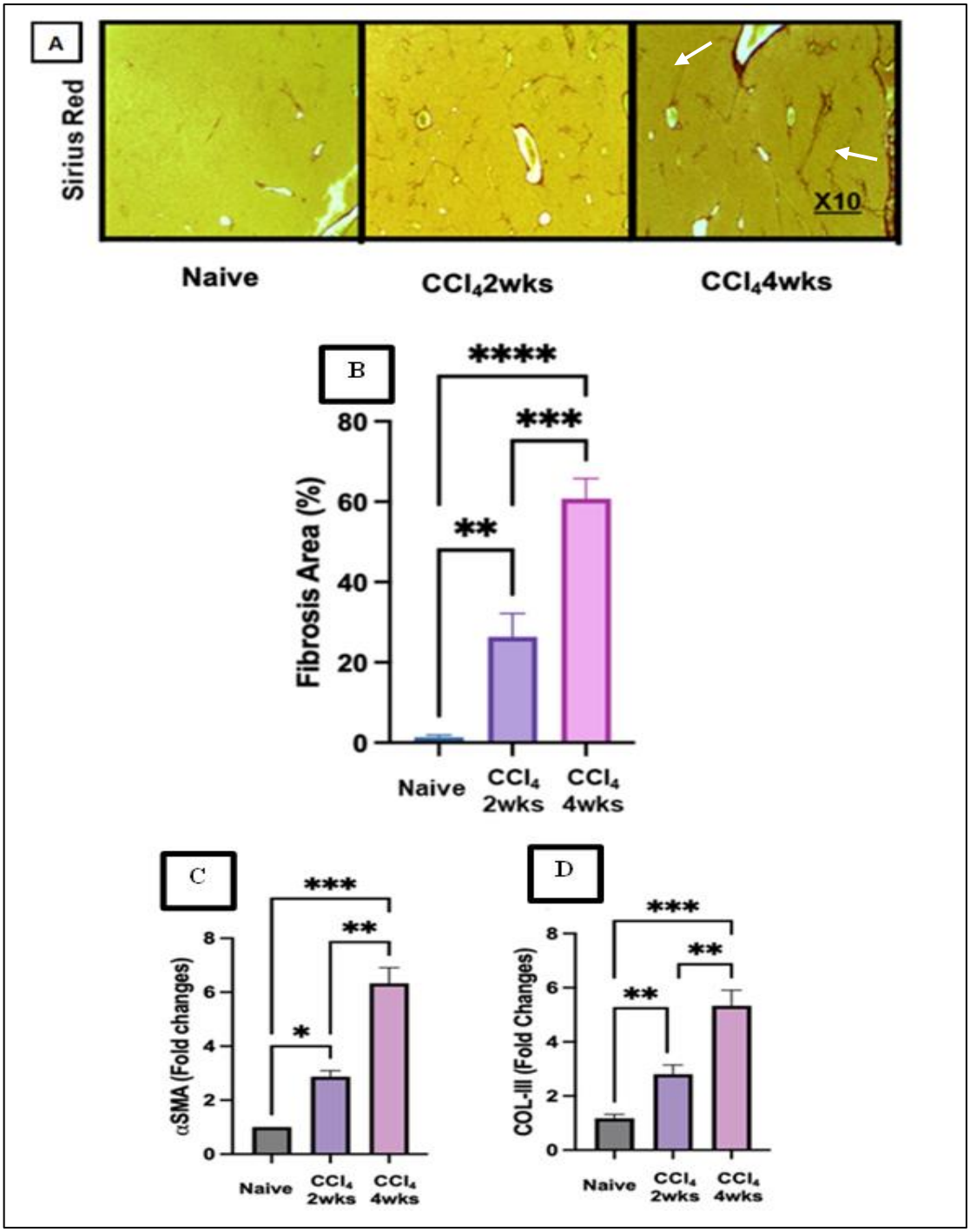
To enhance comprehension of the influence of $CCl_4$ on the evolution of liver damage, the study examined pro-fibrogenic markers using RT-PCR, α-SMA and Collagen III were used as fibrosis indicators.

Figure 6A represents images of Sirius red staining following the acute and chronic effects of $CCl_4$ inductions. Sirius Red staining was conducted on liver fibrosis mice, revealing an elevated collagen deposition in peri-sinusoidal areas with increased fibrous dense tissue in the stained region for both the acute and chronic liver fibrosis models, with a more pronounced effect observed in the latter. The scoring was executed according to the table presented in Appendix A. Moreover, the quantification of collagenous fibers was performed, and the percentage of fibrotic area demonstrated significant collagen depositions in the chronic model.

Additionally, to validate the presence of liver fibrosis in mice induced with $CCl_4$, fibrosis markers were assessed by measuring liver α-SMA (Figure 6C) and Col III (Figure 6D) expression levels through RT-PCR. The research found a substantial increase in α-SMA and Col III levels in both acute and chronic $CCl_4$ models compared to the control group. The acute model demonstrated a 3.1-fold rise in α-SMA levels, whereas the chronic model showed a 6.2-fold increase. The acute model increased Col III levels 3.9-fold, whereas the chronic model increased them 5.8-fold. These results were found to be statistically significant with a p-value of 0.003. The findings derived from both RT-PCR and histological evaluations showed a high degree of similarity.

**Figure 6**

*Histopathological findings of collagen depositions and gene expression of alpha smooth muscle actin (αSMA), collagen type 3 (Col lll)*




**(A)** A 0.1% solution of Sirius red F3B in saturated picric acid stain (Abcam, ab150681) was used for the purpose of visualizing connective tissue of collagen (see arrow). **(B)** represents fibrosis area (%) in naïve, two-weeks $CCl_4$ and four-weeks $CCl_4$**. (C)** gene expression of alpha smooth muscle actin (αSMA) for naïve, acute, and chronic. **(D)** gene

expression of collagen type 3 (Col lll) for naïve, acute, and chronic, N=6, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

### 3.3 Liver fibrosis-induced elevation in metabolic markers

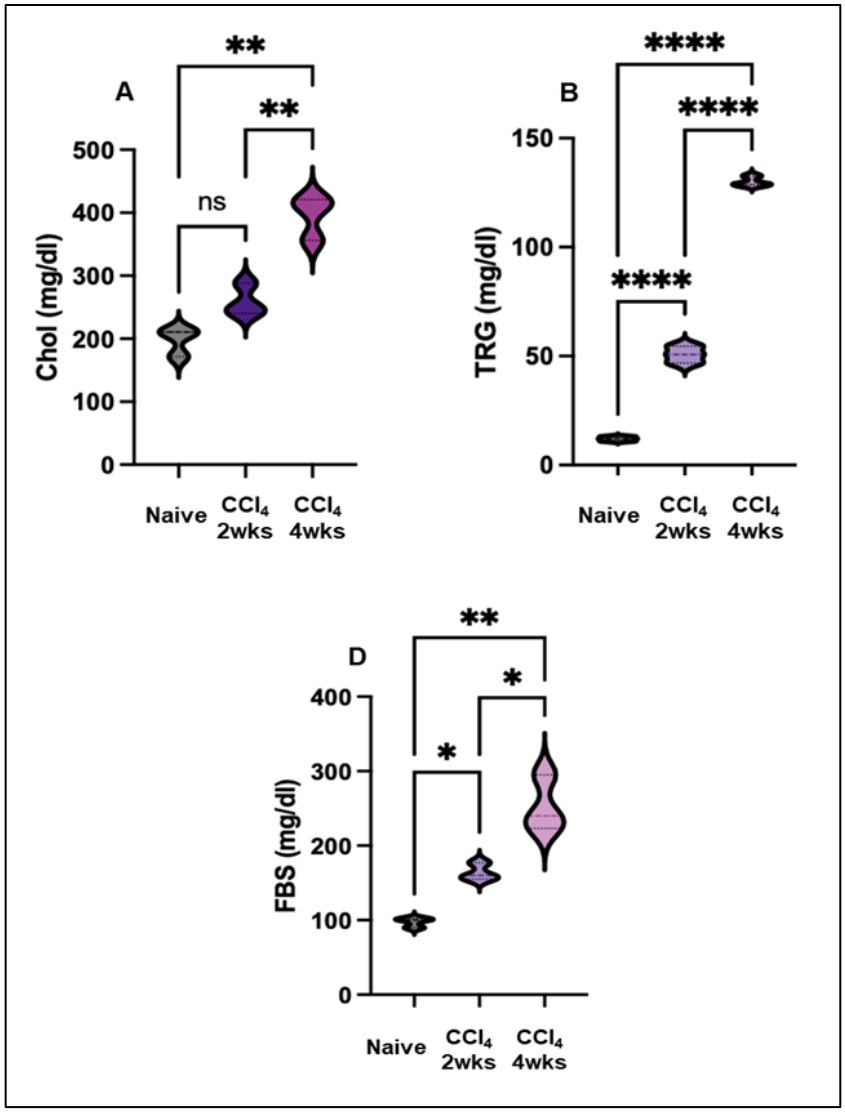
Hyperlipidemia is characterized by elevated lipid levels in the bloodstream and has been linked to pancreatic injury. The pancreas produces enzymes that break down lipids, controlling lipid metabolism (75). In cases of sustained elevation in serum lipid levels, excessive lipids can infiltrate pancreatic cells, leading to the development of lipid droplets within the organ. As a result, this accumulation of lipids disrupts normal cellular functions and can initiate inflammation and oxidative stress within the pancreas, ultimately contributing to pancreatic injury. Consequently, this chronic damage to the pancreas can progressively hinder insulin production and secretion, thereby elevating the susceptibility to diabetes and other associated complications (75).

Subsequently, to further characterize our mouse model, we evaluated metabolic markers related to serum lipids and glucose profiles in both acute and chronic fibrosis models. Significant serum TRG level increases were observed, with average values of 50 and 120 mg/dL, respectively Figure 7B. These results indicate that introducing $CCl_4$ may negatively affect lipid and glucose metabolism, which in turn can adversely affect pancreatic health and overall metabolic balance. The findings presented here offer compelling evidence linking $CCl_4$ administration to disruptions in metabolic homeostasis. The mice administered with $CCl_4$ exhibited significantly increased serum cholesterol levels in both experimental models, with an average of 250 and 400 mg/dL, as illustrated in Figure 7A. Moreover, their FBS were also significantly elevated, averaging 180 and 250 mg/dL, as depicted in Figure 7C. These findings suggest a potential correlation between the administration of $CCl_4$ and the observed alterations in serum cholesterol and fasting blood sugar levels in the experimental mice.

Additionally, further research is warranted to elucidate these findings' underlying mechanisms and implications. The administration of $CCl_4$ has been found to cause disruptions in metabolism, resulting in elevated levels of cholesterol, triglycerides, and fasting blood sugar. Such metabolic changes could potentially harm the pancreas and overall metabolic health.

**Figure 7**

*A-C depict the distribution of serum cholesterol (CHOL), triglycerides (TRG), and fasting blood glucose (FBG) in treatment-naïve acute and chronic liver fibrosis patients*




Note: N=6, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Furthermore, acute liver fibrosis patients exhibited lower average CHOL (A) while also displaying reduced TRG (B) with less variability versus chronic liver fibrosis patients. Additionally, chronic liver fibrosis patients exhibited markedly higher and more variable FBG levels (C) compared to the acute cohort. In summary, significant differences emerged in the central tendencies and dispersion of these biomarkers between the two groups. Consequently, the distinct patterns suggest serum CHOL, TRG, and FBG may differentiate acute versus chronic liver fibrosis states.

### 3.4 Serum C-peptide level in $CCl_4$-induced mice model assisted by ELISA

C-peptide levels have been examined in order to evaluate the efficacy of β-islet cells for treating both acute and chronic liver fibrosis (76). Furthermore, C-peptide levels are considered a reliable indicator of β-islet cell function in clinical settings (69). Moreover, to comprehensively explore the connection between liver injury and pancreatic health, we assessed C-peptide levels as a vital indicator of insulin secretion and calculated the HOMA score as a marker of β-islet cell function and insulin resistance. In situations involving liver injury or liver disease, disruptions in insulin metabolism can lead to elevated concentrations of C-peptide in the bloodstream.

Specifically, in our study, C-peptide levels were found to be significantly elevated in both acute and chronic liver fibrosis models, averaging 1.5 and 2.5 ng/mL, respectively, in contrast to the control group's average of 0.5 ng/mL (Figure 8A). The statistics prove that liver damage could destructively impact glucose stability. Those measurements advise that their glucose management is compromised, potentially due to impaired beta cellular function and heightened insulin insensitivity. As Figure 8B demonstrates, the liver-damaged group exhibited a super decline in β cellular characteristics and an upward thrust in insulin insensitivity.

