NAMIBIA UNIVERSITY
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TITLE: THE EFFECT OF HYPERTENSION COMORBIDITY IN TYPE 2 DIABETES ON INFLAMMATION, CARDIOVASCULAR RISK AND HEPATORENAL FUNCTION

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DECLARATION

I, Ernst JACK GROENEWALD, hereby declare that the work contained in the thesis entitled **"**The effect of hypertension comorbidity in type 2 diabetes on inflammation, cardiovascular risk and hepatorenal function" is my own original work and that I have not previously in its entirety or in part submitted it at any university or other higher education institution for the award of a degree.

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DEDICATION

This thesis is dedicated to all the loved ones I lost during the COVID-19 pandemic.

Eva Jacoba Izaaks (Grandma),

Regina Groenewald (Grandma),

Petronella Dausab-Steenkamp (Mother-in-law),

Zane-Lea Drotsky (My friend),

As well as the ones I have not mentioned,

May you all rest in peace and remain close to my heart forever.

ABSTRACT

Background

The prevalence of non-communicable diseases such as type 2 diabetes mellitus (T2D) has rapidly increased over the years and has exerted a major burden on health systems worldwide. T2D is one of the top ten leading causes of global mortality driven by various T2D-related complications such as cardiovascular disease (CVD) and altered hepatorenal function. Notably, hypertension (HTN) is the most common comorbidity in T2D, with both being independent risk factors for CVD. Although the underlying pathophysiological mechanisms for this bi-directional relationship are multifactorial, chronic inflammation is implicated in the progression of T2D and the pathogenesis of its associated complications. Since HTN is at least in part, an immune-mediated inflammatory disorder, this study aimed at investigating its compounding effects in T2D on inflammation, cardiovascular risk and hepatorenal function.

Methodology

This was a descriptive cross-sectional study that randomly recruited a cohort of one hundred and sixteen (n = 116) outpatients with T2D that visited the Katutura Community Health Centre in Windhoek, Namibia, during the period of September 2020 till December 2020. The diagnoses of T2D were diagnosed by a registered and qualified clinician following the American Diabetes Association guidelines. Venous blood was collected into vacutainer tubes and used for the measurement of haematological and inflammatory indices, glucose and lipid profiles, and renal and liver function tests. Other parameters include the estimated glomerular filtration rate (eGFR), globulins, albumin-to-globulin (A/G) ratio, systemic immune-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR) ratio, and the atherogenic index of plasma (AIP) were calculated using standard formulas. The D'Agostino & Pearson test was used to test for normality and all data were expressed as either mean ± standard deviation (SD) or median and interquartile range [IQR] depending on their distribution. A two-tailed unpaired student's t-test was used to compare the means between the groups for all parametric data. In cases where the variances between the groups were unequal, a Welch's correction was performed. The results were recorded as mean ± SD. For all non-parametric data, the Mann Whitney U test was used, and the results were recorded as median and IQR. Multiple bivariate correlations were performed using Pearson's coefficient. A p-value of <0.05 was considered statistically significant.

Results

A total of 70 patients with a median age range of 43.71 to 49.49 years were included in this study. Half (50%) of these had T2D only (n=35) whist the remaining half had HTN comorbidity (T2D+HTN) (n=35) with a comparable gender distribution between these two groups. Patients with T2D+HTN were significantly older (p=0.0106) and had a longer duration of T2D (p=0.0212) than those with T2D. Although the body mass index (BMI) was comparable between the groups, both groups were overweight. Similarly, the fasting plasma glucose and glycated haemoglobin were comparable between the groups but were above the normal range despite being on various anti-diabetic treatments.

Patients with T2D+HTN had significantly higher inflammatory levels when compared to those with T2D only, denoted by elevated CRP levels (p<0.0001), ESR (p=0.0119), globulins (p=0.0040), NLR (p=0.0494), and SII (0.0298). The white cell indices were all comparable between the groups except for the absolute eosinophil count which was lower in the T2D+HTN group (p<0.0001). The assessment of the red cell indices revealed lower levels of red cell count (p=0.0364) and haematocrit (p=0.0272) coupled with increased red cell distribution width in the T2D+HTN group. Assessment of lipid profiles revealed elevated TG (p=0.0177) and LDL-C (p=0.0189) in the T2D+HTN group in comparison to the T2D group. The AIP was comparable between the groups, despite over two-thirds of the entire cohort being at intermediate to high risk of atherosclerosis. The liver function test showed significantly elevated levels of TP (p=0.0121), T-Bil (p=0.0080), D-Bil (p=0.0133), ALP (p=0.0176), and AST (p=0.0002) in the T2D+HTN group when compared to the T2D group. The assessment of renal function showed lower eGFR levels in the T2D+HTN group accompanied by higher blood urea (p=0.0056) in comparison with the T2D group. Other renal function tests were comparable between the two groups. A correlation analysis showed that CRP was directly associated with urea (r= 0.51, p=0.0078), T-Bil (r= 0.58, p=0.0020), AST (r= 0.50, p=0.0101), and ALP (r= 0.58, p=0.0021), while being indirectly associated with eGFR (r= -0.45, p=0.0203). Furthermore, eGFR was indirectly associated with T-Bil (r= -0.61, p=0.0006), D-Bil (r= -0.73, p=0.0030), AST (r= -0.54, p=0.0012), and ALP (r= -0.59, p=0.0003), while blood urea levels were directly associated with T-Bil (r= 0.94, p<0.0001), D-Bil (r= 0.94, p<0.0001), AST (r= 0.78, p<0.0001), and ALP (r= 0.89, p<0.0001).

Conclusion

Hypertension comorbidity in T2D is associated with exacerbated levels of inflammation and increased CVD risk. Moreover, it is congruent with altered renal and hepatic function. Since these pathophysiological changes are directly associated with inflammation, the use of therapeutic strategies that target and ameliorate inflammation in patients with T2D and HTN comorbidity may be beneficial in lowering cardiovascular risk and restoring hepatorenal function.

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ABBREVIATIONS

Terms/Acronyms/Abbreviations		Definition/Explanation
DM	–	Diabetes mellitus
T1D	–	Type 1 diabetes mellitus
T2D	–	Type 2 diabetes mellitus
HTN	–	Hypertension
T2D+HTN	–	Type 2 diabetes mellitus with comorbid hypertension
IGT	–	Impaired glucose tolerance
NAFLD	–	Non-alcoholic fatty liver disease
NASH	–	Non-alcoholic steatohepatitis
CKD	–	Chronic kidney disease
DKD	–	Diabetic kidney disease
CVD	–	Cardiovascular disease
CVE	–	Cardiovascular events
MACE	–	Major adverse cardiovascular events
RAAS	–	Renin-angiotensin-aldosterone system
IR	–	Insulin resistance
AT	–	Adipose tissue
SBP	–	Systolic blood pressure
DBP	–	Diastolic blood pressure
HbA_1c	–	Glycated haemoglobin
FPG	–	Fasting plasma glucose
CRP	–	C-reactive protein
NLR	–	Neutrophil-to-lymphocyte ratio
SII	–	Systemic immune-inflammation index
TNF-α	–	Tumour necrosis factor-alpha
IL	–	Interleukin
ESR	–	Erythrocyte sedimentation rate
RBC	–	Red blood cells
RDW	–	Red cell distribution width

WCC	–	White cell count
ROS	–	Reactive oxygen species
CETP	–	Cholesterol ester transfer protein
eGFR	–	Estimated glomerular filtration rate
HIF-1α	–	Hypoxia-inducible factor-1 alpha
MCP-1	–	Macrophage chemoattractant protein
TP	–	Total protein
ALB	–	Albumin
T-Bil	–	Total bilirubin
D-Bil	–	Direct bilirubin
GGT	–	Gamma-glutamyl transferase
ALP	–	Alkaline phosphatase
ALT	–	Alanine aminotransferase
AST	–	Aspartate-aminotransferase
LDH	–	Lactate dehydrogenase
FFA	–	Free fatty acids
VLDL	–	Very-low-density lipoprotein
TRL	–	Triglyceride-rich lipoprotein
TG	–	Triglycerides
TC	–	Total cholesterol
LDL-C	–	Low-density lipoprotein cholesterol
HDL-C	–	High-density lipoprotein cholesterol
sd-	–	Small dense
ox-	–	Oxidized

1 CHAPTER ONE: INTRODUCTION

1.1 Background

Diabetes mellitus (DM) is one of the non-communicable diseases that has become a severe global healthcare burden (Yang Wang & Wang, 2020). Currently, the global prevalence of DM is estimated to be 9.3% worldwide, with approximately 60% of these cases from developing countries (Williams et al., 2019). Furthermore, over 90% of DM cases are said to be type 2 diabetes (T2D) (Zhou et al., 2014). This high prevalence is attributed to rapid urbanisation and increased sedentary lifestyle in low-to-medium income countries (Adekanmbi et al., 2019). In fact, the prevalence of T2D in sub-Saharan Africa is at a staggering range of 2 - 15%, with Namibia estimated at 5.1% (Adekanmbi et al., 2019). Notably, patients with T2D are at a 2-4-fold increased risk of developing cardiovascular disease (CVD) (Al-Nozha et al., 2016). This risk is aggravated by the presence of comorbidities like hypertension (HTN). In fact, 71% of individuals with T2D have comorbid HTN (Ekoru et al., 2019). Chronic inflammation has been implicated in the pathogenesis of T2D and its related complications (Tsalamandris et al., 2019). These complications include hyperlipidaemia, liver diseases, chronic kidney disease (CKD), atherosclerosis, and CVD (Akın & Bölük, 2020). Indeed, chronic inflammation may provide a great deal of insight to uncover the underlying mechanisms responsible for the progression of T2D and its related complications.

Chronic inflammation is characterised by persistent immune activation and a 2-3 fold increase in circulating pro-inflammatory mediators such as cytokines, chemokines, and acute phase reactants tumour necrosis factor-alpha (TNF-α), interleukin (IL)-6, and C-reactive protein (CRP)) (Calçada et al., 2014; Pioli & de Faria, 2019). These mediators propagate lipid dysmetabolism by reducing lipoprotein lipase (LPL) activity, a protein responsible for triglyceride (TG) storage and clearance (Esteve et al., 2005). As a result, serum levels of TG and free fatty acids (FFA) are elevated and high-density lipoprotein cholesterol (HDL-C) levels are decreased, driving the pathogenesis of the non-alcoholic fatty liver disease (NAFLD), a disease highly prevalent in T2D as well as in HTN (Lonardo et al., 2018). The increased flux of FFAs to the liver causes a build-up of fat droplets within hepatic tissue. As a coping mechanism, the liver increases its secretion of TG, packaged in very-low-density lipoproteins (VLDL), into circulation. Increased TG levels in circulation stimulate the increased formation of small dense (sd) low-density lipoprotein (LDL) particles via the cholesterol ester transfer protein (CETP). Since sd-LDL particles are highly susceptible to oxidation by reactive oxygen species, they form oxidized (ox) - LDL particles that infiltrate vascular endothelial cells and in turn initiate atherogenesis. Furthermore, the risk of atherosclerosis is increased by constant immune activation, whereby there is an overproduction of reactive oxygen species (ROS) by white blood cells that induces oxidative stress, causing endothelial

dysfunction and may lead to the development of HTN (Ferrannini & Cushman, 2012; Lord et al., 2014). In addition, pro-inflammatory mediators like CRP and TNF-α have an overstimulation effect on the renin-angiotensin-aldosterone system (RAAS) resulting in an increase in vascular tone and, over time, HTN (Dinh et al., 2014). A constant increase in blood pressure (BP) impacts renal function and HTN has been linked as a cause a consequence of a decline in renal function (Vaes et al., 2015). Renal function is also adversely affected by chronic inflammation, whereby the systemic overload of pro-inflammatory mediators activates various transcription factors in the kidney, leading to damage of the renal tubules and capillaries, therefore slowing down the glomerular filtration rate (eGFR) (García-García, 2014). In addition, the kidneys are also a potent source of erythropoietin, a protein that is vital in the production of red blood cells (RBC) via erythropoiesis. Patients with CKD are prone to develop anaemia as a result of blunted erythropoiesis, low levels of RBCs and therefore poor oxygen delivery. Additionally, diabetic anaemia has been linked, not only to the pathogenesis of HTN but also to increased CVD risk.