Such heightened secretion of C-peptide and altered HOMA scores may impose stress on pancreatic β-islet cells, potentially leading to their exhaustion and reduced insulin synthesis over time. Simultaneously, the disruptions in glucose and lipid metabolism induced by liver injury further exacerbate the pancreatic environment. This dysregulation can stimulate inflammatory responses, oxidative stress, and lipid accumulation within the pancreas, ultimately contributing to pancreatic injury and diminished β-islet cell function (32).

Therefore, understanding the intricate relationship between liver injury, C-peptide levels, HOMA score, and pancreatic health may offer valuable insights for devising targeted therapeutic approaches to manage diabetes and related metabolic disorders effectively.

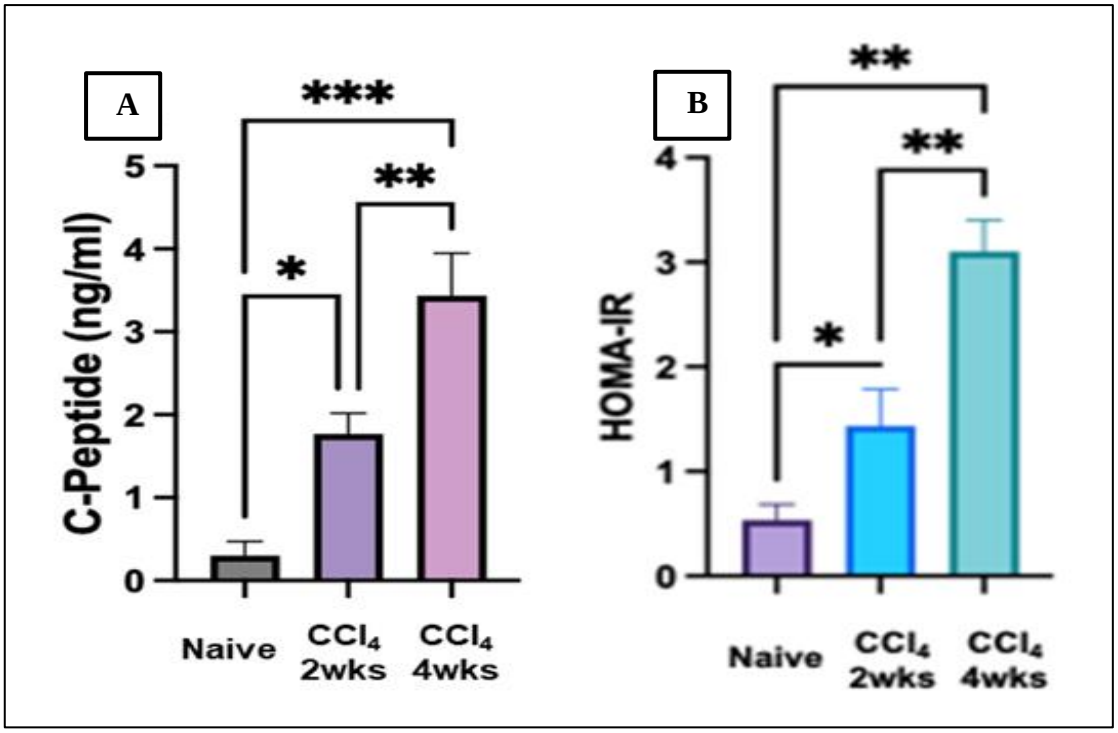
IR was assessed using the following formula:

HOMA-IR: C-peptide (mlU/ml) x FBS (mmol/L) / 22.5

The insulin-based HOMA-IR has been considered the gold standard for assessing IR (76). Several studies propose a threshold of >2 to indicate IR. Upon calculating the IR, we observed that the IR for the naive mice model was below the threshold (>2), and for the acute and chronic liver fibrosis model, it exceeded the threshold, as depicted in Figure 8B.

Figure 8

*Serum C-peptide level in $CCl_4$ induced mice model assist by ELISA*




(A) distribution of C-peptide level for naive, acute and chronic liver fibrosis. (B) distribution of HOMA-IR in naïve, acute and chronic liver fibrosis for evaluating insulin resistance, N=6, *p<0.05, **p<0.01, ***p<0.001.

### 3.5 Pancreatic enzymes were elevated in liver fibrosis mice model, which was associated with apoptosis of β-islet cells

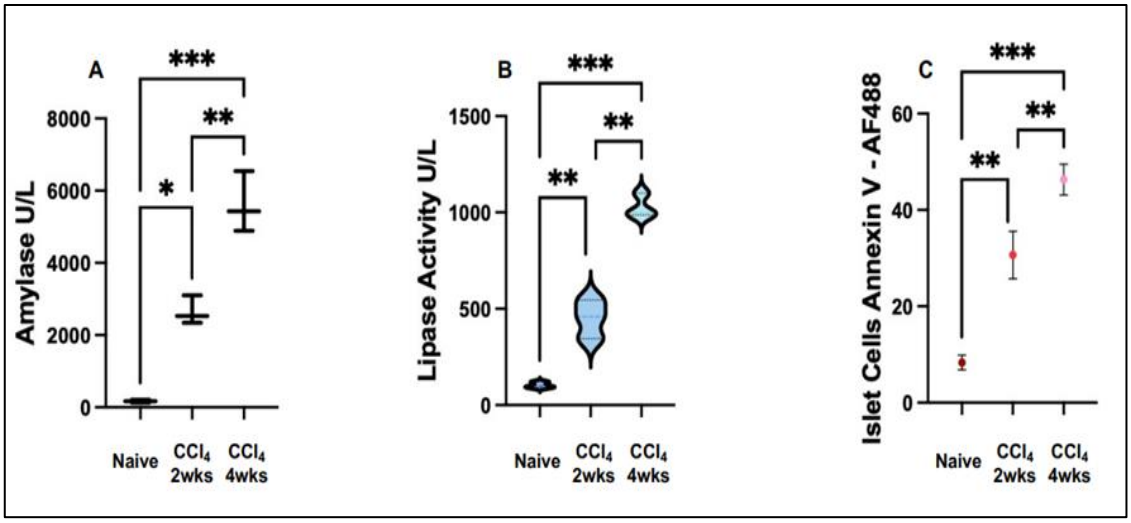
Serum amylase and lipase are the most used techniques in clinical practice due to their simple, quick, low-cost, and widely available assay methods. Serum lipase is highly sensitive for detecting acute pancreatitis, whereas serum amylase is only moderately sensitive (69).

To comprehensively investigate the impact of acute and chronic liver fibrosis on pancreatic health, we evaluated various markers, including amylase, lipase, and islet cell apoptosis. In both acute and chronic liver fibrosis models, there was a significant increase in serum levels of amylase and lipase when compared to the control group. The two models display that patients with liver fibrosis have a significantly higher apoptosis rate in islet cells within the pancreatic tissue, averaging 40%, compared to the control group's average of 10%. The Annexin V examination validates this discovery. Liver fibrosis negatively impacts the pancreas, producing heightened amylase and lipase levels. It is essential to develop focused interventions that reduce the detrimental effects of liver fibrosis on pancreatic health and metabolic balance. Understanding these relationships is vital to achieving this objective.

Our research observes a significant surge in amylase and lipase levels in both acute and chronic models. The control group's amylase levels average 100 U/L, whereas the acute model has an average increase of 2200 U/L. So, in the chronic model, the amylase levels shot up dramatically to an average of 6000 U/L. For comparison, the control group only had an average of 500 U/L lipase. But in the acute model, those lipase levels jumped to 1000 U/L. Even worse, the chronic model had lipase averages spike to 3000 U/L. Basically, these results make it clear that liver fibrosis messes up the pancreas big time. It triggers pancreatic injury and leads to more of these islet cells dying off. So yeah, liver fibrosis has some bad effects on the health of the pancreas. It's like a one-two punch - damaging the liver and then also screwing up the pancreas too. We can see the injury happening and those key cells kicking the bucket.

**Figure 9**

*Distribution of pancreatic injury markers (amylase, lipase and islet cells annexin V – AF488 ) for naive, acute (2-weeks $CCl_4$) and chronic (4-weeks $CCl_4$) liver fibrosis*




**(A)** distribution level of amylase respectively in naive, acute (2-weeks $CCl_4$) and chronic (4-weeks $CCl_4$) liver fibrosis. **(B**) distribution level of lipase respectively in naive, acute (2-weeks $CCl_4$) and chronic (4-weeks $CCl_4$) liver fibrosis. **(C)** Percentages of islet cells apoptosis for naive, acute (2-weeks $CCl_4$) and chronic (4-weeks $CCl_4$) liver fibrosis N=6, *p<0.05, **p<0.01, ***p<0.001.

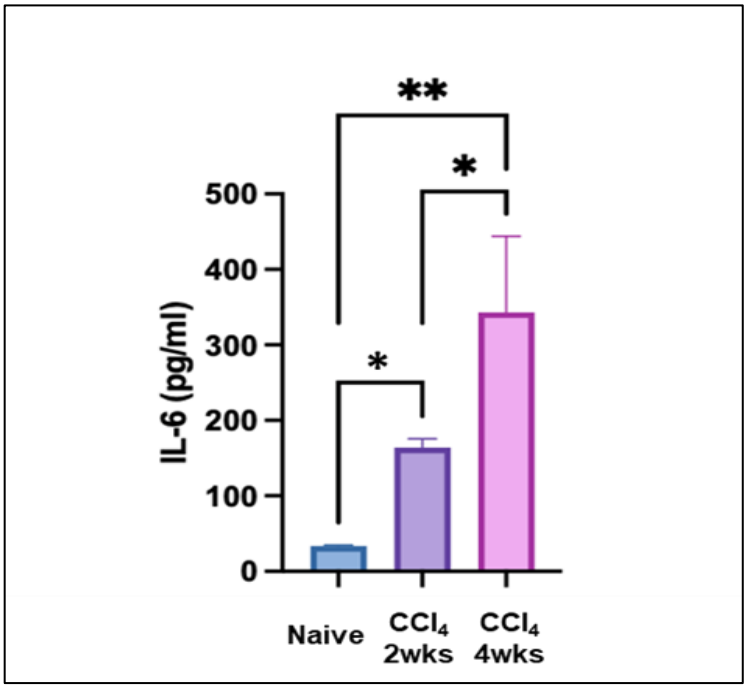
### 3.6 β-islet cells from liver fibrosis mice model showed high expressions of IL-6 and caused deactivation of pancreatic trNK

The levels of IL-6, a cytokine with pro-inflammatory properties (55), were measured in pancreatic β-islet cells obtained from mice models with liver fibrosis and mice models without any pre-existing conditions. In addition, this measurement was conducted to evaluate the impact of acute rise of IL-6 during the first phases of infection. The quantification of IL-6 was performed according to the methods described in the materials and methods section. In Figure 10, the quantification of IL-6 in cell lysates through ELISA is presented.

The research found a substantial increase in IL-6 levels, with a 2.1-fold increase in acute liver fibrosis and a 3.2-fold increase in chronic liver fibrosis, compared to naïve mice ($p<0.05$). A substantial positive association (r) was found between IL-6 levels in β-islet cells and liver fibrosis severity, based on the mean of three repeated experiments.

**Figure 10**

*Expressions of IL-6 level from pancreatic β-Islet cells for naive, acute (2 weeks $CCl_4$) and chronic (4 weeks $CCl_4$) liver fibrosis*




Note: **p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

Due to the elevated levels of IL-6 in the pancreas, our next objective was to determine whether these elevations could potentially disrupt the alterations in pancreatic trNK cell (which could be infiltrating from the liver and/or from the blood) phenotypes. As a result, we proceeded to evaluate the expression of IL-6R on trNK cells and their ability to secrete INF-γ [released by activated NK cells and indicative of their activity], following the methods outlined in the materials and methods section.