Thus, it is evident that chronic inflammation is a key player in the pathogenesis of the metabolic derangement and organ dysfunction observed in patients with T2D. In this study, the impact of comorbid HTN in a T2D population was investigated in order to determine its influence on chronic inflammation and T2D-associated organ complications (liver and renal function). Moreover, it assessed CVD risk in patients with T2D.

1.2 Statement of the research problem

The high global prevalence of patients with T2D has become a major source of morbidity and mortality due to the T2D associated complications (Tancredi et al., 2015). This poses a major threat and burden to public healthcare (J. Liu et al., 2020). Recently, an undesired pro-inflammatory response has gained traction and has been linked to T2D-associated complications such as HTN, CVD, and worsening renal function (Sun et al., 2019a). Previous studies have implicated chronic inflammation in the development of HTN, altered lipid metabolism, liver dysfunction, renal disease, and increased CVD risk, however, the mechanisms involved are incongruent and are yet to be fully elucidated. Research towards understanding the involvement of chronic inflammation in the development and exacerbation of T2D-associated complications could provide new insights on the prevention or mitigation of such complications, allowing for early therapeutic interventions.

Notably, biomarkers of chronic inflammation like CRP, neutrophil-to-lymphocyte ratio (NLR), erythrocyte sedimentation rate (ESR), and systemic immune-inflammation index (SII) have been independently associated with increased risk of CVD and worsening renal function, consequently

mitigating T2D-associated complications comprises of more than effective glycaemic control (Borghetti et al., 2018). Therefore, this study sought to investigate the effect of comorbid HTN and T2D on the levels of inflammation, altered lipid metabolism, metabolic organ dysfunction and CVD risk in a T2D population.

1.3 Research questions

1. What is the effect of HTN comorbidity in T2D on inflammation?
2. Is renal and hepatic function altered in patients with T2D+HTN?
3. What is the cardiovascular risk stratification in patients with T2D?
4. Are there any associations between inflammatory markers, haematological indices, lipid profiles, liver, and renal function tests in patients with T2D+HTN?

1.4 Aim of the research

- To investigate the effect of HTN comorbidity in T2D on inflammation, cardiovascular risk, renal and liver functions.

1.5 Research objectives

- To measure biomarkers of inflammation (CRP, leukocyte count, red cell indices, platelet indices, and ESR), lipid metabolism (TG, LDL-C, HDL-C, and total cholesterol), liver function (total protein, albumin, total and direct bilirubin, alkaline phosphatase, gamma-glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase) and renal function (eGFR, creatinine, urea, sodium, and potassium) in patients with T2D+HTN.
- To determine the atherogenic risk of patients with T2D+HTN.
- To investigate the associations between inflammation, metabolism, and organ function in patients with T2D+HTN.

2 CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Approximately 415 million people worldwide are currently living with diabetes mellitus (DM) and the number is expected to rise to 693 million by 2045 (Kotwas et al., 2021). DM is one of the ten leading causes of mortality worldwide which can be attributed to its various complications like hypertension (HTN) and cardiovascular disease (CVD) (Abbafati et al., 2020). In fact, a staggering 55% of type 2 diabetes mellitus (T2D) associated deaths are related to CVD with a likelihood that at least 70% of patients with T2D will develop cardiovascular complications in their lifetime (Siasos, 2020). Emerging evidence linking T2D, and chronic inflammation has suggested that systemic low-grade inflammation may be an impactful contributor to the pathogenesis and progression of T2D and its related complications. Regardless, the failure of novel T2D treatment and management strategies to circumvent the continued rise in T2D prevalence and mortality creates an enthusiastic need for further research. In recent years the focus has shifted from T2D as a glucose centric condition to a chronic inflammatory disorder (Tsalamandris et al., 2019). Indeed, chronic inflammation has been associated with the risk of T2D incidence and worsening prognosis (Hameed et al., 2015).

Chronic inflammation in T2D disrupts biological pathways such as insulin signalling, lipid metabolism, immune activation, and vascular tonicity (Lonardo et al., 2018; Wiebe et al., 2019). These pathways are inextricably linked to organ function, therefore chronic inflammation in T2D results in organ dysfunction, especially implicating vital organs such as the liver, kidneys, vasculature, and cardiac function (Assulyn et al., 2020; Balta et al., 2016; Burhans et al., 2018; Lonardo et al., 2018; Maio et al., 2021). For instance, elevated pro-inflammatory mediators, such as tumour necrosis factor-alpha (TNF-α), disrupt insulin signalling in adipose tissue (AT) leading to altered lipid metabolism and increased circulating free fatty acids (FFA), classical features of T2D (Du Plessis et al., 2015). This FFAs in circulation builds up in the vascular tissue and promote atherosclerosis (Ghosh et al., 2017). Furthermore, FFAs are readily taken up by hepatic tissue and increase the lipid content of the liver producing fatty liver disease (Sushith et al., 2020). Other effects of increased FFAs in circulation can be seen in the kidneys where lipotoxicity damages renal tissue resulting in a decline in its ability to filtrate waste products from the bloodstream (Yamamoto et al., 2017). Indeed, renal lipotoxicity induces a pro-inflammatory response that leads to increased ROS formation, endothelial dysfunction, oxidative damage, and an over-reactive renin-angiotensin-aldosterone system (RAAS) (Escasany et al., 2019). The destructive effects of chronic inflammation are widespread and may be an effective therapeutic target in mitigating the increased risk of T2D associated complications. Moreover, beyond its negative

effects on the kidney, HTN is another common comorbidity of T2D with 85% of a T2D population presenting with HTN as well (Petrie et al., 2018).

Chronic inflammation is also involved in the pathogenesis and progression of primary HTN and specific markers of inflammation have been associated with the increased risk of developing HTN (Dinh et al., 2014). For instance, elevated levels of C-reactive protein (CRP) are associated with an overstimulated RAAS. Similarly, increased systemic immune-inflammation index (SII) and neutrophil-to-lymphocyte ratio (NLR) are associated with increased blood pressure (BP) (Saylik & Sarıkaya, 2021; Shrivastava et al., 2015). Although the levels of chronic inflammation have been studied in T2D and its comorbidities, the findings are incongruent and require further clarification. It stands to reason that chronic inflammation levels would increase additively as well as their adverse impact on metabolism and organ function in T2D and HTN as compared to T2D alone. Furthermore, such insights may provide new treatment strategies targeting inflammation in T2D to mitigate the risk of T2D related complications. In general, beyond defining DM, including clarifying its characteristic features, the current review explores the implications of chronic inflammation in the pathogenesis of T2D and its associated complications such as altered lipid metabolism, liver function, renal function, and CVD.

2.2 Defining diabetes mellitus and its characteristic features

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterised by chronic hyperglycaemia, inadequate insulin secretion, and decreased insulin sensitivity on insulin-sensitive tissues (Fonseca, 2009). The symptoms of hyperglycaemia often present as frequent headaches, sudden weight loss, difficulty in concentrating, polydipsia, polyuria, and fatigue (Asmat et al., 2016). The diagnostic criteria for DM include fasting plasma glucose (FPG) of ≥7.0 mmol/l (126mg/dl) or 2-hour plasma glucose of ≥11.1 mmol/l (200mg/dl) (See Table 2.1). It is also recommended that glycated haemoglobin (HbA_1c) result of ≥ 6.5% or 48 mmol/mol is indicative of DM (American Association of Diabetes, 2021).

Table 2.1: Diagnostic Criteria of Diabetes Mellitus (Compiled by author, based on the work WHO, 2019)

1. FPG > 7.0mmol/l (126mg/dl). Fasting is defined as no caloric intake for at least 8 hours.
Or
2. 2-hour plasma glucose of ≥ 11.1mmol/l (200mg/dl) during an oral glucose tolerance test (OGTT).

Diabetes mellitus can be classified into various categories, namely type 1 diabetes mellitus (T1D), T2D, gestational diabetes, and other types due to specific causes such as monogenic diabetes, infection-related diabetes, and other genetic syndromes associated with diabetes (Solis-Herrera et al., 2018; World Health Organization, 2019). Apart from the above mentioned, another group has been identified that fall short of the diagnostic criteria of DM, yet their plasma glucose levels are above what is considered normal. They are generally known as individuals with prediabetes or impaired glucose tolerance (IGT).

2.2.1 Prediabetes / impaired glucose tolerance

Impaired glucose tolerance is characterised by an HbA_1c level between 5.7–6.4% (39–47 mmol/mol) or a consistent FPG of 5.6–6.9 mmol/l (100–125 mg/dl) (American Association of Diabetes, 2021). Prediabetes is associated with obesity, dyslipidaemia, and insulin resistance (Luc et al., 2019). Individuals with prediabetes are at increased risk of developing overt DM, however, the intervention of lifestyle modifications such as a low-fat diet, regular exercise and behavioural modifications can reduce the incidence of diabetes by 58% (Mascagni & Simonov, 2002).

2.2.2 Type 1 diabetes

Type 1 diabetes mellitus (T1D), also known as insulin-dependent diabetes, is the most severe form that is often diagnosed early in life (von Scholten et al., 2021). The pathological feature of T1D is the autoimmune attack against the pancreatic beta-cells which results in diminished or total lack of endogenous insulin secretion (Ziegler et al., 2019). As beta-cell death ensues and their number declines, patients with T1D become more dependent on exogenous insulin treatment for survival. Type 1 diabetes is beyond the scope of this review and will not be covered further.

2.2.3 Type 2 diabetes

Type 2 diabetes (T2D) is the most common form of DM accounting for more than 90-95% of diabetic cases worldwide (Henning, 2018). This type is characterised by both insulin resistance (IR) and pancreatic β-cell dysfunction (Tsalamandris et al., 2019).

The pathogenesis of T2D is driven by IR and pancreatic β-cell dysfunction, conditions that are most commonly triggered by a combination of genetic, behavioural, and environmental factors (Sun et al., 2019b). This results in insufficient suppression of hepatic glucose production and a decreased glucose

clearance from circulation (Ozougwu, 2013). Circulating plasma glucose levels are highly regulated since chronic hyperglycaemia may compromise the survival and functionality of cells, tissues and major organs (KAKU, 2010). Therefore, insulin, a peptide hormone, regulates glucose concentrations in plasma and interstitial tissues (Kahn et al., 2014). However, in T2D, inadequate insulin secretion, as well as reduced sensitivity to insulin action, leads to increased glucose concentrations in circulation bringing about a hyperglycaemic state that, if not rectified, can cause severe tissue damage (KAKU, 2010). This damage is notable in diseases such as nephropathy, neuropathy, retinopathy, and peripheral artery disease. The main driver of hyperglycaemia is IR, a condition where cells become less responsive to the action of insulin and unable to absorb circulating glucose (Shimobayashi et al., 2018). Insulin resistance is more prevalent in obesity and may occur before the onset of T2D (Wu & Ballantyne, 2020). In addition, chronic inflammation in T2D is induced by chronic hyperglycaemia and in the setting of an obese state (Antonopoulos & Tousoulis, 2017; Pradhan, 2001). Understanding the mechanisms involved may give insight into the pathogenesis of T2D and the progression towards its related complications. Moreover, chronic inflammation provides a great deal of insight to uncover the underlying mechanisms responsible for the progression of T2D and its related complications (B. Patel et al., 2015).