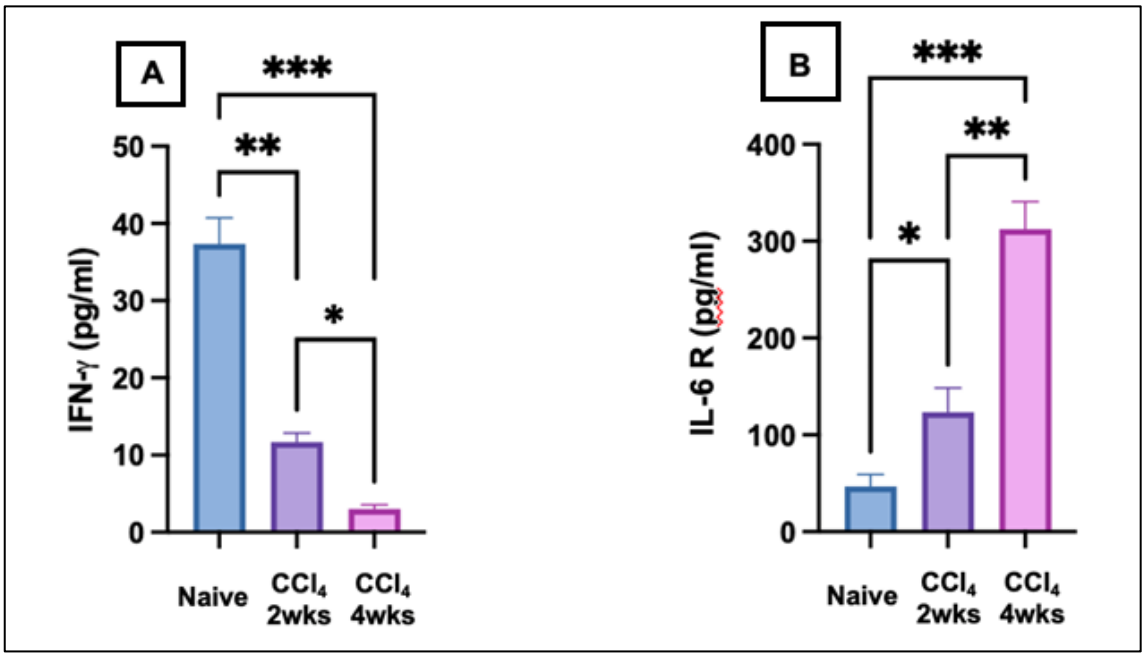
Figure 11A presents the percentage of IL-6R expression on trNK cells for the naive, acute (2 weeks $CCl_4$) and chronic (4 weeks $CCl_4$) liver fibrosis groups. The figure shows a 2.5-fold increase in IL-6R expression in acute liver fibrosis and a 6.3-fold rise in chronic liver fibrosis, both statistically significant ($p<0.05$), compared to naive animals. Additionally, trNK cells from fibrosis mice had lower IFN-γ levels, which were linked to liver disease severity. Specifically, the levels of IFN-γ were measured to be 38±6.8 pg/ml in the naïve state, 12±3.4 pg/ml in the acute model, and 7±2.1 pg/ml in the chronic $CCl_4$ model, with a statistically significant difference ($p<0.005$).

We calculated the correlation coefficient (r) based on the mean of three repeated experiments. The correlation coefficients reveal a substantial linear association between IL-6R and the severity of liver fibrosis, along with a significant inverse association between IFN-γ and the severity of liver fibrosis.

Our data unequivocally indicates that β-Islet cells (which were previously demonstrated to have a high apoptosis rate, as shown in Figure 10) exhibit elevated secretions of IL6. This, in turn, triggers an up-regulation of IL-6R on trNK cells. This up-regulation partially affects the activity of trNK cells, leading to their diminished capacity to produce enough IFN-γ.

**Figure 11**

*The distribution of IFN-γ levels and IL-6 R expression.*




Note: The quantities of IFN-γ and IL-6R were evaluated by using the Human IL-6R Quantikine ELISA Kit (R&D; D6066) and the Human IFN-γ Quantikine ELISA Kit (R&D; 285-IF).

**(A)** the distribution of IFN-γ level for naive, acute (2- weeks $CCl_4$) and chronic (4-weeks $CCl_4$) liver fibrosis. **(B)** showed the percentage of IL-6R of NK cells for naive, acute (2-weeks $CCl_4$) and chronic (4-weeks $CCl_4$) liver fibrosis, N=6, *p<0.05, **p<0.01, ***p<0.001.

# Chapter Four

# Discussion

## 4.1 Introduction

Twelve-week-old male mice, housed within a barrier facility, carbon tetrachloride ($CCl_4$; twice a week for two and four weeks intraperitoneally (i.p.). This injection regimen was designed to induce liver fibrosis. Each group consisted of six mice. On sacrifice day, mice were weighed, anaesthetized intramuscularly with 5% inhaled isoflurane for 10 seconds, and cervical dislocated. The experimental groups had 6 mice.

This study came to identify the private studies and the hypothesis that has been conducted and that clearly investigate the effect of liver fibrosis on NK cells. Several assessments were conducted to see if liver injury could affect the activity of NK cells in the pancreas. NK cells are one of the major and important type of immune cell. We hypothesized a link between liver injury, pancreatic health, and NK cells. To assess for liver injury, we did several tests including histopathological changes in liver tissue and tested liver function enzymes. To study the pancreas, we measured serum insulin levels and isolated for NK cells from the pancreas. By doing all these tests, we anticipated to better understand how liver fibrosis is connected to problems with the pancreas. The immune cells in the pancreas might help explain why liver damage can lead to pancreatic damage over time.

### 4.1.1 Liver and metabolic profile assessments in serum

The liver assists in strengthening the immune system and modulating blood volume while also directing macronutrient metabolism.

Specifically, the liver serves as a critical hub for various physiological processes that are essential to maintaining homeostasis. The liver regulates endocrine signaling pathways that govern growth and is accountable for sustaining lipid and cholesterol homeostasis; therefore, considering the liver's integral role in these critical functions, it is plausible that hepatic conditions like fibrosis could disrupt metabolic equilibrium and consequently produce cascading effects on the endocrine pancreas. Furthermore, as the liver maintains lipid and cholesterol balance, fibrosis may interfere with this process, leading to imbalances that impact the interconnected pancreatic system. Overall, as a vital center for physiological processes, impairment of liver function through fibrosis can plausibly cause

metabolic disturbances, which in turn can negatively affect pancreatic endocrine activities through complex, interlinked pathways. Ultimately, the liver and pancreas are closely intertwined, so hepatic pathology may propagate systemic dysregulation. Furthermore, the liver's capacities to synthesize, break down, and release glucose hold pivotal importance in the postnatal metabolic transition. These processes enable glucose to be stored as glycogen during feeding and synthesized via the gluconeogenic pathway during fasting. Moreover, the liver plays a pivotal function in lipid metabolism, since it is accountable for the synthesis and release of VLDL, as well as the absorption of fatty acids and lipoproteins. It packages surplus lipids for storage and secretion in various tissues, including adipose tissue. Importantly, given its role in producing most blood-secreted proteins, the liver significantly contributes to protein and amino acid metabolism (77).

TRG and other lipids abnormally accumulate in hepatocytes in cases of non-alcoholic fatty liver disease (78), which may be triggered by IR (26). Hypertriglyceridemia is a condition characterized by elevated levels of triglycerides in the blood. This condition is influenced by the presence of larger VLDL particles, as well as increased concentrations of tiny, dense LDL and reduced levels of HDL cholesterol. The process of transferring cholesterol ester and triglyceride molecules between LDL or HDL particles and TRL particles takes place in the presence of hypertriglyceridemia (78). The increased susceptibility associated with tiny, dense LDL particles stems from their ability to readily penetrate the arterial intima, hence expediting the buildup of cholesterol inside atherosclerotic plaques. The reduced quantity of HDL particles reported in individuals with NAFLD might potentially influence the regulation of cholesterol levels inside the body.

The existing body of research suggests that serum obtained from individuals with NAFLD has a reduced capacity for HDL-mediated removal of cholesterol from cultured macrophages, in comparison to serum collected from healthy individuals (78). A significant factor impacting VLDL secretion equilibrium is insulin signaling. Individuals with FLD experience elevated basal VLDL production, and the suppression of insulin dependent VLDL secretion is compromised (78). Liver injury is worsened because of damage to the hepatocyte membrane, leading to the release of transaminase enzymes into the bloodstream (79). A study has demonstrated that high ALT levels, linked to decreased hepatic insulin sensitivity, predict the onset of T2DM (26).

Triglycerides and large VLDL particles have been associated with increased overall and central obesity, along with a heightened risk of T2DM. Elevated lipid levels in the bloodstream, commonly referred to as hyperlipidemia, have been connected to pancreas injury. The pancreas plays a pivotal role in lipid metabolism control by producing enzymes that break down lipids. With chronically elevated serum lipid levels, surplus lipids can infiltrate pancreatic cells, leading to the formation of lipid droplets within the organ. This accumulation of lipids impairs normal cellular function, triggering inflammation and oxidative stress in the pancreas, ultimately resulting in pancreas damage. Prolonged pancreas injury can lead to reduced insulin synthesis and secretion, thereby elevating the risk of diabetes and other associated disorders.

As results indicated that AST and ALT levels were slightly elevated in cases of acute liver fibrosis. In the murine model produced by $CCl_4$, the presence of chronic fibrosis resulted in a significant elevation in AST and ALT levels as compared to the untreated type. ALT levels increased to 80±12.5 and 175±42.3 IU/ml, respectively, in contrast as compared to 46±8.6 IU/ml in the naïve mice. A similar pattern of results was obtained in serum AST levels. This notable increase was observed in the mice with chronic $CCl_4$-induced fibrosis, which clearly demonstrated substantial damage, as shown in the histological findings of the chronic model (Figure 5A).

Our results revealed a significant increase in serum cholesterol and triglyceride levels. A statistically significant association was found between elevated TRG and the progression of liver fibrosis stages ($p<0.0001$). Moreover, a robust correlation between increased cholesterol levels and liver fibrosis was detected in the chronic model ($p<0.01$). Additionally, these effects were accompanied by a substantial rise in FBS. Therefore, the data provides compelling evidence linking $CCl_4$ administration to disrupted lipid and glucose metabolism. This potentially has implications for pancreatic health and overall metabolic balance.

### 4.1.2 Genetic expression of alpha-smooth muscle actin (α-SMA) and collagen type 3 (Col lll) in livers

HSCs play a pivotal role in liver fibrosis progression. Specifically, HSC activity is dictated by α-SMA expression, an intracellular microfilament protein. A study by Shteyer et al. showed α-SMA expression in liver lobules and portal tracts was meaningfully correlated with fibrosis scores. Overall, the findings demonstrated significantly lower fibrosis scores in cases with reduced smooth muscle expression (80). The research found α-SMA and Col III expression in both acute and chronic liver fibrosis, as indicated in the panel profile. The graphic shows the distribution of α-SMA levels in acute and chronic liver fibrosis. Notably, an evident increase in α-SMA levels is observed in the chronic fibrosis model, showing a linear correlation where higher liver fibrosis is associated with elevated α-SMA expression. This correlation is statistically significant ($p<0.01$). Furthermore, in cases of chronic liver fibrosis, α-SMA gene expression is twofold higher than in acute fibrosis.

Elevated collagen creation and degradation are features of alcoholic liver disease, which become more unbalanced as the disease progresses. Fibrosis advances due to a net increase in fibrillar collagens. A patient-controlled biopsy study on individuals with alcohol-related liver disease (ALD) discovered that patients with ALD had higher levels of type III collagen formation and degradation as the condition worsens and becomes more imbalanced. The progression of fibrosis is facilitated by a net increase in fibrillar collagens. As fibrosis advanced to higher levels, the production of type III, IV, and type V collagen increased more than their breakdown did. Collagen type III is positively linked with the fibrosis stage in multivariable analyses that control for inflammatory activity and steatosis score ($P=0.001$)(80). Results indicate a positive correlation between liver fibrosis and collagen III ($p<0.001$). All metrics correlate with fibrosis severity.

### 4.1.3 Serum C-peptide level in $CCl_4$-nduced mice model assist by ELISA

The effectiveness of β-islet cells in treating acute and chronic liver fibrosis has been assessed by examining C-peptide levels (76). The proinsulin cleavage product connecting peptide (C-peptide) is a 31-amino acid short linear molecule. t serves as a measure of endogenous insulin secretion (76). Within the Golgi complex of pancreatic beta cells, C-peptide is separated from proinsulin, contributing to the creation of the insulin molecule.

Post-creation, both insulin and C-peptide are stored in the secretory vesicles of β-islet cells in an equimolar ratio until triggered by high glycemia. Upon release into the portal circulation, insulin and C-peptide exhibit distinct kinetics. Insulin has a half-life of approximately 3 minutes, while C-peptide has a half-life of about 30 minutes. Due to its stability and resistance to the liver's first-pass metabolism, C-peptide can be reliably used as an indicator of β-islet cell function in clinical settings (62).