2.3 A general overview of inflammation

Inflammation is an essential protective biological immune response, localized to the site of injury or infection, to facilitate healing, tissue repair and the restoration of homeostasis (L. Chen et al., 2018). Once activated, an inflammatory response results in increased vasodilation and vascular permeability enabling the migration of immune cells and pro-inflammatory mediators to the site of injury (Nyambuya et al., 2019). The type and degree of inflammation is dependent on the trigger and its duration (Medzhitov, 2010). The clinical presentation of inflammation is swelling, redness, heat, and pain, however, these symptoms are not always visible when inflammation occurs sub-clinical (Burhans et al., 2018). Generally, inflammation can be divided into two phases, namely the acute phase and the chronic phase. Acute inflammation is the first and immediate immune response by the innate immune system that is short-lived and subsides after neutralisation of the threat and restoration of homeostasis (Das et al., 2019).

The inflammatory response is regulated by the balance between pro- and anti-inflammatory cells and mediators, the latter being responsible for deactivating the inflammatory response, clearing debris, and discarding waste products (Burhans et al., 2018) (See Figure 2.1). When the equilibrium between pro- and anti-inflammatory cells and mediators are disrupted by a continuous stimulus, the inflammatory response is constantly triggered and prolonged, turning acute inflammation into chronic inflammation. Chronic inflammation has been linked to many chronic diseases such as dyslipidaemia,

T2D, and HTN (Tsalamandris et al., 2019; Wiebe et al., 2019). In fact, it has been debated whether or not chronic inflammation is the driver of the progression of T2D and its associated complications.

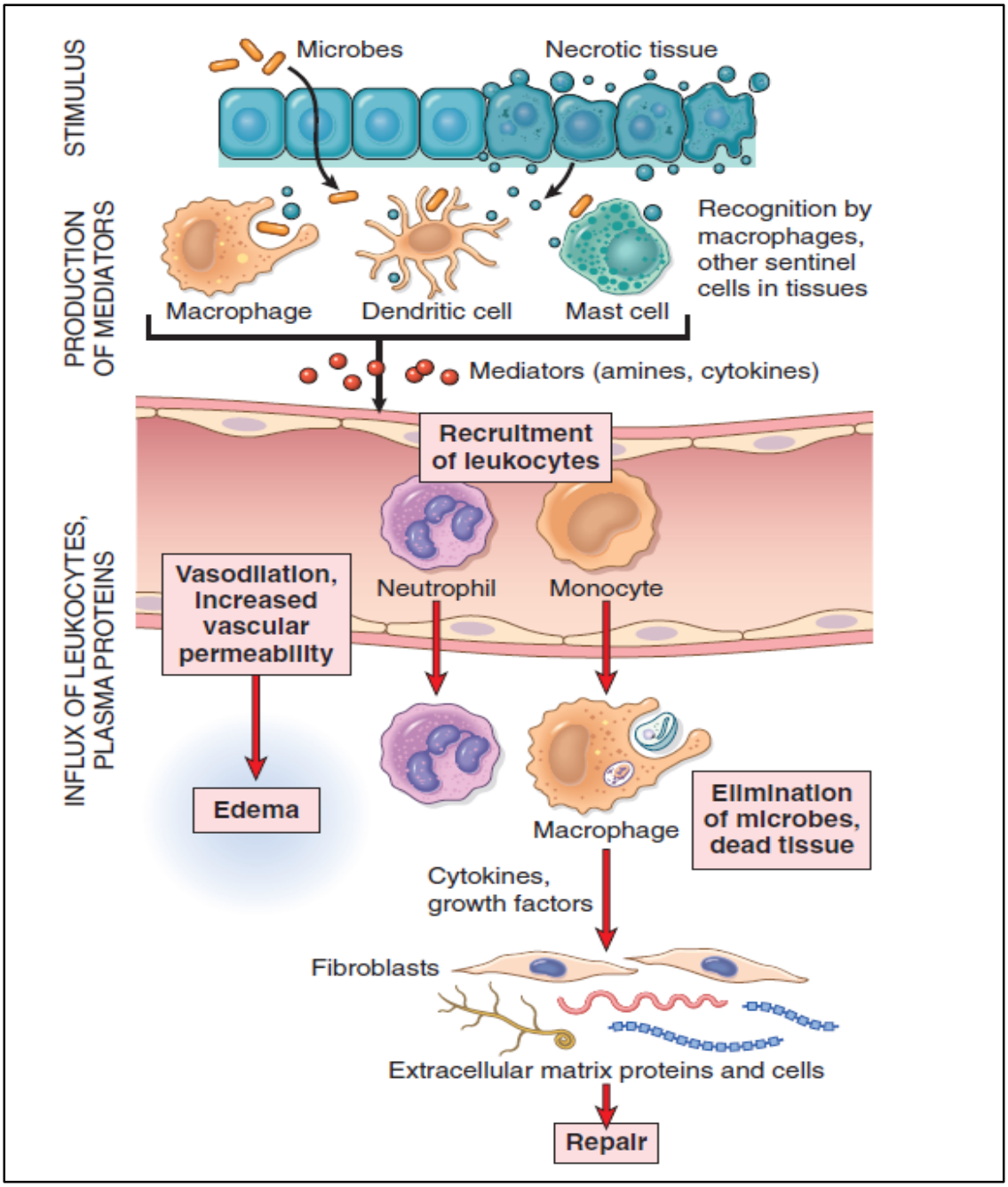


Figure 2.1: Sequence of events in an inflammatory response. Macrophages and other immune cells recognize a stimulus and release pro-inflammatory cytokines and chemokines. As a result, vasodilation and increased vascular permeability allow for the migration of leukocytes and plasma proteins to the site of inflammation. Further production of cytokines and growth factors leads to tissue repair and restoration of homeostasis. (Adapted from Kumar et al., 2018)

2.3.1 The effects of chronic inflammation in type 2 diabetes mellitus

Chronic inflammation is a long-term reaction to inflammatory stimuli that is characterised by continuous activation of immune cells long after infection or injury (L. Chen et al., 2018). It is most commonly low-grade, thus going unnoticed until its damaging effects manifests clinically. Biochemically, it is presented by elevated levels of pro-inflammatory immune cells and mediators. These mediators include cytokines and adipokines such as adiponectin, leptin, interleukin (IL) -6, IL-1β and TNF-α, and chemokines such as macrophage chemoattractant protein (MCP) -1 (Hameed et al., 2015). Another pro-inflammatory mediator, CRP, is an acute-phase reactant that is strongly upregulated by IL-6 and TNF-α. While circulating IL-6 and TNF-α stimulate the hepatic synthesis and secretion of CRP, it can also be produced locally in lymphocytes and monocytes during the formation of atherosclerotic lesions (Shrivastava et al., 2015).

C-reactive protein has been accepted as a reliable biomarker of inflammation and an independent predictor of CVD risk with levels >3.00 mg/L associated with worse cardiovascular prognosis in clinical practice (Stephen et al., 2012). In fact, CRP levels above normal at baseline is strongly associated with major cardiovascular events independent of other CVD risk factors like blood pressure, lipids and glycaemic status (Cardoso et al., 2016). Elevated CRP levels in T2D has been widely reported, even more so during hyperglycaemia and in obesity (Chang & Yang, 2016). Moreover, elevated CRP levels have been positively associated with increased FPG and HbA_1c levels (Seo & Shin, 2021). Another marker of inflammation, erythrocyte sedimentation rate (ESR) has also been associated with the glycaemic status of patients with T2D as well as a strong predictor of increased future major adverse cardiovascular events (MACE) risk (Charles-Schoeman et al., 2019). The value of ESR as a marker of underlying chronic inflammation has also been studied in patients with T2D, however, variable controversies exist as to its value in indicating underlying inflammation in T2D. Furthermore, in the studies that have examined the levels of ESR in a T2D population, contradictory evidence exists. For instance, one study showed that ESR levels were elevated in patients with T2D compared to non-diabetics, whilst another showed no such difference (Elimam et al., 2019; Owiredu et al., 2018). The reason for the incongruent results may be due to the low number of participants enrolled in these studies. Further investigation as to the levels of ESR in T2D may elucidate this controversy. In addition, increased inflammation is accompanied by a rise in immune activation characterised by an elevated leukocyte count.

The involvement of leukocytes in the progression of T2D associated complications lie in their ability to initiate endothelial dysfunction that leads to atherosclerotic disease (Maio et al., 2021). Specifically,

macrophages are central to the formation of atherosclerotic plaque formation whereby they form proatherogenic foam cells from the uptake of oxidised or glycated metabolites. These foam cells are embedded into the vascular wall and later form atherosclerotic lesions or plaques (Soran et al., 2016). Furthermore, neutrophils have an affinity to infiltrate such plaques and cause their dislodgement into circulation (Balta et al., 2016). The evidence concerning leukocyte levels in T2D are variable with some studies reporting elevated levels whilst others do not (Harish Kumar et al., 2017; Umeji et al., 2019). A more accurate marker of systemic inflammation is the NLR which combines the neutrophil count with the lymphocyte count. In fact, NLR has been independently associated with T2D and HTN and, even with a leukocyte count within the normal range, has been associated with atherosclerotic events (Balta et al., 2016).

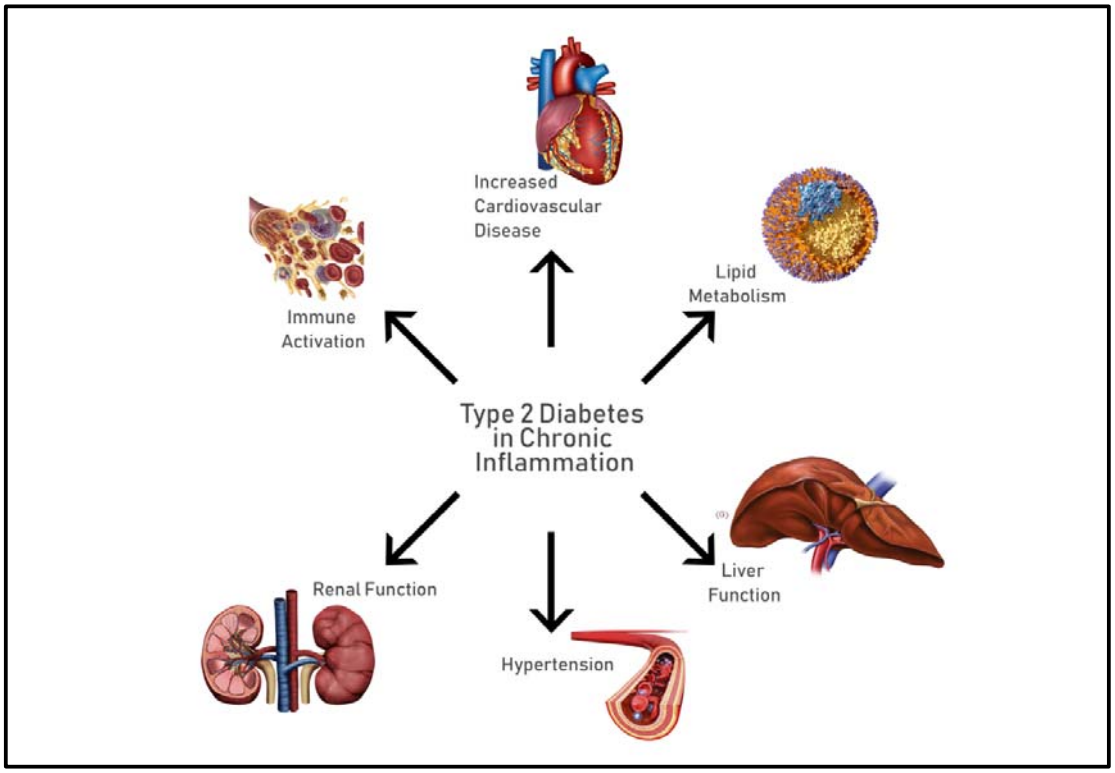


Figure 2.2: An overview of the impact of chronic inflammation in T2D on organ function and metabolic processes. Chronic inflammation is implicated in the complications associated with T2D. It alters lipid metabolism in the adipose tissue and in the liver. In addition, it triggers an inflammatory response in the liver that leads to various fatty liver diseases. In the kidneys, it leads to endothelial dysfunction, immune cell aggregation, oxidative stress, and glomerular damage. Similarly, in the vasculature, it activates the endothelial cells as well as the RAAS resulting in vascular damage and elevated blood pressure. Lastly, chronic inflammation has been linked to an increased risk of atherosclerosis and CVD (Created by the author).

A novel indicator of systemic inflammation, SII, has been developed in recent years that take into account the NLR and platelet counts (J. Xu et al., 2021). Initially, it was used as a prognostic marker in patients with hepatocellular carcinoma (HCC), however, its use has since been extended to include

determining target organ damage and CVD risk (Saylik & Sarıkaya, 2021). Its value in determining CVD disease risk is its association with carotid intima-media thickness, showing that it may be prognostic of atherosclerotic disease (Çırakoğlu & Yılmaz, 2021). Its use in research related to T2D is limited with only two studies published relating it to diabetic depression and the development of diabetic macular oedema (Elbeyli et al., 2021; Jie Wang et al., 2021). Since it is a reliable marker for assessing CVD risk, its use in assessing T2D related complications may provide further insight into the involvement of chronic inflammation in such complications.

Evidently, chronic inflammation is involved in the pathogenesis and progression of T2D, however, the mechanisms involved are not yet clearly defined. Generally, two theories have been proposed to induce and perpetuate chronic inflammation in T2D, namely hyperglycaemia and obesity. Indeed, both hyperglycaemia and obesity have been associated with chronic inflammation independently.

2.3.2 The pathogenesis of chronic inflammation in type 2 diabetes

Hyperglycaemia is the hallmark of T2D and, if left untreated, leads to the accelerated formation and accumulation of advanced glycation end products (AGEs). When AGEs interact with their specific receptor, advanced glycation end products receptor (RAGE) on the surfaces of immune cells and tissues, the expression of pro-inflammatory cytokines are stimulated and the generation of reactive oxygen species (ROS) is upregulated (Daryabor et al., 2020). This ROS activates the transcription factor nuclear factor-κB (NF-κB), which further stimulates leukocytes to produce more ROS whereby its overproduction induces local and systemic oxidative stress (Petrie et al., 2018). The NF-κB increases the transcription of pro-inflammatory genes and so perpetuate the inflammatory response (L. Chen et al., 2018). In addition, increased levels of glucose also upregulate the expression of pro-inflammatory mediators like ROS, TNF-α, IL-6, and MCP-1 in mononuclear cells (Shi et al., 2019). Moreover, the free radical oxygen in ROS reduces the bioavailability of nitric oxide (NO), a potent vasodilator, which might explain the increasing prevalence of HTN among patients with T2D (Jabarpour et al., 2019).

The normal physiological response to elevated glucose levels is increased insulin secretion that stimulates cells, such as adipose tissue (AT), hepatocytes, and muscle cells, to take up glucose for storage or as an energy source (Galicia-Garcia et al., 2020). Though, in T2D, these cells become IR. Chronic inflammation results in elevated pro-inflammatory responses and elevated TNF-α and IL-6. These two cytokines are overexpressed in AT and muscle cells where they disrupt the insulin signalling cascade by downregulating genes essential for insulin action and increased serine phosphorylation of

insulin receptor substrate-1 (IRS-1) (Shi et al., 2019). When cells become unresponsive to the action of insulin, glucose can't enter. This is known as insulin resistance and results in obesity as adipocytes expand beyond normal (See Figure 2.3).

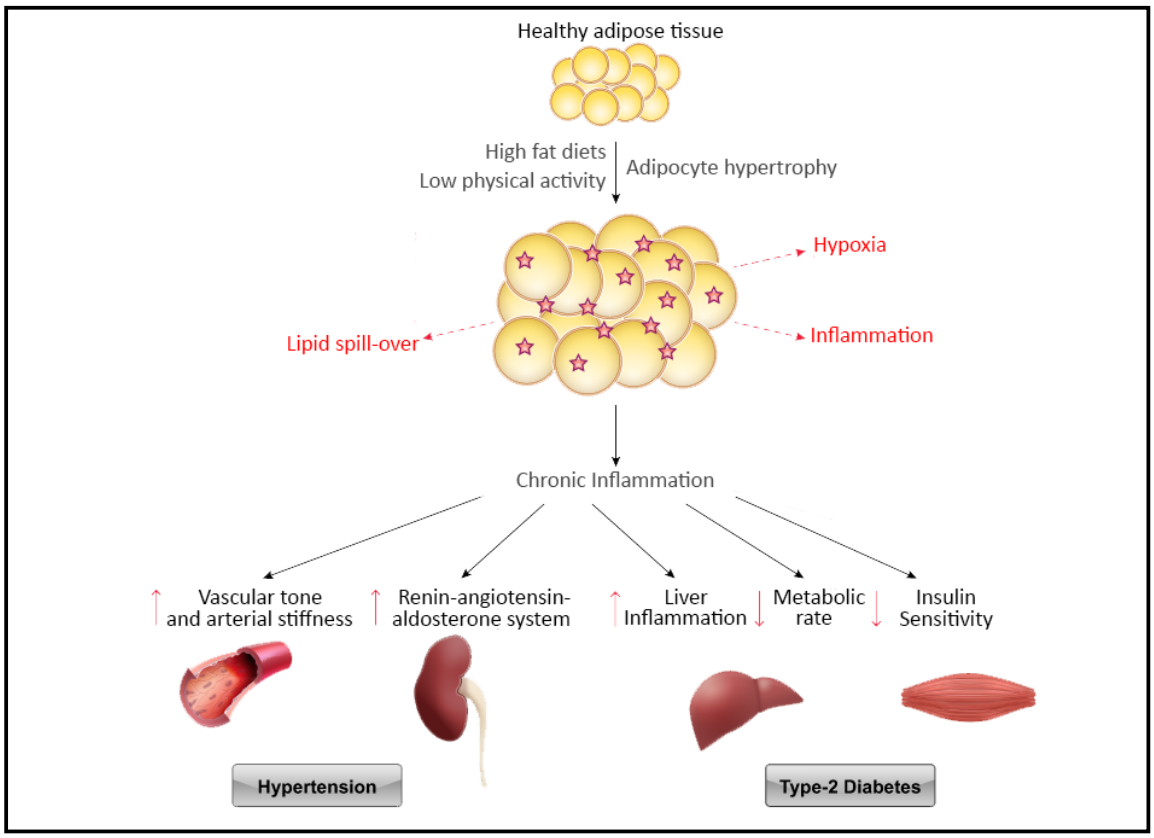


Figure 2.3: The process of adipose tissue induced inflammation and its effects. Over-nutrition, a high-fat diet and decreased physical activity led to the disproportionate expansion of adipocytes. This hypertrophy coupled with inadequate vasculature and limited blood supply leads to tissue hypoxia. Hypoxia triggers inflammation by the release of TNF-α and IL-6 from resident macrophages. These two cytokines facilitate the recruitment and accumulation of other leukocytes that produce reactive oxygen species that cause oxidative damage to surrounding tissues. The inflammatory response becomes chronic because the persistent stimulus is persistent. The result of adipose tissue induces chronic inflammation is increased vascular tone and arterial stiffness as well as increased RAAS activity that may develop into overt hypertension. Another consequence is increased liver inflammation, reduced metabolic rate and increased IR. (Adapted from Burhans et al., 2018)

Obesity occurs as a result of an imbalance between calorie intake and energy expenditure, leading to cellular stress and decreased metabolic flexibility within adipocytes (Calçada et al., 2014). Adipose tissue becomes dysfunctional with disproportionate enlargement of AT in the subcutaneous tissue and the deposition of ectopic fat on vital body organs such as the liver and heart (Dewidar et al., 2020). As AT hypertrophy sets in, inadequate vasculature and limited oxygen supply cause tissue hypoxia and eventually fibrosis and necrosis (Trayhurn, 2013). The inflammatory process is initiated by AT release of pro-inflammatory adipokines like TNF, IL-6, HIF-1-α, and chemokines that facilitate the recruitment

and accumulation of leukocytes to the site of inflammation (R. Liu & Nikolajczyk, 2019). Leukocytes are activated and amplify inflammation by releasing more cytokines, especially TNF and IL-6, which spill over into the general circulation where they promote systemic inflammation (Teichert et al., 2014). The consistent exposure to the trigger, that being AT expansion, hypertrophy, and hypoxia, turns an acute inflammatory response into chronic inflammation in the AT.

The effects of chronic inflammation in T2D are far-reaching, affecting major metabolic pathways and organ systems. The degree or severity of such effects may be dependent on the duration of T2D and the presence of comorbid complications, such as HTN. The underlying pathogenic mechanisms involved may be attributed to elevated levels of CRP in circulation. This is evident in the absence of CRP in the vascular wall of healthy patients, however, CRP levels are elevated in the vascular wall of patients with atherosclerosis (Thiele et al., 2014). Furthermore, CRP induces endothelial dysfunction by inhibiting the action of endothelial nitric oxide (eNOS) therefore suppressing the production of nitric oxide (NO), a potent vasodilator. Nitric oxide protects the vascular wall from shear stress-induced damage resulting from increased blood flow (Maio et al., 2021). At reduced NO levels, increased blood flow leads to increased shear stress and vascular damage. Additionally, CRP promotes monocyte recruitment, increased ROS production, and exacerbation of inflammation that may lead to overt HTN (Teixeira et al., 2014) (see Figure 2.4). Several studies have implicated chronic inflammation in the pathology of hepatic and renal dysfunction, altered lipid metabolism, and increased immune activation that induces oxidative stress. The consequences are fatty liver disease, worsening renal function and CVD (See figure 2.3) (Dharmalingam & Yamasandhi, 2018; Patrick et al., 2021).

2.4 The pathophysiology of liver complications in type 2 diabetes mellitus

The critical role of the liver is found in its function as a regulator of energy homeostasis, with the ability to detect and react to imbalances in nutrition. A great deal of this regulation is based on its control over lipid and glucose metabolism. In T2D, both these processes are disrupted resulting in metabolic dysfunction (Sargsyan & Herman, 2019). The exact mechanisms responsible are not yet clear, nevertheless, chronic inflammation and IR have been implicated in the pathogenesis of liver diseases such as abnormal liver enzymes, cirrhosis, hepatocellular carcinoma, acute liver failure, and non-alcoholic fatty liver disease (NAFLD) (Tolman et al., 2007; Watt et al., 2019). Non-alcoholic fatty liver disease is highly prevalent in T2D with about 55% of patients with T2D are likely to develop NAFLD, even more so amongst obese individuals with T2D (Younossi et al., 2019). In obesity, the lipid metabolism is altered and the hepatic involvement may be clarified by its function as a regulator of lipid metabolism, both within hepatic tissue and externally in other tissues (Farooque et al., 2021). In

addition, chronic inflammation alters lipid metabolism in the liver, muscle and AT through the increased expression of TNF-α (Makoveichuk et al., 2017).