The findings of our study reveal a significant association between IR and liver fibrosis in both the acute and chronic experimental models ($p<0.001$). The elevated levels of HOMA, which is an indicator of reduced beta cell function, establish a link between the effectiveness of β-islet cells and chronic liver fibrosis ($p<0.05$). Consequently, the null hypothesis is rejected, leading us to infer that metabolic changes resulting from liver fibrosis could potentially impact the activity of pancreatic β-islet cells.

Based on the study findings and the obtained results, which reveal significantly elevated C-peptide levels in both acute and chronic liver fibrosis models, a noteworthy decrease in β-islet cell function and an increase in insulin resistance were observed in the liver injury group, indicating disrupted glucose homeostasis (Figure 8B). The heightened secretion of C-peptide and altered HOMA score could potentially place stress on pancreatic beta cells, ultimately leading to their depletion and reduced insulin synthesis over time. Simultaneously, the changes induced by liver injury in glucose and lipid metabolism further exacerbate the pancreatic environment.

Chronic liver disease is linked to dyslipidemia, insulin resistance, and elevated glucose levels (hyperglycemia). Due to insulin resistance, β-islet cells are exposed to prolonged periods of high glucose concentrations. Once the conventional glycolysis process becomes saturated, excess glucose is channeled into alternative pathways generating ROS, leading to oxidative stress. Additionally, it has been shown that an overabundance of lipids (hyperlipidemia) may induce oxidative stress reactions in β-islet cells, leading to lipotoxicity and subsequent harm (32). Pancreatic islet cells produce and release the pro-inflammatory cytokine IL-1β more strongly. Local inflammation may reduce islet mass, insulin output, and beta-cell death (18).

Conversely, β-islet cells possess the capability to detect excess FA and generate chemokines, which attract Ly6C+ monocytes and macrophages to the islets, potentially

positioning them as early responders (26). β-islet cell death induced by NEFA and cytokines, as observed in multiple studies, is influenced by the inflammatory impact of NEFA on islets through the stimulation of local macrophages and activation of TLR4 (28). Furthermore, the drawing of macrophages to islets is facilitated by the secretion of chemokines, including MCP-1 and IL-8.

Only elevation of NEFA in individuals corresponds to insulin resistance only when accompanied by increased plasma fetuin-A levels. Fetuin-A derived from fatty liver exerts a direct, TLR4-independent influence on beta cells, leading to reduced GSIS. Moreover, it intensifies pancreatic inflammation through a TLR4-dependent mechanism by provoking a pro-inflammatory response in islet macrophages and monocytes. These circumstances could potentially accelerate beta cell loss, causing them to lose their insulin-producing capacity (81). This dysregulation can facilitate inflammatory reactions, oxidative stress, and lipid accumulation within the pancreas, potentially resulting in pancreatic damage and diminished β-islet cell function (32). Understanding the intricate interplay between liver injury, C-peptide levels, HOMA score, and pancreatic well-being holds the potential to provide valuable insights for developing targeted therapeutic strategies to effectively manage diabetes and associated metabolic disorders.

### 4.1.4 β-islet cells from liver fibrosis mice model showed high expressions of IL-6

IL-6 is a multipurpose pro-and anti-inflammatory molecule. And is produced by a variety of cell types, including most immune cells, endothelial cells, skeletal and smooth muscle cells, adipocytes, β-islet-cells, hepatocytes, and others. Hepatocytes, certain endocrine cells, and leukocytes have membrane-bound IL-6R (82). This entity controls the immune system, bone metabolism, endocrine system, energy metabolism, glucose homeostasis, lipid metabolism, and many other physiological functions. IL-6 coordinates the rapid immune response to an infectious stimulus during acute inflammation. IL-6 helps produce anti-inflammatory cytokines including IL-1ra, IL-8, and IL-10, as well as acute phase reactants like CRP. Metabolic illnesses with elevated cytokine levels, such as IL-6, TNF-α, and IL-1, have continuous low-level inflammation (55). A study conducted on experimental rats with liver injury unveiled significant alterations in the pancreas and liver. Pathogenic alterations were identified, as shown by elevations in oxidative indicators such as malondialdehyde, as well as inflammatory markers TNF-α and IL-6 (28).

IL-6 was first characterized as a pro-inflammatory cytokine based on early investigations, which observed its rapid increase during the early phases of infection or in reaction to non-infectious triggers. In the situations, macrophages and monocytes exhibit the secretion of IL-6 at levels that are much greater, up to 1000 times, compared to the baseline levels seen in the circulation. Increases in pro-inflammatory cytokines like IL-1 and TNF-α precede IL-6 increases and are key inducers. Chronic liver fibrosis, persistent low-grade inflammation is related to metabolic syndrome, obesity, and T2DM. Within this context, there is an observed elevation in systemic cytokine levels (namely IL-1, TNF-α, and IL-6) and CRP, with an approximate two- to three-fold increase (55).

It is generally acknowledged that IL-6 controls glucose homeostasis; however, it remains unclear whether this regulation serves a positive or negative purposes (55). The hepatocyte-specific expression of a constitutively active IKK-β in mice is associated with increased IL-6 production and insulin resistance. Hepatic IR stems from this IL-6 increase. In vitro studies using human hepatocarcinoma cells and primary hepatocytes from mice have demonstrated that IL-6 induces insulin resistance by inhibiting the tyrosine phosphorylation of IRS-1 through the SOCS-3 pathway (83). Infusions of IL-6 during tests on mice have been observed to impede insulin action, whereas inhibiting IL-6 signaling enhances hepatic IS. Research suggests that IL-6 may have a role in the development of hepatic insulin resistance, maybe via an increase in the phosphorylation of SOCS-3. The observed elevation in phosphorylation of SOCS-3 leads to its interaction with IRS, promoting their targeting for proteasomal destruction, thereby impeding the process of insulin-dependent glucose absorption (55).

Due to increased islet apoptosis, rheumatoid arthritis patients have higher glucose metabolism, poor insulin sensitivity, and impaired islet-cell activity. Overexpression of pro-inflammatory cytokines may be the cause. RA patients had much greater IL-6 levels than controls. Due to the significant connection between IL-6 and caspase-3, this abnormal glucose metabolism was followed by increased islet death, which decreased insulin production (84). IL-6 also increases lipolysis in adipocytes and free fatty acid release, which damages mitochondria, GLUT2, and insulin sensitivity (84).

Additional studies reveal potentially beneficial effects of increased IL-6. Specifically, it was found that IL-6 promotes increased GLP-1 synthesis, which raises insulin secretion

(55). Moreover, evidence indicates that IL-6 regulates adipose tissue and lipid metabolism physiologically. Human studies show a single IL-6 infusion elevates lipolysis and oxidation throughout the body at both high and low doses, without raising adrenaline or insulin, implicating IL-6 alone. While the precise molecular mechanism inducing adipose lipolysis remains unclear, it may involve AMPK. However, IL-6 is known to activate AMPK, causing oxidation (55).

Our results demonstrated a significant association between liver fibrosis and IL-6. This relationship may stem from chronic low-grade inflammation induced by metabolic syndrome, obesity, and T2DM, which leads to liver fibrosis and 2-3-fold IL-6 increases. Thus, our findings imply chronic liver fibrosis could potentially increase IL-6 levels, causing IR and elevated glucose. Moreover, the impact of heightened IL-6 on β-islet cell dysfunction and apoptosis merits consideration.

Despite its intricacy, our comprehension of IL-6's role in glucose homeostasis remains incomplete. Additionally, it is intriguing to ponder whether harnessing the effects of acute IL-6 treatment could provide a therapeutic approach to lower blood sugar.

Further investigation is required to delve into the mechanisms through which IL-6 exerts its effects, enabling us to address this question effectively.

### 4.1.5 Interferon-gamma and IL-6R

Innate immunity is supported by NK cells, which are a subgroup of lymphocytes responsible for lysing target cells and secreting cytokines. NK cell activity is governed by a multitude of activating and inhibitory receptors. Activated NK cells release IFN-γ, which plays an immunoregulatory role by not only further activating NK cells but also fostering cytokine secretion alongside cytotoxic granules (85).

A study investigation using experimental models revealed a decrease in NK cells in the peripheral circulation. The observed decline may be ascribed to the active migration of these cells towards the pancreas and associated lymph nodes. The results of this study indicate that these cells play a significant role in the pathogenesis of the illness. Within the islets, they serve as cytotoxic agents targeting β-islet cells, hence playing a role in the development of the illness. Pancreatic NK cells also showed higher activation marker expression, a more mature phenotype, and faster proliferation than other mouse strains.

These attributes substantiate their function in facilitating the demise of T-cells. The findings from investigations involving human subjects have generated conflicting outcomes. The research argues that the observed decrease in NK cells over a one-year period of monitoring may be attributed to their functional decline. Interestingly, a separate study noted that the decrease in NK cell activation among individuals with T1DM occurs approximately a year after the disease's onset (52).

This contrasts with nude mouse research that found more NK cells invading tumors in the peripheral blood and tissues of esophageal squamous cell carcinoma patients. Through the STAT3 signaling pathway, IL-6 inhibits NK cell activity. STAT3 signaling by IL-6 or IL-8 predominantly impairs NK cells by primary ESCC cells (58). Another study demonstrated that peritoneal fluid from endometriosis patients inhibited NK cell differentiation and cytotoxicity in comparison to peritoneal fluid from control subjects ($p<0.05$). Elevated IL-6 levels were also observed in the peritoneal fluid of endometriosis patients ($P=0.01$), and these levels were inversely correlated with NK cell cytolytic activity ($rs=-0.558$, $P=0.03$). Moreover, the downregulation of granzyme B coincided with the reduction of NK cell cytolytic activity due to IL-6 ($p<0.05$) (86). The pro-inflammatory cytokine IL-6 strongly opposed NK cell function (87).

The results of this study support the researchers' hypothesis. They looked at the relationship between liver scarring and natural killer cell activity. NK cells are a type of immune cell. The researchers measured levels of a protein called IFN- γ, which is produced by NK cells when they are active. We found a significant inverse correlation between liver fibrosis and IFN-γ levels ($p<0.005$). This means patients exhibiting liver fibrosis had lower levels of IFN- γ, indicating less natural killer cell activity. The study also found a statistically significant positive link between liver fibrosis and IL-6 levels ($p<0.05$). People with obesity, diabetes and other conditions often have chronically high IL-6. This inflammation may explain the 2-3 times higher IL-6 in people with liver scarring. In summary, the study shows liver fibrosis is associated with reduced NK cell activity and increased inflammation. This helps explain how liver problems could eventually contribute to pancreatic dysfunction and diabetes over time. Through the STAT3 signaling pathway, IL-6 may also inhibit NK cell activity. This inhibition accelerates pancreatic disease. Clusters of abdominal obesity, dyslipidemia, hyperglycemia, and hypertension are collectively referred to as the metabolic syndrome

(16). Obesity, along with its associated chronic low-grade inflammatory state, has been recognized as a primary factor contributing to the onset of the metabolic syndrome and its associated pathophysiological effects (17).

Steatogenesis in NAFLD primarily arises due to an escalation in the hepatic esterification of FFAs. These FFAs originate from dysfunctional or inflamed white adipose tissue as well as de novo lipogenesis (21). The liver accumulates FA due to decreased VLDL synthesis, increased FFA transfer from the liver, and increased IR and hyperinsulinemia. This causes hepatic TRG accumulation and obesity (22). Increased lipolysis plays a role in exacerbating hepatic insulin resistance by triggering hepatic gluconeogenesis and encouraging hepatic lipid synthesis through FFA esterification. This process furnishes hepatocytes with an excessive supply of lipogenic substrates (including glucose and NEFA) as well as hormones, manifesting as IR – a notable characteristic of chronic infection (hyperinsulinemia) (21, 23).

Muscle IR enhances de novo lipogenesis by augmenting the supply of glucose to the liver. Hepatic insulin resistance has a direct association with the storage of ectopic lipids within the liver (21). The liver's release of hepatokines or other endocrine mediators can impact insulin action, secretion, and glucose metabolism. Steatotic hepatocytes release Fetuin-A, which blocks the insulin receptor and causes IR. Meanwhile, the reduction in adiponectin production, stemming from disturbances in secretory adipocytes due to increased ECM caused by fibrosis, exerts a secondary influence on insulin sensitivity.