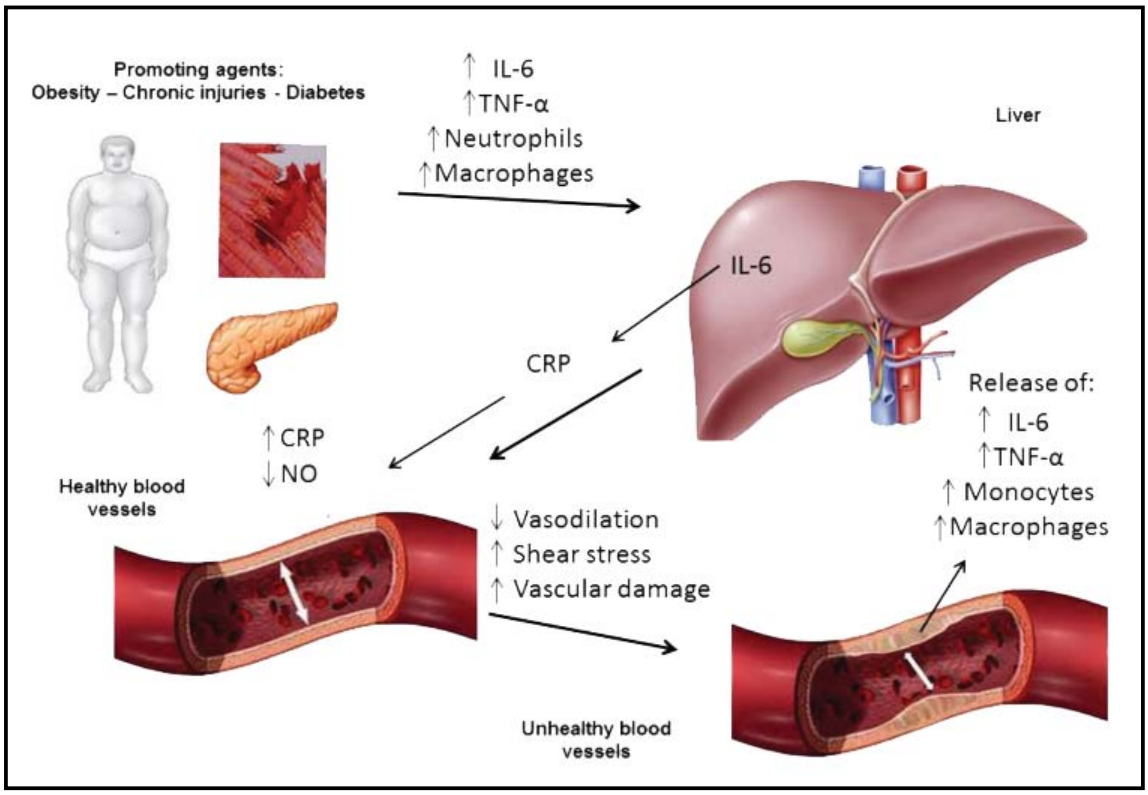


Figure 2.4: Aetiology of the inflammatory process. Low-level chronic inflammation increases the concentrations of markers and of inflammatory cells, leading to increased production of C-reactive protein (CRP) by the liver, in response to interleukin-6 (IL-6), which provokes a reduction in vasodilation and an increase in vascular damage. TNF-α – Tumour necrosis factor-alpha; IL-6 – Interleukin-6; CRP – C-reactive protein; NO – nitric oxide; ET-1 – Endothelin-1. (Adapted from Teixeira et al., 2014)

2.4.1 The link between altered lipid metabolism and chronic inflammation

Altered lipid metabolism in T2D is presented by reduced levels of high-density lipoprotein cholesterol (HDL-C) and elevated levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), commonly known as diabetic dyslipidaemia. In a study conducted in Ethiopia, 65% of a diabetic cohort presented with abnormal lipid levels and this was mirrored by several other studies. Levels of pro-inflammatory cytokines have been associated with abnormal lipid levels, thus implicating chronic inflammation in disrupting lipid metabolism. Specifically, one study found that elevated CRP has been positively associated with elevated TC, TG, and LDL-C, but not with HDL-C whilst another study found an inverse relationship between CRP and HDL-C (Rathore et al., 2017). The mechanisms involved are not clearly defined, however, TNF-α has been implicated in interfering with the lipolysis of triglyceride-rich lipoproteins (TRL) (Soran et al., 2016).

Chronically elevated TNF-α inhibits the action of endothelial lipoprotein lipase (LPL), an enzyme that catalyses the breakdown of TG from circulating TRLs to fatty acids for cellular utilization (Soran et al., 2016). Its inhibition results in increased concentration of TRLs, such as chylomicrons and very-low-density lipoproteins (VLDL), in circulation (Makoveichuk et al., 2017). Contrastingly, an intracellular enzyme, hormone-sensitive lipase (HSL), breaks down fats from intracellular lipid stores into free fatty acids (FFA) by hydrolysis and subsequently secretes them into the bloodstream (Hsiao et al., 2013). Hormone-sensitive lipase is regulated by insulin and is present during increased energy demands, however, in an IR state, HSL remains uninhibited and consistently increases circulating FFA concentrations (Mersmann, 1998). Furthermore, the increase of TRLs in the bloodstream upregulates the expression and activity of cholesterol ester transfer protein (CETP) (Hirano, 2018). Elevated TRL in circulation readily exchanges TGs for cholesterol esters from LDL-C and HDL-C via the CETP forming of small-dense (sd)-LDL and -HDL particles (Vaziri, 2014) (See figure 2.5).

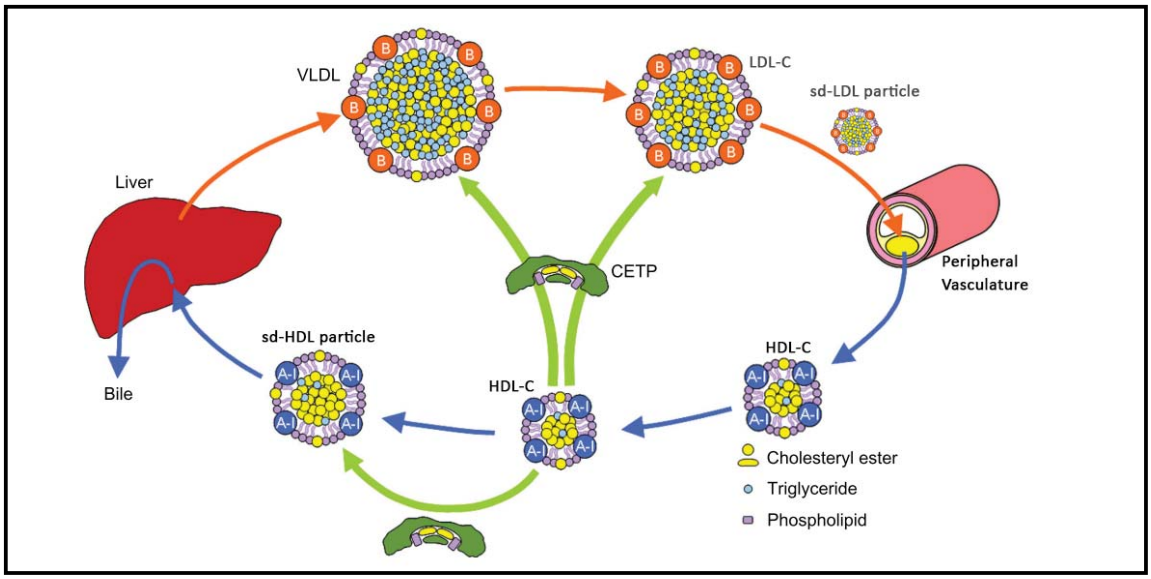


Figure 2.5: Cholesterol transport in the formation of sd-LDL and sd-HDL particles in a high triglyceride environment. The increase in circulating TRLs stimulates the increased expression and activity of CETP. In turn, CETP facilitates the exchange of triglycerides in TRLs for cholesterol from LDL-C and HDL-C to form sd-LDL and sd-HDL particles. The sd-HDL undergoes a conformational change rendering it dysfunctional and is swiftly removed from circulation by hepatic lipase whilst sd-LDL particles evade removal and infiltrate peripheral vasculature. (Adapted from Shinkai, 2012)

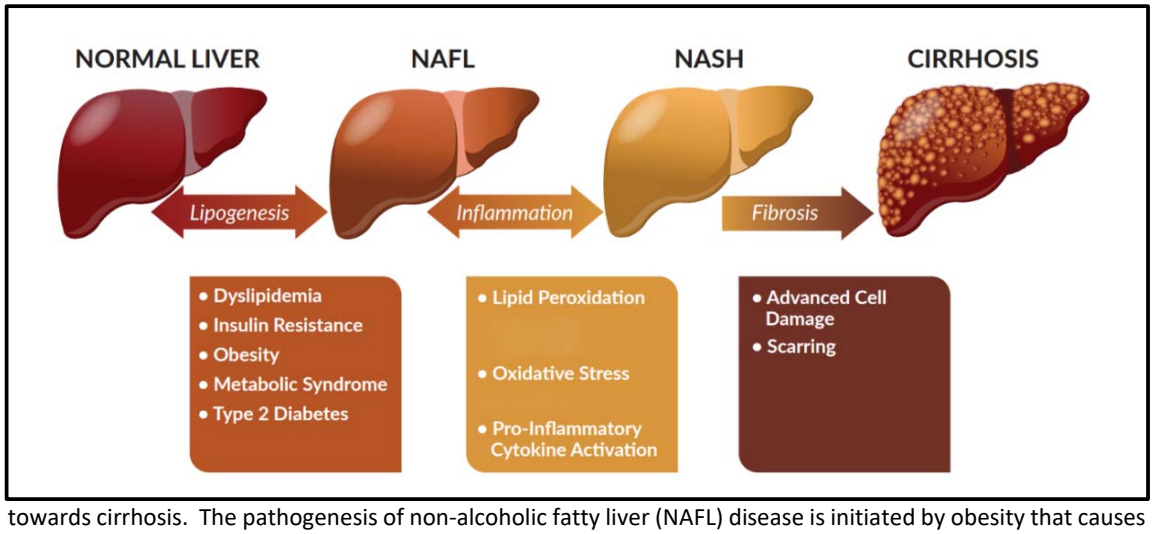
Small-dense HDL undergoes a conformational change that drastically decreases its anti-inflammatory and anti-oxidant properties (Hui et al., 2019). This dysfunctional sd-HDL are swiftly hydrolysed by hepatic lipase, catabolized and cleared from the plasma (Parhofer, 2011). Moreover, increased TG has been associated with reduced HDL-C supporting the increased activity of CETP with elevated TG levels (Dayimu et al., 2019). The sd-LDL particles are very vulnerable to glycation and oxidation after which they are taken up by macrophages to form foam cells, which is the basis of atherosclerosis (Soran et

al., 2016). Small dense-LDL and oxidised (ox)-LDL in circulation are embedded into vascular walls where they lead to endothelial activation and vascular inflammation. The atherogenic potential of sd-LDL and ox-LDL is also seen in patients with HTN, increasing their risk for CVD proportionally (Ivanova et al., 2017). Similarly, NAFLD is also a risk factor for CVD and about 50% of patients with HTN have NAFLD (Ma et al., 2021). Type 2 diabetes with comorbid HTN may have further destructive effects on liver function and CVD risk. Interestingly, it has been proposed that the increased CVD risk in T2D can be attributed to inadequate management of blood lipid levels and blood pressure control (Vazquez-Benitez et al., 2015). This requires further research and is yet to be elucidated.

2.4.2 The adverse effects of chronic inflammation and altered lipid metabolism on hepatic function in type 2 diabetes

Adipose tissue inflammation results in increased levels of TNF-α and FFAs in circulation, which aggravates IR in hepatic tissue. The mechanism whereby FFAs augment insulin signalling is by impairing the activation of the IRS-phosphoinositol-3 kinase, an enzyme in the insulin signalling cascade (Trayhurn, 2013). The main source of hepatic FFA comes from circulation where they are subsequently metabolised (Du Plessis et al., 2015). Free fatty acids in the liver are either oxidized for energy or re-esterified into TG for storage or exported in VLDLs (Dharmalingam & Yamasandhi, 2018).