In addition, IL-6 can exacerbate the dysfunction of mitochondria, GLUT2, and insulin sensitivity. This is achieved by promoting lipolysis in adipocytes and the subsequent release of FFA (56).

In liver fibrosis, metabolic disorders such as dyslipidemia, inflammation, IR, and hyperglycemia are well documented (20). Glycolysis inside the cell does not occur due to IR. Increased lipolysis promotes hepatic IR by stimulating hepatic gluconeogenesis and driving hepatic lipid synthesis through the esterification of FFAs, which supplies the hepatocyte with an excess of lipogenic substrates, (including glucose and NEFA) (21, 23).

Glucose transporter 4 (GLUT4) belongs to the glucose transporter protein family. It responds to insulin signaling and involves membrane trafficking (88). The surface of NK cells is GLUT4. It transports glucose, glucosamine, and dehydroascorbic acid. It is expressed in leukocytes, heart, fat, and skeletal muscle, which transport glucose through insulin. When insulin binds to its receptor, GLUT4 swiftly moves to the plasma membrane, increasing glucose absorption (89). In cases of liver fibrosis where IR is present and inhibits insulin-mediated glucose uptake, deficient cellular metabolism becomes a critical factor in the impaired development of NK cells (47). And from our results there is a positive correlation between IR and decrease the activity of NK cells.

Metabolism has emerged as a captivating aspect in distinguishing NK cell functional outcomes. The strongest cytotoxic NK cells use glycolysis and OxPhos to generate glucose. Glucose serves as a primary energy source for NK cells, undergoing processing in both the cytosol and mitochondria via aerobic glycolysis (47). In contrast, hypoxic glycolysis-restricted regulatory NK cells survive. This indicates limited OxPhos and glycolysis for regulatory actions. The key to lifespan is flexibility and resilience. Reduced levels of reactive oxygen species increased mitochondrial respiratory capacity and membrane potential, and the elimination of defective mitochondria all contribute to enhanced mitochondrial fitness in memory NK cells. It seems that different metabolic patterns, and not only phenotypic, are responsible for the wide range of functional outcomes seen in NK cells (48).

Donnelly et al. studied the effect of inhibiting glycolysis or mTOR on NK cell responses to TLR3 ligand poly(I:C) activation in vivo. The administration of either pathway inhibitors dramatically lowered the percentage of IFN- γ -+ NK cells in mice 24 hours after poly(I:C) injection. Metabolic changes, such as alterations in protein folding or post-translational modification, could potentially impact the stability and synthesis rate of IFN-γ. Protein glycosylation is essential for the stability and folding of proteins, including IFN-γ (49).

The emergence of defective NK cells has been linked to a wide variety of chronic illnesses, including obesity, and there is growing evidence that impaired cellular metabolism plays a major role in this process (47). Previous research has established a connection between insufficient physical activity or an unfavorable metabolic state and

diminished NK cell functionality (70). The metabolic inefficiency seen in this context has been ascribed to the accumulation of lipids inside NK cells, which is mediated by pPPAR. This lipid accumulation subsequently alters the expression of genes, resulting in diminished signaling of MYC and mTORC1, as well as lower rates of OXPHOS. TGFβ has the ability to directly impede the metabolic activity of NK cells via many methods, one of which involves the suppression of mammalian targets of mTORC1 signaling (47). And this what our results showed, as there is an inverse relationship between increased triglyceride and decreased NK activity.

The presence of GLUT4 protein on NK cell membranes correlates with their function. The aforementioned observation of diminished GLUT4 expression on NK cells in individuals diagnosed with colon cancer is indicative of a phenomenon whereby the cytotoxicity of NK cells is hampered within this particular patient cohort (89).

The assessment of GLUT4 expression on NK cells in persons with DT2M, since it serves as an indirect indicator of the cytotoxic capacity of these cells, might potentially enhance the precise identification of individuals who are susceptible to cancer development (89). Many investigations have indicated that abnormalities in GLUT4 translocation are directly connected to IR (90). A well-established association exists between the expression of GLUT4 and the extent of IR (90). NK cell activity demonstrates a noticeable linear decrease with rising blood glucose levels. Insufficient insulin levels could potentially contribute to reduced NK cell activity. Additionally, NK cell activity exhibited a significant correlation with HOMA-B, a measure of cell function (70).

### 4.1.6 Annexin V and pancreatic enzymes

Annexin V, a Ca++-binding protein that binds to phosphatidylserine residues on apoptotic cells, is used as a marker. Annexin V has been detected on cytoplasmic detritus, most likely arising as a result of apoptotic blebbing (91). The use of annexin V in the identification and tracking of apoptosis within cellular cytotoxicity is a sensitive qualitative method that has been recently adapted for measuring xenogeneic cell-mediated cytotoxicity (92).

In a previous study investigating the role of the IL-6 cytokine on pancreatic β-islet cells using PCR microarrays, the researchers evaluated changes in apoptotic pathway gene

expression profiles during IL-6 administration to uncover potential pro-apoptotic pathways. They found that continuous IL-6 treatment of β-islet cells reduced cell viability. Furthermore, the participation of the STAT-3 signalling pathway in the induction of β-islet death by IL-6 was found (93). Our results demonstrated an increase in annexin-V, indicating an increase in apoptotic β-islet islet cells. Furthermore, the findings revealed an elevation in IL-6 levels, which decreased cell viability and induced β-islet cell apoptosis via the STAT-3 signaling pathway. Thus, a positive correlation between liver fibrosis and β-islet cell apoptosis exists ($p<0.05$).

AP is a severe condition with far-reaching immediate and long-term consequences, and it cannot be regarded as self-limiting. Upper stomach pain, amylase/lipase readings over three times normal, and cross-sectional imaging findings are diagnostic criteria (94). The findings strongly suggest a link between liver fibrosis and pancreatic injury, as evidenced by elevated amylase and lipase levels alongside increased islet cell apoptosis.

A recent global systematic review identified hypertriglyceridemia as the cause of AP in 9% of cases, positioning it as the third most prevalent cause (94). Our results align with this, revealing a positive correlation ($p<0.0005$) between elevated triglyceride levels in cases of liver fibrosis, potentially indicating a role in pancreatitis onset. In conclusion, our study establishes a frequent occurrence of elevated pancreatic enzymes and β-islet cell apoptosis in liver fibrosis, both of which are linked to the severity and progression of this condition.

No published studies were discovered that elucidated the impact of IL-6 cytokine on pancreatic NK cell activity within a liver fibrosis model. Similarly, other studies did not delve into this association in the context of liver fibrosis. As a result, this study has shed light on the potential metabolic pathway that could connect liver fibrosis with pancreatic injury through the mediation of NK cells.

Based on our findings, we deduce that the decline in pancreatic NK cell activity might stem from the influence of the IL-6 cytokine and metabolic dysregulation, both of which impact GLUT4. These regulatory efforts might be significant. Thus, NK cells may not mediate cytotoxicity against a variety of activated immune cells or regulate immune response and homeostasis. As a result, the progression of pancreatic injury may occur. A study identified an independent prognostic factor, HOMA-IR (62), as the sole predictor

of AP severity. Our HOMA-IR findings, therefore, align with indications of pancreatic damage. The findings propose that the reduction in NK cell activity is intricately involved in the pathophysiology of pancreatic damage within the liver fibrosis model. NK cells emerge as a pivotal component in the interplay between liver fibrosis and pancreatic injury, functioning to regulate immunity and thwart the advancement of fibrosis and pancreatic cancer.

## 4.2 Limitation

Other metabolic markers of histopathological findings of lipid accumulation using the oil red staining technique were not performed because of limitations in budget.

Markers of pancreatic NK cells exhaustions and additional activation markers could give clear picture on NK cell phenotypic alteration in liver fibrosis model.

## 4.3 Recommendations and conclusion

### 4.3.1 Recommendations

- More research is necessary to examine how IL-6 affects glucose and lipid metabolism.
- Periodical testing of IL-6 with C-peptide in patients with liver fibrosis as an indicator of beta islet cell status.
- Further research to examine pancreatic NK cells phenotype in liver fibrosis model.
- Given the absence of published studies exploring the effect of IL-6 in the context of liver fibrosis on NK cell activity in the pancreas, and the lack of comprehensive understanding in other related studies, it is strongly recommended to conduct additional studies in this area.

### 4.3.2 Conclusion

Liver fibrosis induces pancreatic injury, as evidenced by elevated amylase and lipase levels and increased islet cell apoptosis. A link exists between liver fibrosis and dysregulation of lipid and glucose metabolism, which may implicate pancreatic health and overall metabolic homeostasis. In the liver fibrosis model, the decrease in pancreatic NK cell activity could stem from the IL-6 cytokine effect and metabolic dysregulation. Hence, regulatory activities can be achieved. Thus, it cannot mediate cytotoxicity against

various activated immune cells or serve a crucial physiological role in regulating immune responses and maintaining homeostasis, consequently impacting the progression of pancreatic injury. It can be inferred from our findings that the decrease in NK cell activity is involved in the pathophysiology of pancreatic damage in the liver fibrosis model. NK cells are a key factor in the crosstalk between liver fibrosis and pancreatic damage, functioning to prevent fibrosis promotion and pancreatic cancer.

# List of abbreviation

| Abbreviation | Meaning |
|---|---|
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| AMPK | Adenosine monophosphate –activated protein kinase |
| αSMA | Alpha-Smooth Muscle Actin |
| AST | Aspartare aminotransferase |
| BM | Bone marrow |
| CCK | Cholrcystokinnin |
| $CCl_4$ | Carbon tetrachloride |
| CRP | C reactive protein |
| CY | Cyanine Dyes |
| DAMPs | Danger-Associated Molecular Patterns |
| DEPC | Diethyl pyrocarbonate |
| DMEM | Dulbeccos Modified Eagle Medium |
| DNL | De-novo lipogenesis |
| ECM | Extracellular Matrix |
| EtOH | Ethanol |
| FA | Fatty acid |
| FBS | Fasting blood sugar |
| FFAs | Free Fatty Acids |
| FLD | Fatty liver disease |
| GK rates | Goto- Kakizaki rates |
| GLP-1 | Glucagon like peptide -1 |
| GSIS | Glucose-stimulated insulin secretion |
| H&E | Hematoxylin and Eosin |
| HBV | Hepatitis B virus |
| HCV | Hepatitis C virus |
| HDL | High density lipoprotein |
| HFD | High fat diet |
| HGF | Hepatic growth factor |
| HIF1α | Hypoxia inducible factor 1 α |
| HLA-DR | Human leukocyte antigen- DR isotype |
| HOMA-IR | Homeostatic Model Assessment for Insulin Resistance |
| H2O2 | Hydrogen peroxide |
| HSCs | Hepatic stellate cells |
| ILCs | Innate Lymphoid Cells |
| ILC | Innate lymphoid cells |

| Abbreviation | Meaning |
|---|---|
| IL-17 | Interleukin 17 |
| IL-1β | Interleukin 1 beta |
| IL-13 | Interleukin 13 |
| IL-33 | Interleukin 33 |
| IL-6 | Interleukin 6 |
| IRS | Insulin receptor substrate |
| LDL | Low density lipoprotein |
| LSM | Laser Scanning Microscopy |
| MCD | Methionine/choline deficient |
| MCP-1 | Monocyte Chemoattractant Protein-1 |
| MMPs | Matrix metalloproteinase |
| NASH | Nonalcoholic steatohepatitis |
| NAFLD | Nonalcoholic fatty liver disease |
| NEFA | Hepatokines non-esterified fatty acids |
| NF-κB | Nuclear factor kappa B |
| NIH | National institutes of health |
| NK cell | Natural Killer Cell |
| NLRP3 | NLR family pyrin domain containing protein 3 |
| NPCs | Non-Parenchymal cells |
| PBS | Phosphate Buffer Saline |
| PDGF | Platelet-derived growth factor |
| PMN elastase | Polymorphonuclearelastase |
| PP | Pancreatic polypeptide |
| RIP | Rate insulin promoter |
| ROS | Reactive oxygen species |
| SFAs | Saturated fatty acids |
| SFFAs | Saturated free fatty acids |
| SOCS3 | Suppressor of cytokine signaling 3 |
| STAT3 | Signal transducer and activator of transcription 3 |
| TC | Total cholesterol |
| TGs | Triglycerides |
| TGF-β | Transforming growth factor beta |
| Th cell | T helper cell |
| TNFα | Tumor necrosis factor alpha |
| VLDL | Very low-density lipoprotein |
| WAT | White adipose tissue |
| WD | Western diet |

# References


1. Roehlen N, Crouchet E, Baumert TF. Liver Fibrosis: Mechanistic Concepts and Therapeutic Perspectives. Cells. 2020;9(4).

2. Mehta KJ, Farnaud SJ, Sharp PA. Iron and liver fibrosis: Mechanistic and clinical aspects. World J Gastroenterol. 2019;25(5):521-38.

3. Altamirano-Barrera A, Barranco-Fragoso B, Méndez-Sánchez N. Management strategies for liver fibrosis. Annals of hepatology. 2017;16(1):48-56.

4. Campana L, Iredale JP. Regression of Liver Fibrosis. Seminars in liver disease. 2017;37(1):1-10.

5. Cui N, Hu M, Khalil RA. Biochemical and Biological Attributes of Matrix Metalloproteinases. Progress in molecular biology and translational science. 2017;147:1-73.

6. An P, Wei LL, Zhao S, Sverdlov DY, Vaid KA, Miyamoto M, et al. Hepatocyte mitochondria-derived danger signals directly activate hepatic stellate cells and drive progression of liver fibrosis. 2020;11(1):2362.

7. Schwabe RF, Tabas I, Pajvani UB. Mechanisms of Fibrosis Development in Nonalcoholic Steatohepatitis. Gastroenterology. 2020;158(7):1913-28.

8. Pei K, Gui T, Kan D, Feng H, Jin Y, Yang Y, et al. An Overview of Lipid Metabolism and Nonalcoholic Fatty Liver Disease. Biomed Res Int. 2020;2020:4020249.

9. Torok NJ. Dysregulation of redox pathways in liver fibrosis. American journal of physiology Gastrointestinal and liver physiology. 2016;311(4):G667-g74.

10. Dhar D., Baglieri J, Kisseleva T, Brenner DA. Mechanisms of liver fibrosis and its role in liver cancer. Experimental biology and medicine (Maywood, NJ). 2020;245(2):96-108.

11. Wu J, Zhang L, Shi J, He R, Yang W, Habtezion A, et al. Macrophage phenotypic switch orchestrates the inflammation and repair/regeneration following acute pancreatitis injury. EBioMedicine. 2020;58:102920.

12. So-Armah K, Freiberg M, Cheng D, Lim JK, Gnatienko N, Patts G, et al. Liver fibrosis and accelerated immune dysfunction (immunosenescence) among HIV-infected Russians with heavy alcohol consumption - an observational cross-sectional study. BMC gastroenterology. 2019;20(1):1.

13. Albillos A, Martin-Mateos R, Van der Merwe S, Wiest R, Jalan R, Álvarez-Mon M. Cirrhosis-associated immune dysfunction. Nat Rev Gastroenterol Hepatol. 2022;19(2):112-34.

14. Tsuchida T, Lee YA, Fujiwara N, Ybanez M, Allen B, Martins S, et al. A simple diet- and chemical-induced murine NASH model with rapid progression of steatohepatitis, fibrosis and liver cancer. Journal of hepatology. 2018;69(2):385-95.

15. Boll M, Weber LW, Becker E, Stampfl A. Pathogenesis of carbon tetrachloride-induced hepatocyte injury bioactivation of CCI4 by cytochrome P450 and effects on lipid homeostasis. Zeitschrift fur Naturforschung C, Journal of biosciences. 2001;56(1-2):111-21.

16. Engin A. The Definition and Prevalence of Obesity and Metabolic Syndrome. Advances in experimental medicine and biology. 2017;960:1-17.

17. Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators of inflammation. 2010;2010.

18. Sun K, Tordjman J, Clément K, Scherer PE. Fibrosis and adipose tissue dysfunction. Cell metabolism. 2013;18(4):470-7.

19. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes research and clinical practice. 2014;105(2):141-50.

20. Patel S, Jinjuvadia R, Patel R, Liangpunsakul S. Insulin Resistance is Associated With Significant Liver Fibrosis in Chronic Hepatitis C Patients: A Systemic Review and Meta-Analysis. Journal of clinical gastroenterology. 2016;50(1):80-4.

21. Ballestri S, Nascimbeni F, Romagnoli D, Baldelli E, Targher G, Lonardo A. Type 2 Diabetes in Non-Alcoholic Fatty Liver Disease and Hepatitis C Virus Infection--Liver: The "Musketeer" in the Spotlight. International journal of molecular sciences. 2016;17(3):355.

22. Zaman CF, Sultana J, Dey P, Dutta J, Mustarin S, Tamanna N, et al. A Multidisciplinary Approach and Current Perspective of Nonalcoholic Fatty Liver Disease: A Systematic Review. Cureus. 2022;14(9):e29657.

23. Lonardo A, Adinolfi LE, Restivo L, Ballestri S, Romagnoli D, Baldelli E, et al. Pathogenesis and significance of hepatitis C virus steatosis: an update on survival strategy of a successful pathogen. World journal of gastroenterology. 2014;20(23):7089-103.

24. Bhattamisra SK, Koh HM, Lim SY, Choudhury H, Pandey M. Molecular and Biochemical Pathways of Catalpol in Alleviating Diabetes Mellitus and Its Complications. Biomolecules. 2021;11(2).

25. Ying W, Fu W, Lee YS, Olefsky JM. The role of macrophages in obesity-associated islet inflammation and β-cell abnormalities. Nat Rev Endocrinol. 2020;16(2):81-90.

26. Hsieh PS, Hsieh YJ. Impact of liver diseases on the development of type 2 diabetes mellitus. World journal of gastroenterology. 2011;17(48):5240-5.

27. Ruzittu S, Willnow D, Spagnoli FM. Direct Lineage Reprogramming: Harnessing Cell Plasticity between Liver and Pancreas. Cold Spring Harbor perspectives in biology. 2020;12(7).

28. López-Bermudo L, Luque-Sierra A, Maya-Miles D, Gallego-Durán R, Ampuero J, Romero-Gómez M, et al. Contribution of Liver and Pancreatic Islet Crosstalk to β-Cell Function/Dysfunction in the Presence of Fatty Liver. Frontiers in endocrinology. 2022;13:892672.

29. Wang J, Wang H. Oxidative Stress in Pancreatic Beta Cell Regeneration. 2017;2017:1930261.

30. Gerst F, Wagner R, Kaiser G, Panse M, Heni M, Machann J, et al. Metabolic crosstalk between fatty pancreas and fatty liver: effects on local inflammation and insulin secretion. Diabetologia. 2017;60(11):2240-51.

31. Bhattamisra SK, Koh HM. Molecular and Biochemical Pathways of Catalpol in Alleviating Diabetes Mellitus and Its Complications. 2021;11(2).

32. Mayerle J, Sendler M, Hegyi E, Beyer G, Lerch MM, Sahin-Tóth M. Genetics, Cell Biology, and Pathophysiology of Pancreatitis. Gastroenterology. 2019;156(7):1951-68.e1.

33. Ying W, Fu W, Lee YS, Olefsky JM. The role of macrophages in obesity-associated islet inflammation and β-cell abnormalities. 2020;16(2):81-90.

34. Rugivarodom M, Geeratragool T. Fatty Pancreas: Linking Pancreas Pathophysiology to Nonalcoholic Fatty Liver Disease. 2022;10(6):1229-39.

35. Váncsa S, Németh D, Hegyi P, Szakács Z, Hegyi PJ, Pécsi D, et al. Fatty Liver Disease and Non-Alcoholic Fatty Liver Disease Worsen the Outcome in Acute Pancreatitis: A Systematic Review and Meta-Analysis. Journal of clinical medicine. 2020;9(9).

36. Wu D, Zhang M, Xu S, Wu K, Wang N, Wang Y, et al. Nonalcoholic Fatty Liver Disease Aggravated the Severity of Acute Pancreatitis in Patients. 2019;2019:9583790.

37. Liu W, Du JJ, Li ZH, Zhang XY, Zuo HD. Liver injury associated with acute pancreatitis: The current status of clinical evaluation and involved mechanisms. World journal of clinical cases. 2021;9(34):10418-29.

38. Wei X, Yao W, Li H, Qian J, Xie Y, Zhang Z, et al. B and NK Cells Closely Correlate with the Condition of Patients with Acute Pancreatitis. 2019;2019:7568410.

39. Li Z, Yu X, Werner J, Bazhin AV, D'Haese JG. The role of interleukin-18 in pancreatitis and pancreatic cancer. Cytokine & growth factor reviews. 2019;50:1-12.

40. Manohar M, Kandikattu HK, Verma AK, Mishra A. IL-15 regulates fibrosis and inflammation in a mouse model of chronic pancreatitis. American journal of physiology Gastrointestinal and liver physiology. 2018;315(6):G954-g65.

41. Shimizu K. Mechanisms of pancreatic fibrosis and applications to the treatment of chronic pancreatitis. Journal of gastroenterology. 2008;43(11):823-32.

42. Thomas D, Radhakrishnan P. Tumor-stromal crosstalk in pancreatic cancer and tissue fibrosis. 2019;18(1):14.

43. Hühn MH, Hultcrantz M, Lind K, Ljunggren HG, Malmberg KJ, Flodström-Tullberg M. IFN-gamma production dominates the early human natural killer cell response to Coxsackievirus infection. Cellular microbiology. 2008;10(2):426-36.

44. Zitti B, Bryceson YT. Natural killer cells in inflammation and autoimmunity. Cytokine & growth factor reviews. 2018;42:37-46.

45. Tosello-Trampont A, Surette FA, Ewald SE, Hahn YS. Immunoregulatory Role of NK Cells in Tissue Inflammation and Regeneration. Frontiers in immunology. 2017;8:301.

46. Gianchecchi E, Delfino DV, Fierabracci A. Natural Killer Cells: Potential Biomarkers and Therapeutic Target in Autoimmune Diseases? Frontiers in immunology. 2021;12:616853.

47. O'Brien KL, Finlay DK. Immunometabolism and natural killer cell responses. 2019;19(5):282-90.

48. Poznanski SM, Ashkar AA. What Defines NK Cell Functional Fate: Phenotype or Metabolism? Frontiers in immunology. 2019;10:1414.

49. Mah AY, Cooper MA. Metabolic Regulation of Natural Killer Cell IFN-γ Production. Critical reviews in immunology. 2016;36(2):131-47.

50. Liu Z, Shen Y, Cui N, Yang J. Clinical observation of immunity for severe acute pancreatitis. Inflammation. 2011;34(5):426-31.

51. Ye R, Mai Z, Pan X, Cai S, Deng L. Acute fatty liver of pregnancy causes severe acute pancreatitis and stillborn fetus: A case report. Medicine (Baltimore). 2021;100(16):e25524.

52. Gomez-Muñoz L, Perna-Barrull D, Villalba A, Rodriguez-Fernandez S, Ampudia RM, Teniente-Serra A, et al. NK Cell Subsets Changes in Partial Remission and Early Stages of Pediatric Type 1 Diabetes. Frontiers in immunology. 2020;11:611522.

53. Gardner G, Fraker CA. Natural Killer Cells as Key Mediators in Type I Diabetes Immunopathology. Frontiers in immunology. 2021;12:722979.

54. Folias AE, Penaranda C, Su AL, Bluestone JA, Hebrok M. Aberrant innate immune activation following tissue injury impairs pancreatic regeneration. PloS one. 2014;9(7):e102125.

55. Lehrskov LL, Christensen RH. The role of interleukin-6 in glucose homeostasis and lipid metabolism. Seminars in immunopathology. 2019;41(4):491-9.

56. Jin H, Ning Y, Zhou H, Wang Y. IL-6 Promotes Islet β-Cell Dysfunction in Rat Collagen-Induced Arthritis. 2016;2016:7592931.

57. Butturini E, Carcereri de Prati A, Mariotto S. Redox Regulation of STAT1 and STAT3 Signaling. International journal of molecular sciences. 2020;21(19).

58. Wu J, Gao FX, Wang C, Qin M, Han F, Xu T, et al. IL-6 and IL-8 secreted by tumour cells impair the function of NK cells via the STAT3 pathway in oesophageal squamous cell carcinoma. Journal of experimental & clinical cancer research : CR. 2019;38(1):321.

59. Salgado AL, Carvalho L, Oliveira AC, Santos VN, Vieira JG, Parise ER. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. Arq Gastroenterol. 2010;47(2):165-9.