Figure 2.6: The pathogenesis of fatty liver disease and the risk factors that influence the disease progression



towards cirrhosis. The pathogenesis of non-alcoholic fatty liver (NAFL) disease is initiated by obesity that causes hepatic insulin resistance. Consequently, lipid metabolism is altered leading to an accumulation of fats within hepatocytes. As fats accumulate within hepatic tissue, lipid peroxidation produces oxidative stress that triggers an inflammatory response marked by an increase of pro-inflammatory cytokines, therefore establishing non-alcoholic steatohepatitis (NASH). Oxidative damage coupled with exacerbated inflammation produces fibrotic tissue that proceed towards cirrhosis with advanced cellular damage and tissue scarring. (Adapted from Cayman Chemicals, 2017)

In T2D, this process is altered by an increased hepatic uptake of FFAs and its inability to sufficiently export FFAs in VLDLs. This impairment is perpetuated by increased TNF-α that stimulates hepatic de novo lipogenesis (Shi et al., 2019). Moreover, IR prevents the inhibition of gluconeogenesis, a process by which the liver converts non-carbohydrate substrates into glucose. The increased intrahepatic glucose further stimulates de novo lipogenesis (Titchenell et al., 2017). Consequently, lipids build up in hepatocytes forming and growing into fat droplets that overtake the cells. The build-up of excess hepatic fats is the result of an imbalance between FFA import, increased hepatic lipogenesis, and VLDL secretion and is known as hepatic steatosis (Ipsen et al., 2018). These fat droplets are highly susceptible to oxidation by ROS, creating an oxidative stress environment that damages cell membranes resulting in hepatocyte death which triggers an inflammatory response (Watt et al., 2019). In the absence of excessive alcohol consumption, steatosis and inflammation combined are called steatohepatitis and, if not treated, causes necrosis, fibrosis and later cirrhosis of hepatic tissue (Toshikuni et al., 2017). From the time of steatosis until cirrhosis, NAFLD can be diagnosed (See figure 2.6).

2.4.3 Liver enzymes as biomarkers in assessing hepatic disease and beyond

The most effective diagnostic criteria for hepatic injury or damage is a liver biopsy and an x-ray of the liver, however, these techniques are invasive and expensive (Berzigotti et al., 2021). There is a need for the development of more accessible techniques with high efficacy in assessing liver damage. In the laboratory setting, biochemical markers testing for liver function have been used and may present the best option in the absence of an x-ray or biopsy. These biomarkers are liver enzymes such as alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate-aminotransferase (AST), urea, and bilirubin (Berzigotti et al., 2021). Alanine aminotransferase and AST facilitate the metabolism of proteins and are released into the bloodstream at an increased level upon liver injury or damage (Sookoian et al., 2016). Furthermore, AST in combination with ALT has been associated with hepatocyte necrosis (Padda et al., 2011). It stands to reason that since the liver is a major regulator of glucose concentrations, elevated levels of AST and ALT would indicate liver damage and predict incident T2D over time.

Though previous studies have examined the relationship between these biomarkers and the pathogenesis of T2D, the evidence seems to be inconsistent (Shibabaw et al., 2019; Song et al., 2018). While some studies showed the predictive value of elevated ALT and AST in incident T2D, others found no such association (André et al., 2005; Fraser et al., 2009). Similarly, GGT had the same contradictory evidence (Vozarova et al., 2002; Wannamethee et al., 2005). Furthermore, ALT and GGT are suggestive of systemic and hepatic insulin resistance (Bonnet et al., 2011). Gamma-glutamyl transferase enzyme, mainly found in the liver at low levels, has also been observed to be elevated in liver disease, however,

just like AST and ALT, it is best used in combination with other liver function biomarkers. A possible explanation for these inconsistencies is that GGT, AST, and ALT have low specificity, since their increased levels may also indicate muscle damage or coronary injury (Song et al., 2018). In the past when direct measurement of liver fat accumulation by ultrasonography was not possible, elevated levels of AST, ALT and GGT as indicators of a fatty liver (Riley & Kahn, 2006). They may still be useful in developing countries where access to ultrasonography is not possible, however, continued research is required to elucidate their use in this manner. Low levels of urea as a biomarker has been observed in patients with end-stage chronic liver, however, its levels may be influenced by muscle mass, nutrition, and urea cycle disorders making it unreliable in assessing liver function in patients with other underlying illnesses (Majumdar & Pinzani, 2016).

Alkaline phosphatase, a liver enzyme mainly found in the liver and bone marrow, has also been reported to indicate liver injury, mainly damage to the biliary epithelial cells or canalicular membrane, however, its use in T2D has not been clarified (Fu et al., 2020). While there are studies indicating elevated levels of ALP in T2D, a causal relationship has not been established (Deepika et al., 2016). This makes the use of ALP in detecting liver injury non-specific since it may originate from either liver injury or bone disease.

Bilirubin is a liver enzyme produced by the catabolism of heme in the heme oxygenase (HO) system. The HO system is a catabolic process that clears waste products produced by the destruction of aged or atypical red blood cells (RBC) (Humphreys, 2015). Bilirubin is used as a common liver function indicator with elevated levels suggestive of overall liver damage (Fu et al., 2020). It is divided into three subgroups, namely direct bilirubin (D-BIL), indirect bilirubin (I-BIL), and total bilirubin (T-BIL), which is the combined value of D-BIL and I-BIL (Lai et al., 2018). The levels of T-BIL in T2D remain controversial and are yet to be clarified. For example, one study found that T-BIL levels had no association with T2D whilst another showed that decreased T-BIL was associated with the increased risk of T2D incidence (Jing Wang et al., 2017; Žiberna et al., 2021). The reason for these incongruent findings might be explained by the study population characteristics or the study methodology employed. Most studies used only T-BIL to assess the association with T2D, however, when assessing liver function, the clinical implication of D-BIL and T-BIL differ. For example, elevated D-BIL has been associated with an increased risk of T2D incidence while T-BIL showed no association (Jing Wang et al., 2017). Other confounding factors that influence the results of these studies included age, ethnicity, and duration of T2D. Younger patients with IGT or newly onset T2D showed elevated T-BIL levels while older patients with prolonged T2D had lower levels (Min Yang et al., 2019).

Likewise, contrasting T-BIL levels were also found in different ethnic groups such that a Dutch T2D population had a mean T-BIL level of 10 µmol/L whilst an Asian T2D population had an average T-BIL level of 20 µmol/L (Abbasi et al., 2015; Jung et al., 2014). There is a need for T-BIL reference levels to be stratified per ethnic or age group as well as the duration of T2D. The mechanisms involved in increased T-BIL levels in IGT and newly onset T2D are currently under investigation and increased HO system activity may provide some insight. Heme oxygenase-1 (HO-1) is an enzyme involved in the first step of the HO system and elevated levels of HO-1 is a strong predictor of metabolic inflammation in obese IR individuals (Hosick & Stec, 2012). Therefore, it can be postulated that increased HO-1 levels in IGT or newly onset T2D lead to the elevation of T-BIL. In contrast, lower T-BIL levels in longstanding T2D indicates that the HO system has adapted to long-term oxidative stress induced by chronic inflammation, therefore, resulting in a decreased expression of HO-1 and in turn, lower T-BIL levels (Drummond et al., 2019). Bilirubin has protective antioxidant and anti-inflammatory effects and elevated levels of bilirubin has an inverse correlation with oxidative stress and CVD risk (Kunutsor et al., 2015). The opposite is also true in that increased levels of ROS and oxidative stress in vasculature leads to lower levels of bilirubin (Jing Wang et al., 2017). This makes bilirubin a possible biomarker of vascular damage and atherosclerosis. In fact, low levels of bilirubin have been associated with an increased risk of HTN (Hosick & Stec, 2012). Blood pressure is, in part, regulated by the renal system, therefore vascular dysfunction, such as atherosclerosis and HTN, as a result of chronic inflammation will affect renal function as well.

The disruption in hepatic metabolism and general lipid metabolism has a systemic effect on other organs such as the kidneys. Recently, research studies have eluded to the fact that NAFLD is an independent risk factor for renal disease and although the mechanism involved has not been clarified, concomitant T2D may be the link between the two (Byrne & Targher, 2020). In fact, 20-55% of patients with NAFLD have CKD (Marcuccilli & Chonchol, 2016). This is clear in T2D where the progression of diabetic kidney disease (DKD) is linked to chronic inflammation, hyperglycaemia and altered lipid metabolism.

2.5 An overview of renal function in T2D

The main function of the kidneys is to filter blood and by doing so, dispose of waste products and excess fluids (Webster et al., 2017; Wen & Crowley, 2018). It is also involved in glycaemic control by the reabsorption of glucose (Wen & Crowley, 2018) (See figure 2.5).

The kidneys are highly sensitive and any alterations to their structure or function may have serious consequences. For example, damage to the glomeruli may reduce the kidney's filtration ability thereby

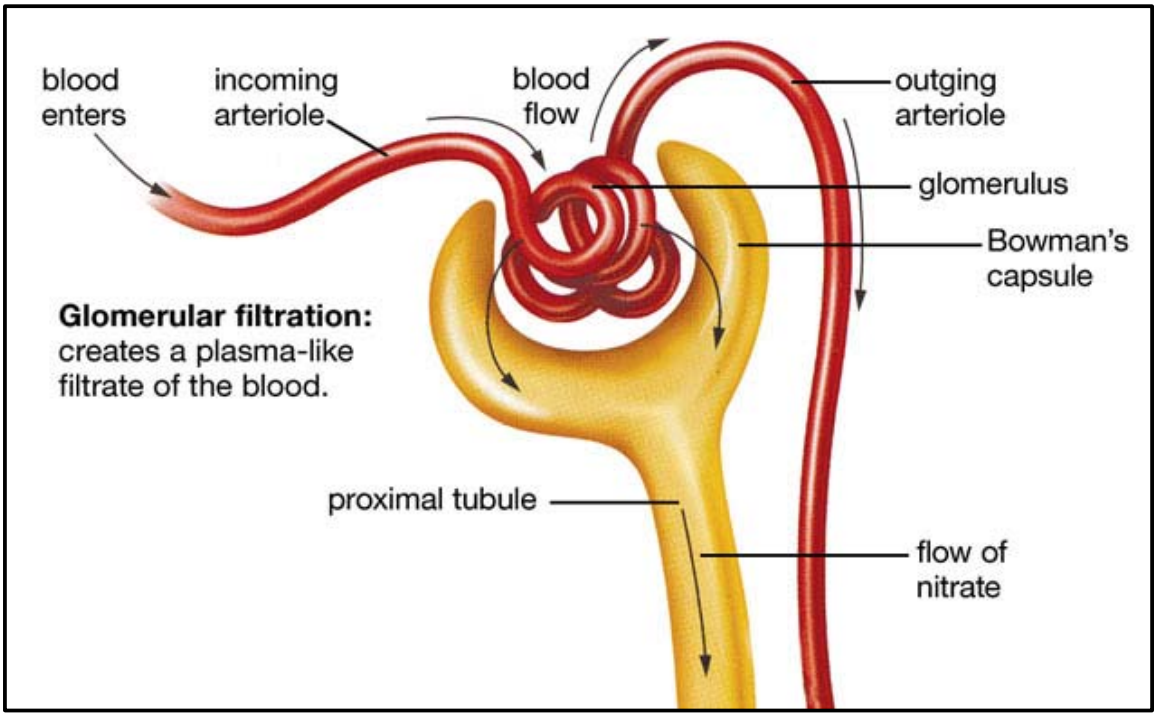
allowing for the build-up of toxic chemicals in the bloodstream that damage other organs. Furthermore, a decline in renal function, denoted by estimated eGFR, is highly prevalent among T2D (Gheith et al., 2016). In fact, worsening eGFR has been associated with uncontrolled glycaemia in T2D with 40% of patients with T2D likely to develop diabetic kidney disease (DKD) (Hussain et al., 2021). Current guidelines define chronic kidney disease as a consistent eGFR of <60mL/min/1.73 m^2 for a minimum of 3 months and an eGFR of < 15mL/min per 1.73 m^2 denotes end-stage renal disease (de Boer et al., 2020). It has been widely accepted that hyperglycaemia is a major key player in the pathogenesis of DKD and that a high concentration of circulating glucose causes renal injury (Shareed et al., 2020). Indeed, during a hyperglycaemic event, the high concentration of glucose in the kidneys activates the endothelium causing the microcirculation to narrow and become clogged, damaging the glomeruli and stimulating an inflammatory response (Hussain et al., 2021). The dysfunction in the glomeruli leads to a higher retainment of salt and water resulting in hypervolemia, oedema and weight gain (Pecoits-Filho et al., 2016). There is also a decrease in the disposal of waste products causing its build up in the vascular tissue and promoting atherosclerosis. Undoubtedly, hyperglycaemia is responsible for renal injury, however, contradictory evidence exists as to the involvement of hyperglycaemia in the progression of DKD and the decline in renal function in patients with T2D. For instance, it has been shown that regardless of glycaemic control, patients with T2D still showed a decline in renal function (Y. Chen et al., 2020; Coca et al., 2012). Factors under investigation for contributing to this decline are chronic inflammation, hyperlipidaemia, and obesity (DeFronzo et al., 2021).