60. Isokuortti E, Zhou Y, Peltonen M, Bugianesi E, Clement K, Bonnefont-Rousselot D, et al. Use of HOMA-IR to diagnose non-alcoholic fatty liver disease: a population-based and inter-laboratory study. Diabetologia. 2017;60(10):1873-82.

61. Chatterjee A, Basu A, Das K, Singh P, Mondal D, Bhattacharya B, et al. Hepatic transcriptome signature correlated with HOMA-IR explains early nonalcoholic fatty liver disease pathogenesis. Ann Hepatol. 2020;19(5):472-81.

62. Tso TK, Huang HY, Chang CK, Liao YJ, Huang WN. Clinical evaluation of insulin resistance and beta-cell function by the homeostasis model assessment in patients with systemic lupus erythematosus. Clinical rheumatology. 2004;23(5):416-20.

63. Cho SK, Huh JH, Yoo JS, Kim JW, Lee KJ. HOMA-estimated insulin resistance as an independent prognostic factor in patients with acute pancreatitis. Sci Rep. 2019;9(1):14894.

64. Váncsa S, Németh D, Hegyi P, Szakács Z. Fatty Liver Disease and Non-Alcoholic Fatty Liver Disease Worsen the Outcome in Acute Pancreatitis: A Systematic Review and Meta-Analysis. 2020;9(9).

65. Huang C, Iovanna J, Santofimia-Castaño P. Targeting Fibrosis: The Bridge That Connects Pancreatitis and Pancreatic Cancer. 2021;22(9).

66. Kumagi T, Terao T, Yokota T, Azemoto N, Kuroda T, Imamura Y, et al. Early Detection of Pancreatic Cancer in Patients With Chronic Liver Disease Under Hepatocellular Carcinoma Surveillance. Mayo Clinic proceedings. 2019;94(10):2004-10.

67. Park JH, Hong JY, Han K, Kang W, Park JK. Increased risk of pancreatic cancer in individuals with non-alcoholic fatty liver disease. Scientific reports. 2022;12(1):10681.

68. Rezende AQM, Gestic MA. Is there a link between non-alcoholic fatty liver disease aspects and pancreatic cancer? Results of a case-matched study. 2021;48:e20202913.

69. Cho SK, Huh JH. HOMA-estimated insulin resistance as an independent prognostic factor in patients with acute pancreatitis. 2019;9(1):14894.

70. Kim JH, Park K, Lee SB, Kang S, Park JS. Relationship between natural killer cell activity and glucose control in patients with type 2 diabetes and prediabetes. 2019;10(5):1223-8.

71. Grancini V, Trombetta M, Lunati ME, Zimbalatti D, Boselli ML, Gatti S, et al. Contribution of β-cell dysfunction and insulin resistance to cirrhosis-associated diabetes: Role of severity of liver disease. J Hepatol. 2015;63(6):1484-90.

72. Hsieh PS, Chan JY, Shyu JF, Chen YT, Loh CH. Mild portal endotoxaemia induces subacute hepatic inflammation and pancreatic beta-cell dysfunction in rats. Eur J Clin Invest. 2008;38(9):640-8.

73. Mxinwa V, Dludla PV, Nyambuya TM, Mokgalaboni K, Mazibuko-Mbeje SE, Nkambule BB. Natural killer cell levels in adults living with type 2 diabetes: a systematic review and meta-analysis of clinical studies. BMC Immunol. 2020;21(1):51.

74. Li N, Liu F, Yang P, Xiong F, Yu Q, Li J, et al. Aging and stress induced β cell senescence and its implication in diabetes development. Aging (Albany NY). 2019;11(21):9947-59.

75. Hong H, Xu Y, Xu J, Zhang J, Xi Y, Pi H, et al. Cadmium exposure impairs pancreatic β-cell function and exaggerates diabetes by disrupting lipid metabolism. Environment international. 2021;149:106406.

76. Tekoh BN, Mbono-Samba EAE, Etoa-Etoga MC, Eko MA, Amazia F, Agoons BB, et al. Correlation between insulin-based and C-peptide based homeostatic model assessment of insulin resistance in adults without diabetes in a sub-Saharan African setting: a cross-sectional study. 2022;15(1):322.

77. Trefts E, Gannon M, Wasserman DH. The liver. Curr Biol. 2017;27(21):R1147-r51.

78. Heeren J, Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. Mol Metab. 2021;50:101238.

79. Ghanim M, Amer J, Salhab A, Jaradat N. Ecballium elaterium improved stimulatory effects of tissue-resident NK cells and ameliorated liver fibrosis in a thioacetamide mice model. Biomed Pharmacother. 2022;150:112942.

80. Thiele M, Johansen S. Progressive alcohol-related liver fibrosis is characterised by imbalanced collagen formation and degradation. 2021;54(8):1070-80.

81. Hashemi E, Malarkannan S. Tissue-Resident NK Cells: Development, Maturation, and Clinical Relevance. Cancers (Basel). 2020;12(6).

82. Kristiansen OP, Mandrup-Poulsen T. Interleukin-6 and diabetes: the good, the bad, or the indifferent? Diabetes. 2005;54 Suppl 2:S114-24.

83. Kim JH, Bachmann RA, Chen J. Interleukin-6 and insulin resistance. Vitamins and hormones. 2009;80:613-33.

84. Jin H, Ning Y, Zhou H, Wang Y. IL-6 Promotes Islet β-Cell Dysfunction in Rat Collagen-Induced Arthritis. J Diabetes Res. 2016;2016:7592931.

85. Bryceson YT, Fauriat C, Nunes JM, Wood SM, Björkström NK, Long EO, et al. Functional analysis of human NK cells by flow cytometry. Methods in molecular biology (Clifton, NJ). 2010;612:335-52.

86. Kang YJ, Jeung IC, Park A, Park YJ, Jung H, Kim TD, et al. An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression. Hum Reprod. 2014;29(10):2176-89.

87. Lee HS, Leem G. Peripheral natural killer cell activity is associated with poor clinical outcomes in pancreatic ductal adenocarcinoma. 2021;36(2):516-22.

88. Vargas E, Podder V, Carrillo Sepulveda MA. Physiology, Glucose Transporter Type 4. StatPearls. Treasure Island (FL): StatPearls Publishing



89. Piątkiewicz P, Bernat-Karpińska M, Miłek T, Rabijewski M, Rosiak E. NK cell count and glucotransporter 4 (GLUT4) expression in subjects with type 2 diabetes and colon cancer. Diabetology & metabolic syndrome. 2016;8:38.

90. Herman R, Kravos NA. Metformin and Insulin Resistance: A Review of the Underlying Mechanisms behind Changes in GLUT4-Mediated Glucose Transport. 2022;23(3).

91. Pellicciari C, Bottone MG, Biggiogera M. Detection of apoptotic cells by annexin V labeling at electron microscopy. European journal of histochemistry : EJH. 1997;41(3):211-6.

92. Aubry JP, Blaecke A, Lecoanet-Henchoz S, Jeannin P, Herbault N, Caron G, et al. Annexin V used for measuring apoptosis in the early events of cellular cytotoxicity. Cytometry. 1999;37(3):197-204.

93. Oh YS, Bae GD, Park EY, Jun HS. MicroRNA-181c Inhibits Interleukin-6-mediated Beta Cell Apoptosis by Targeting TNF-α Expression. Molecules (Basel, Switzerland). 2019;24(7).

94. Szatmary P, Grammatikopoulos T, Cai W, Huang W, Mukherjee R, Halloran C, et al. Acute Pancreatitis: Diagnosis and Treatment. 2022;82(12):1251-76.

# Appendices

## Appendix A

## Liver Histological Evaluation

| Item | Definition | Score/Code |
|---|---|---|
| Steatosis | | |
| Grade | Low- to medium-power evaluation of parenchymal involvement by steatosis | |
| | <5% | 0 |
| | 5%-33% | 1 |
| | >33%-66% | 2 |
| | >66% | 3 |
| Location | Predominant distribution pattern | |
| | Zone 3 | 0 |
| | Zone 1 | 1 |
| | Azonal | 2 |
| | Panacinar | 3 |
| Microvesicular steatosis* | Contiguous patches | |
| | Not present | 0 |
| | Present | 1 |
| Fibrosis | | |
| Stage | | |
| | None | 0 |
| | Perisinusoidal or periportal | 1 |
| | Mild, zone 3, perisinusoidal | 1A |
| | Moderate, zone 3, perisinusoidal | 1B |
| | Portal/periportal | 1C |
| | Perisinusoidal and portal/periportal | 2 |
| | Bridging fibrosis | 3 |
| | Cirrhosis | 4 |
| Inflammation | | |
| Lobular inflammation | Overall assessment of all inflammatory foci | |
| | No foci | 0 |
| | <2 foci per 200× field | 1 |
| | 2-4 foci per 200× field | 2 |
| | >4 foci per 200× field | 3 |
| Microgranulomas | Small aggregates of macrophages | |
| | Absent | 0 |
| | Present | 1 |
| Large lipogranulomas | Usually in portal areas or adjacent to central veins | |
| | Absent | 0 |
| | Present | 1 |
| Portal inflammation | Assessed from low magnification | |
| | None to minimal | 0 |
| | Greater than minimal | 1 |
| Liver cell injury | | |
| Ballooning* | | |
| | None | 0 |
| | Few balloon cells | 1 |
| | Many cells/prominent ballooning | 2 |
| Acidophil bodies | | |
| | None to rare† | 0 |
| | Many | 1 |
| Pigmented macrophages | | |
| | None to rare† | 0 |
| | Many | 1 |
| Megamitochondria* | | |
| | None to rare† | 0 |
| | Many | 1 |

# Appendix B

# Approval from Faculty of Graduate Studies



Reload Page

**نموذج تحديد عنوان الأطروحة و المشرف**

*** يجب توفر جميع الشروط التالية لتحديد عنوان الأطروحة و المشرف :
- أن يكون مسار الطالب أطروحة ** الشرط متحقق **
- أن يتم الطالب 12 ساعة . ** الشرط متحقق ** ** عدد الفصول أقل أو يساوي 4 **
- أن لا يكون الوضع الدراسي للطالب "مفصول من البرنامج". ** الشرط متحقق **
- المعدل التراكمي للطالب أكبر أو يساوي من 2.8 ** الشرط متحقق **

| | | | |
|---|---|---|---|
| اسم الطالب : | ديانا غانم عدنان ابو عره | رقم التسجيل : | 11952620 |
| اسم البرنامج : | ماجستير الكيمياء الحيوية السريرية | مسار الدراسة: | أطروحة |
| عدد الساعات المعتمدة التي انجزت حتى الان: | 30 | المعدل التراكمي: | 3.39 |
| الوضع الدراسي : | ترك | | |
| عنوان الطالب : | عقابا-طوباس | رقم الهاتف المحمول : | 0597159506 |
| البريد الالكتروني : | dianaabuarra78@gmail.com | | |
| لغة الرسالة : | انجليزي | | |
| عنوان الأطروحة باللغة العربية : | تغيرات الخلايا المناعية البنكرياسية في نموذج الفئران الحيوانية لتليف الكبد | | |
| عنوان الأطروحة باللغة الانجليزية: | Pancreatic Immune Cell Alterations in an Animal Mice Model of Liver Fibrosis | | |
| النسخة الإلكترونية من مقترح الاطروحة : | doc.11952620-2 | | |