2.5.1 The impact of chronic Inflammation on renal function

Apart from hyperglycaemic induced inflammation in the kidneys, the systemic spill over effect of chronic inflammation results in pro-inflammatory cytokines, like TNF-α, in circulation affecting the kidneys (See figure 2.7). As such, the blood passing through the kidneys contain elevated levels of TNF-α that infiltrate renal cells and tissues. Tumour necrosis factor-alpha facilitate the recruitment and accumulation of macrophages via the up-regulation of macrophage chemoattractant protein-1 (MCP-1) expression (Satirapoj et al., 2018). Macrophages produce more pro-inflammatory cytokines and ROS that amplify inflammation. The endothelium is activated and ROS creates a state of oxidative stress within the kidneys that lead to further renal injury (Matoba et al., 2019). Endothelium activation in the kidneys promotes the development of microvascular remodelling such as atherosclerotic changes that limits blood flow to the kidneys and in turn reduce access to oxygen. The kidneys require a high amount of blood flow to meet its equally high oxygen demand for ATP generation in order to function successfully. With modified vasculature, limited oxygen supply induces tissue hypoxia (Deng et al., 2006). In a healthy individual, increased HIF-1-α synthesis would upregulate the expression of

erythropoietin, however, in T2D this is not the case. Although HIF-1-α concentration increases, the genes involved in erythropoietin does not (Persson & Palm, 2017). Consequently, the concentration of RBC is reduced and therefore the oxygen supply is further decreased (DeFronzo et al., 2021). The underlying mechanisms resulting in reduced erythropoiesis have not been elucidated, however, chronic inflammation has been implicated whereby it suppresses the expression of the genes encoding for erythropoietin resulting in reduced erythropoiesis and a reduced RBC count. Another mechanism proposed involved the increased expression of hepcidin, a major regulator of iron metabolism. Increased levels of hepcidin inhibit the intestinal absorption of iron as well as the release of iron from macrophages. Iron is required in the synthesis of haemoglobin, a protein that provides RBCs the ability to carry and deliver oxygen to vital tissues. Reduced levels of iron disrupt erythropoiesis that results in reduced or deformed RBCs that ultimately leads to tissue hypoxia. Hypoxia provides signals for pro-inflammatory mediators that promote renal inflammation most likely leading to the development of fibrotic tissue (M. Liu et al., 2017). Chronic inflammation in T2D provides a persistent trigger for renal inflammation, perpetuating renal injury and a decline in renal function.

Figure 2.7: An overview of the filtration process in the renal bowman's capsule. Blood enters the afferent



arteriole and flows into the capillaries of the glomerulus in the Bowman's capsule. Here the blood is filtered, and the glomerular filtrate is collected in the Bowman's capsule and processed in the nephron to form urine. The remainder of the blood flows out in the efferent arteriole. (Adapted from www.medicalbiochemist.com)

2.5.2 The effects of altered lipid metabolism on renal function

Obesity has been independently linked to a decline in renal function, evidenced by the presence of glomerulosclerosis and glomerular hypertrophy commonly found in obese individuals without T2D

(García-Carro et al., 2021). The mechanisms involved in linking obesity to renal function are not yet fully understood, however, lipotoxicity as a result of obesity may provide some clarity. In an obese state, FFA concentrations are elevated in circulation and a large number of lipids, including FFAs, have been found in the renal podocytes and mesangial cells, where lipotoxicity has led to renal injury (Yamamoto et al., 2017). In addition, the lipid profile in T2D is highly atherogenic, marked by increased levels of ox-LDL (Taskinen & Borén, 2015). In the kidneys, mesangial cells express ox-LDL receptors and when activated, upregulate the production and secretion of MCP-1 and IL-6 (Roumeliotis et al., 2021). Interleukin-6 and MCP-1 serve as chemotaxis, recruiting macrophages that penetrate the glomeruli. In the presence of elevated CRP, the uptake of oxidised (ox)-LDL particles by macrophages are highly promoted, forming foam cells that perpetuate inflammation and trigger the formation of atherosclerotic lesions.

A significant association exists between CRP and ox-LDL (Harmon et al., 2016). Furthermore, ox-LDL facilitates the increased renal infiltration of monocytes into glomeruli endothelium and tubular epithelium where they have damaging effects and propagate an inflammatory response leading to apoptosis of podocytes, nephron loss and interstitial fibrosis (DeFronzo et al., 2021).

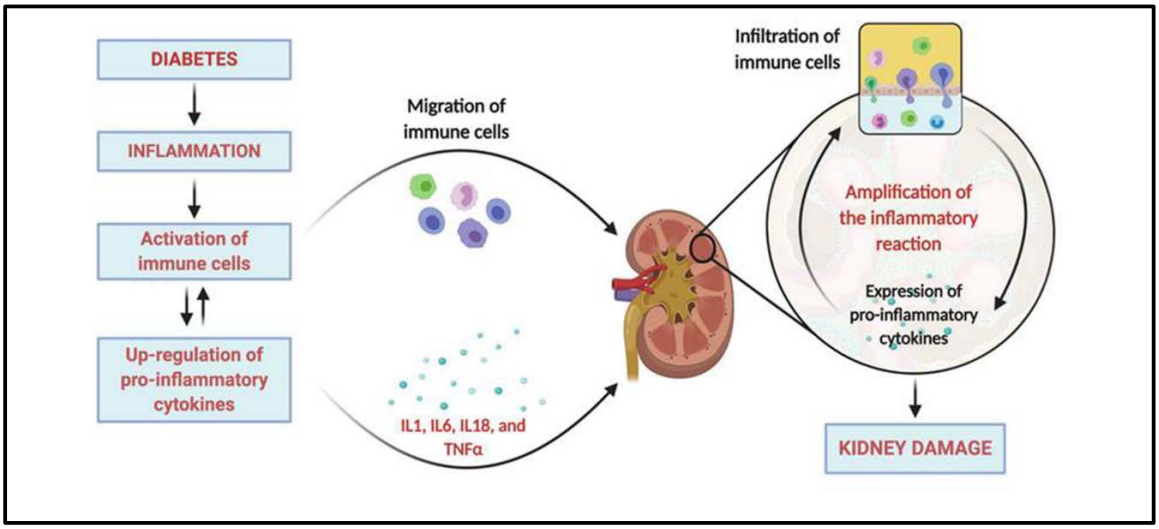


Figure 2.8: The process of immune cell and pro-inflammatory cytokine infiltration of the kidneys. Chronic inflammation in type 2 diabetes is marked by increased levels of pro-inflammatory cytokines and immune activation spill over into general circulation. These immune cells and cytokines travel to the micro-circulation of the kidneys and infiltrate the renal cells and tissues where they propagate and amplify an inflammatory response that induces renal damage. (Adapted from Donate-Correa et al., 2021)

Diabetes and HTN are the leading causes of end-stage renal disease and worsening renal function has a high prevalence among patients with HTN (Weldegiorgis & Woodward, 2020). A key player in the pathogenesis of HTN is the chronic activation of the RAAS which upregulates the synthesis of angiotensin II. Angiotensin II in circulation directly stimulates MCP-1 expression in the kidneys, leading

to an inflammatory response (Pérez-Morales et al., 2019). Indeed, angiotensin II as a driver of chronic inflammation has been associated with a DKD (Stephens et al., 2020). It is clear that inflammation in both T2D and HTN is a driver of worsening renal function, however, the question remains whether there is a further decline in renal function in comorbid T2D and HTN than in isolated T2D.

2.6 The impact of hypertension and type 2 diabetes mellitus on the risk of cardiovascular disease

Cardiovascular disease exists in a milieu of metabolic derangement and organ dysfunction. Patients with T2D are at an increased risk of atherogenic disease and CVD. This risk is heavily influenced by risk factors such as dysglycaemia, chronic inflammation, oxidative stress, and endothelial dysfunction. In fact, with HTN being the most prevalent comorbidity in T2D, these aetiological factors are associated with HTN as well. Therefore, it is rational to speculate that the CVD risk in patients with T2D and comorbid HTN is compounded, however, this requires further investigation to be confirmed. The addition of other T2D associated complications such as CKD and fatty liver disease would further aggravate such a risk. Furthermore, when assessing CVD risk in such patients, it proves imperative to involve the above-mentioned factors. In doing so, the threat that CVD poses might be mitigated by a wholistic approach to the treatment and management strategies in patients with T2D and comorbid HTN. (See figure 2.8).

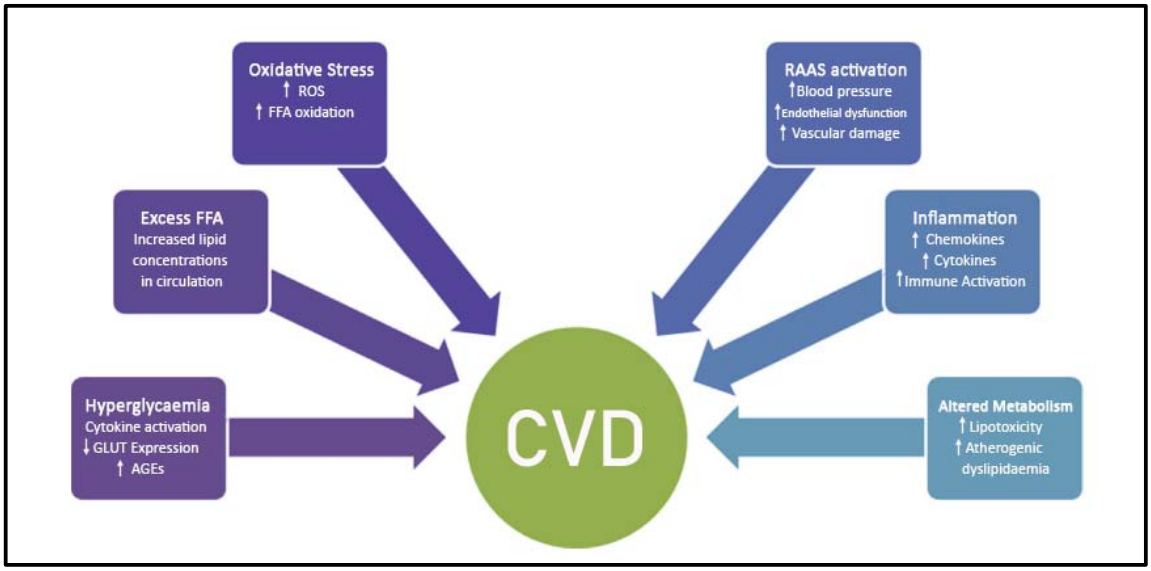


Figure 2.9: Pathophysiological risk factors of cardiovascular disease in T2D. In addition to hyperglycaemia, the combination of ROS, increased β-oxidation with elevated FFAs leading to lipotoxicity, cytokine damage, and the activation of the RAAS leading to accelerated endothelial dysfunction and vascular damage. All of these increase the cardiovascular disease risk in T2D. (Adapted from Kovacic et al., 2014)

2.7 Conclusion

The influence of chronic inflammation in T2D is widespread, affecting organ function and metabolic processes throughout the body, therefore facilitating a disturbance in homeostasis. Both T2D and HTN share underlying inflammatory aetiologies. With HTN as the most prevalent comorbidity in patients with T2D, the possibility exists of an increased risk of Worsening renal function, liver failure and dyslipidaemia brought on by the compounding effect of coexisting T2D and HTN. This remains unclear and requires further investigation. Therefore, it is the intention of this study to evaluate the levels of chronic inflammation in a T2D cohort and the effects of chronic inflammation on metabolic parameters. This study also evaluated the cardiovascular risk and renal function in the same cohort to assess whether chronic inflammation has a causal effect or not. Lastly, the biomarker levels of liver function, renal function, chronic inflammation, dyslipidaemia, and cardiovascular risk in the T2D is compared to a cohort of T2D and comorbid HTN for any statistically significant differences.