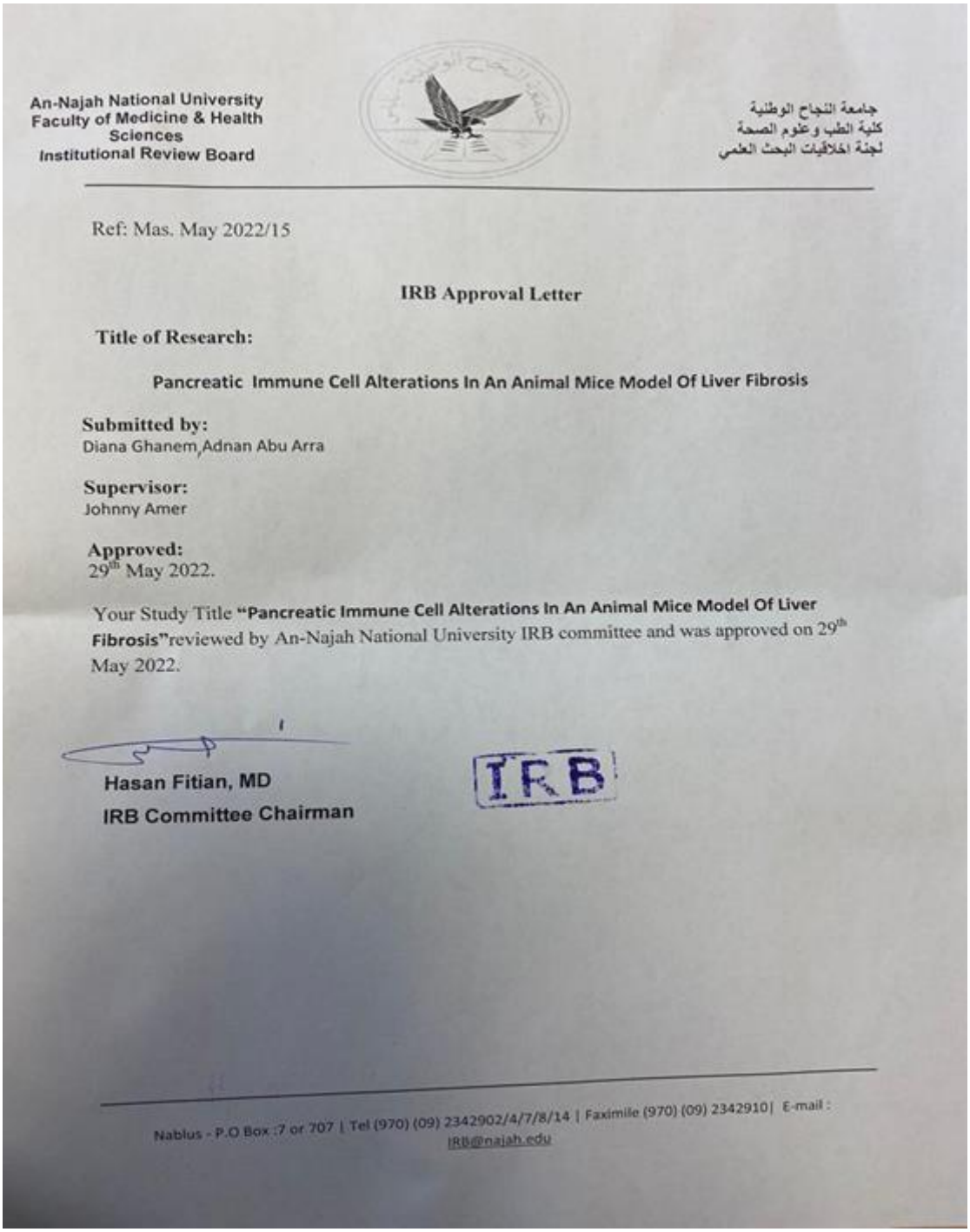
| | | | |
|---|---|---|---|
| رقم المشرف الأول : | 3586 | اسم المشرف الأول: | جوني يعقوب نصري عامر |
| المشرف الثاني : | يعمل في جامعة النجاح: نعم<br>رقم المشرف الثاني: 3586<br>اسم المشرف : جوني يعقوب نصري عامر<br>رتبة المشرف : استاذ مساعد | | |

| | | | | | |
|---|---|---|---|---|---|
| ملاحظة المشرف : | nothing | | | التاريخ : | 2022-05-11 |
| ملاحظة المنسق : | موافق | رأي المنسق : | موافق | التاريخ : | 2022-05-16 |
| ملاحظة رئيس القسم : | موافق | رأي رئيس القسم : | موافق | التاريخ : | 2022-05-17 |
| ملاحظة مدقق الدراسات : | تعرض على مجلس الكلية | رأي مدقق الدراسات : | موافق / عبء الاشراف : أدنى من الحد | التاريخ : | 2022-05-17 |
| ملاحظة عميد الدراسات العليا : | لا مانع | رأي عميد الدراسات العليا: | موافق | التاريخ : | 2022-05-17 |

| قرار مجلس الكلية | | | | |
|---|---|---|---|---|
| تم تغيير العنوان من قبل مجلس الكلية : | لا | | | |
| عنوان الأطروحة باللغة العربية : | تغيرات الخلايا المناعية البنكرياسية في نموذج الفئران الحيوانية لتليف الكبد | | | |
| عنوان الأطروحة باللغة الانجليزية: | PANCREATIC IMMUNE CELL ALTERATIONS IN AN ANIMAL MICE MODEL OF LIVER FIBROSIS | | | |
| رقم المشرف: | 3586 | اسم المشرف: | جوني يعقوب نصري عامر | |
| المشرف الثاني : | يعمل في جامعة النجاح: ---------- | | | |
| فصل الاعتماد : | الثاني | سنة الاعتماد : | 2021 ** ملاحظة : مثال العام الدراسي 2021-2022 يتم ادخاله على شكل 2021 | |
| رقم جلسة الكلية: | 418 | | | |
| تاريخ جلسة الكلية: | 15/5/2022 | | | |

# Appendix C

# IRB approval



An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref: Mas. May 2022/15

**IRB Approval Letter**

**Title of Research:**

**Pancreatic Immune Cell Alterations In An Animal Mice Model Of Liver Fibrosis**

**Submitted by:**
Diana Ghanem, Adnan Abu Arra

**Supervisor:**
Johnny Amer

**Approved:**
29th May 2022.

Your Study Title **"Pancreatic Immune Cell Alterations In An Animal Mice Model Of Liver Fibrosis"** reviewed by An-Najah National University IRB committee and was approved on 29th May 2022.

**Hasan Fitian, MD**
**IRB Committee Chairman**

IRB

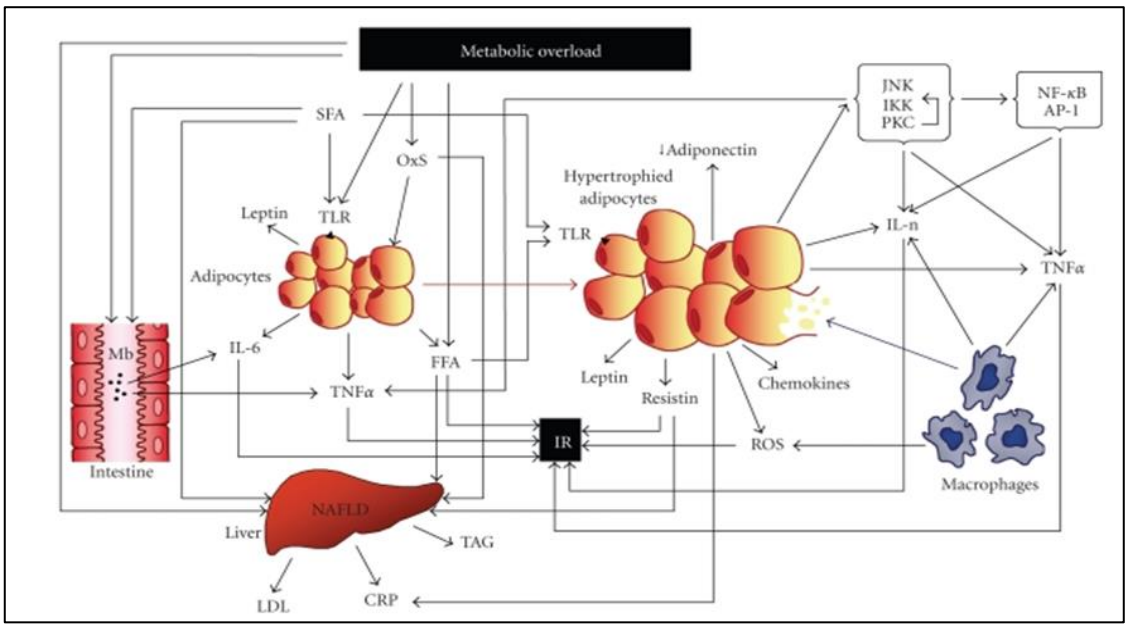
Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910| E-mail : IRB@najah.edu

## Appendix D

## Figures

**Figure 4**

*Overview of the complex interplay between obesity-inflammation-metabolic syndrome*




Adipose tissue is affected by metabolic overload, which causes organelle stress, adipokine and ROS generation, and kinase activation that amplifies the transcription of inflammatory genes and obstructs insulin communication. Adipocyte rupture caused by hyperthrophy attracts and activates macrophages, which significantly intensify the inflammatory process by generating more reactive oxygen species (ROS) and inflammatory cytokines. Adiponectin is produced at a lower level. The overabundance of adipose tissue and increased FFA concentration, or SFA, Adipocytes ensconced within a constrictive ECM “shell” lose their proper functions within fat pads and become more prone to necrosis , Adipose tissue with compromised metabolism often exhibits fibrosis, so FFA can build up in the liver and other organs(17).

جامعة النجاح الوطنية
كلية الدراسات العليا

# تغيرات الخلايا المناعية البنكرياسية في نموذج الفئران الحيوانية لتلف الكبد

إعداد
ديانا أبو عرة

إشراف
د. جوني عامر

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في برنامج الكيمياء الحيوية السريرية بكلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس- فلسطين.

2023

# تغيرات الخلايا المناعية البنكرياسية في نموذج الفئران الحيوانية لتلف الكبد

**إعداد**

**ديانا أبو عرة**

**إشراف**

**د. جوني عامر**

## الملخص

**الخلفية:** تليف الكبد مرتبط بأمراض التمثيل الغذائي ويعتبر عامل خطر للإصابة بالتهاب البنكرياس الحاد والزيادة من شدته. ان الهدف الرئيسي لهذا البحث هو فحص تأثير تليف الكبد على تعديل الخلايا القاتلة الطبيعية في البنكرياس والتغيرات التي تحدث على المسارات الجزيئية في نموذج الفئران لإصابة الكبد.

**المنهاجية:** تم تحفيز حدوث تضرر الكبد في الفئران من خلال حقن رابع كلوريد الكربون، تم استخدام نموذجين: نموذج اصابة حاده (أسبوعين) ونموذج ذو اصابة مزمنة (أربعة أسابيع). تم تخدير الفئران وجمع السيرم لتقييم انزيمات الكبد وتقييم تضرر البنكرياس من خلال الأميليز والليباز. فحص موت الخلايا المبرمج لخلايا بيتا من خلال فحص annexin V وتم فحص مقاومة الأنسولين-الببتيد C ومعدل السكر في الدم وفحص نسيج الكبد واستخدامه لقييم حدوث الالتهاب والتليف. وتم فحص α-SMA و Collagen III من خلال تقنية (RT-PCR) وأيضًا تقييم مستويات IL-6 من خلايا بيتا البنكرياسية من تليف الكبد الساذج والحاد والمزمن. كما تم عزل الخلايا القاتلة الطبيعية المقيمة في أنسجة البنكرياس وتقييم نشاطها من خلال تقييم انتاجها لـ IFN-γ ومستقبلات IL-6.

**النتائج:** في النموذج المزمن لتليف الكبد، وجد ان هناك تدهور في خصائص نسيج الكبد مقارنه مع النموذج الحاد. كشفت مستويات مصل ALT وAST، بالإضافة إلى التقييمات الأيضية للكوليسترول والدهون الثلاثية والببتيد C وسكر الدم وفحوصات تقييم تليف الكبد، ووجدت الدراسة علاقة إيجابية ما بين هذه العوامل ودرجة تليف الكبد. هناك ارتفاع في إنزيمات البنكرياس في نموذج الفئران لتليف الكبد

وارتبطت بموت خلايا بيتا المبرمج. وجدنا علاقه عكسـية بارِزه ما بين مسـتويات IL-6 لخلايا بيتا وشـدة تليف الكبد وتسـببت في زيادة تعبيرات مسـتقبلات IL-6 على الخلايا القاتلة الطبيعية ، والتي أثرت جزئيًا على نشاطها و عدم قدرتها على إنتاج كميات كافية من IFN-γ.

**الخلاصة:** يؤدي تليف الكبد إلى اصابة البنكرياس، كما يتضح من ارتفاع مستويات الأميليز والليباز وزيادة موت خلايا بيتا المبرمج. هناك ارتباط بين تليف الكبد وحدوث خلل في التمثيل الغذائي للدهون والجلوكوز، مما قد يكون له آثار على صحة البنكرياس. استخدام مضادات IL-6 و/ اوR IL-6 قد يحسن من فعالية الخلايا القاتلة الطبيعية في البنكرياس والكبد كذلك ويؤخر من تطور تليف الكبد وكذلك التهاب البنكرياس.

**الكلمات المفتاحية:** البنكرياس؛ الخلايا القاتلة الطبيعية؛ تليف الكبد؛ نموذج الفئران.