3 CHAPTER THREE: METHODOLOGY

3.1 Study design and population

This was a descriptive cross-sectional study that included a cohort of clinically known outpatients with type 2 diabetes (T2D) who visited the Katutura Community Health Centre in the Windhoek urban areas in Namibia during the period of September 2020 till December 2020. The study setting was chosen based on Namibia's most populated urban city. The study randomly recruited a total of one hundred and sixteen adult patients (n=116) of both genders. All of the patients were confirmed cases of T2D diagnosed by clinicians based on the American Diabetes Association guidelines (ADA, 2020). This study forms part of a bigger study that received ethical clearance from the Namibian University of Science and Technology (FHAS 1/2020) (Appendix A) and the Ministry of Health and Social Services (17/3/3 MN) (Appendix B). The study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki – 2013) (World Medical Association, 2013). Computer-generated codes were used as identification of the study participants to protect their anonymity and confidentiality.

3.1.1 Inclusion criteria

This study included adult (>18 years) patients with known cases of T2D. All patients were clinically diagnosed with T2D using the American Diabetes Association guidelines and had no active or recent infections prior to enrolment, were over 18 years of age, fasting, and gave informed consent.

3.1.2 Exclusion criteria

Participants were excluded if they were pregnant, had an active infection, were under the age of 18 years, or had an underlying condition preventing their participation. Furthermore, patients without diagnosed T2D were also excluded. Since this study involved blood collection, it would have been unethical to collect blood from pregnant patients or patients with illnesses such as bleeding disorders, inadvertently endangering their lives. Furthermore, there was a need to eliminate confounding factors that may have affected the outcomes of this study.

3.1.3 Sample size calculation

The minimum sample size for this study was calculated using G*power version 8.01 software. The effect size was calculated from a previous study reporting on a similar population and the following assumptions were considered (Liu et al., 2001). Student two-tailed t-test analysis was chosen since the estimated differences in this study will be between the two groups. Therefore, the study power

was set at 80%, an alpha value set at 0.05, effect size on 0.0728 and group allocation ratio 1:1. Based on these, a minimum sample of 31 patients is required per group. Therefore, taking into account at least a 5% attrition rate, we estimated the sample size to be 35 in each group leading to a total sample size of 70 patients with T2D.

3.2 Sample Collection

Venous blood was collected from the included fasting patients by a qualified registered nurse with three different types of tubes. These were 4.5mL ethylenediaminetetraacetic acid (EDTA), 4.0mL sodium fluoride and a 6mL serum separator tube (SST). Blood drawn in the EDTA tube was used for haematological parameters, erythrocytes sedimentation rate (ESR), and glycated haemoglobin HbA_1c) analysis, whilst in the sodium fluoride tube was used for fasting plasma glucose (FPG) and the SST tube was used for C- reactive protein (CRP) and lipids profiles (total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL)-c, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein/ cholesterol ratio (HDL/Cholesterol ratio). Samples were transported to the laboratory within the time frame of 2-3 hrs. A temperature of 2-8 °C was used and analysis was performed within 3hrs of collection. The SST tubes and sodium fluoride tubes were spun at 3000rpm for 10 minutes before analysis in order to separate serum from the red blood cells (RBC).

3.3 Clinical measurements

All clinical measurement was performed by qualified nurses and included weight, height, and blood pressure. Other clinical data collected and recorded were age, gender, duration of T2D diagnosis and treatment regimen. Body mass index (BMI) was calculated using the formula; BMI = $\frac{Body\ mass\ (Kg)}{\mathrm{h}eight\ \ m2}$ (Bays et al., 2007).

3.4 Laboratory measurements

All laboratory tests were performed and measured by a researcher at the Namibian Institute of Namibia (NIP), an ISO 15189 of 2012 accredited laboratory in Windhoek, Namibia. The measured parameters and the methods of testing used are indicated in Table 3.1. Calibrations and quality control were performed on all instruments before sample analysis in accordance with the laboratory's standard operating procedures and manufacture specifications. The haematological parameters included a complete blood count and were measured using a Sysmex 1000 XN automated analyser. The biochemical parameters included inflammatory profiles (CRP and ESR), glucose profiles (FPG) and (HbA_1c), lipid profiles (total cholesterol, triglycerides, LDL-C, HDL-C), liver function tests (total bilirubin (T-Bil), direct bilirubin (D-Bil), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine

aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)), and renal function profiles (potassium, sodium, urea, creatinine).

The inflammatory parameters, CRP and ESR, were analysed using Alinity c analyser and Test 1 THL Alifax S.p.A, respectively. The FPG and HbA_1c were analysed using the Cobas c501 analyser. The liver function, renal function, and lipid profiles were analysed using the Alinity c analyser. All measurements were conducted according to the manufacturer's instructions. Other parameters were calculated using standard formulas and included estimated glomerular filtration rate (eGFR), globulins, albumin-to-globulin (A/G) ratio, systemic immune-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR) ratio, and the atherogenic index of plasma (AIP). The formulas used are shown in Table 3.2.

3.5 Statistical analysis

The D'Agostino & Pearson test was used to test for normality and all data were expressed as either mean ± standard deviation (SD) or median and interquartile range [IQR] based on their distributions. For all parametric data, a two-tailed unpaired student's t-test was used to compare the means between the groups. In cases where the variances between the groups were unequal, a Welch correction was performed. The results were recorded as mean ± SD. For all non-parametric data, the Mann Whitney U test was used, and the results were recorded as median and IQR. Multiple bivariate correlations were performed using Pearson's coefficient. A p-value of <0.05 was considered statistically significant. All statistical analyses will be performed using GraphPad Prism version 8 software (GraphPad Software Inc., CA, USA).

Table 3.1: The analytes and parameters measured, including the manufacturers

Analytes	Analysis method	Analyser
Full Blood Count	Turbidimetric/ Immunoturbidimetry	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Fasting plasma glucose	Photometric	Cobas c501 (Roche, Basel, Switzerland)
Glycated haemoglobin	Photometric	Cobas c501 (Roche, Basel, Switzerland)
Erythrocyte Sedimentation rate	Westergren method	Test 1 THL Alifax S.p.A (Italy)
C-reactive protein	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Total Cholesterol	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Triglycerides	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
High-density lipoprotein cholesterol	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Low-density lipoprotein cholesterol	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Total Protein	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Albumin	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Total Bilirubin	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Direct Bilirubin	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Alkaline phosphatase	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Gamma-glutamyl transferase	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Alanine aminotransferase	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Aspartate aminotransferase	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Lactate dehydrogenase	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Potassium	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Sodium	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Urea	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)
Creatinine	Photometric, Potentiometric	Alinity c analyser (Abbot, Illinois, USA) (Sysmex Corporation, Kobe, Japan)

Table 3.2: Formulas used to calculate biochemical parameters

Parameter	Formula	Reference
eGFR CKD-EPI (ml/min/1.73m^2)	Calculate with an online calculator	(*GFR Calculator - CKD-EPI*, n.d.)
Globulins (g/L)	Total protein - albumin	(Jolles et al., 2014)
A/G Ratio	$\frac{Albumin\ (g/l)}{Globulins\ (g/l)}$	(Malawadi & Adiga, 2016)
SII	$\frac{Platelet\ (10^9/l) x\ Neutrophil\ (10^9/l\ )}{Lymphocyte\ (10^9/l)}$	(Chan Li et al., 2018)
NLR	$\frac{Neutrophil\ (10^9/l\ )}{Lymphocyte\ (10^9/l)}$	(Hashim Almalki et al., 2020)
AIP	$log\ 10 \frac{Triglycerides\ (mmol/l)}{High-density\ Lipoproteins\ (mmol/l)}$	(Z. Li et al., 2018)

A/G- Albumin-to-Globulins; **SII**-systemic immune-inflammation index; **NLR**- Neutrophil-to-lymphocyte ratio; **AIP** – Atherogenic index of plasma

4 CHAPTER FOUR: RESULTS

4.1 Characteristics of included participants

A total of 70 outpatients with type 2 diabetes mellitus (T2D) with a median age range of 43.71 to 49.49 years and similar gender distribution among the groups with a male to female ratio of 0.56 were included in the study. The clinical characteristics of all included participants are summarised in Table 4.1. Half (50%) of the included participants (n=35) had T2D and hypertension (T2D+HTN) whilst the remaining half had T2D only (n=35). Patients in the T2D group were younger (42.71 ± 10.88) than those in the T2D+HTN group (49.49 ± 10.67), p=0.0106. All study participants self-reported as black Africans and were from a similar socio-economic and ethnic background. The duration of T2D differed significantly between groups with the T2D+HTN group having a longer duration than the T2D group (p=0.0212). All included participants were on anti-diabetic treatment and 93% of them were on metformin, 28% on insulin, and 8.5% on sulphonylureas. Furthermore, 25.7% of all patients were on a combination of two or more of these medications with 18.6% on metformin and insulin, 5.7% on metformin and sulphonylureas, and 1.5% on metformin, insulin and sulphonylureas.

4.2 Clinical Parameters and glucose profiles

As expected, the T2D+HTN group had significantly elevated systolic (p=0.0040) (Figure 4.1A) and diastolic (p=0.0149) (Figure 4.1B) blood pressures (BP) when compared to the T2D group (Table 4.1). Although the body mass index (BMI) was comparable between the two groups (p>0.05), both groups' mean BMI indicated an overweight status (>25 kg/m^2) (Figure 4.1C, Table 4.1). The fasting plasma glucose (FPG) and glycated haemoglobin (HbA_1c) levels were comparable between the groups (Table 4.1, Figure 4.1D and E) but were still above the normal range despite being on treatment.

Table 4.1: Clinical Characteristics of included patients (n=70)

Parameter	T2D (n=35)	T2D+HTN (n=35)	*p*-value
Clinical Characteristics			
Age	42.71 ± 10.88	49.49 ± 10.67	**0.0106**
Male n (%)	13 (37%)	10 (29%)	0.4452
Systolic blood pressure (mm/Hg)	127.10 ± 15.07	140.20 ± 21.24	**0.0040**
Diastolic blood pressure (mm/Hg)	82.34 ± 9.17	88.66 ± 11.79	**0.0149**
Body mass index (kg/m^2)	27.52 ± 5.13	28.96 ± 5.41	0.2552
Duration of type 2 diabetes (years)	3.69 ± 2.87	6.40 ± 6.10	**0.0212**
Glucose profiles			
Glycated haemoglobin (%)	8.58 ± 1.45	8.31 ± 1.54	0.4587
Fasting plasma glucose (mmol/L)	10.12 ± 3.55	9.53 ± 2.93	0.4454
Treatment regimens			
Metformin, n (%)	31 (89%)	34 (97%)	0.1638
Insulin, n (%)	9 (26%)	11 (31%)	0.5967
Suphonylurea, n (%)	0 (0%)	6 (17%)	**0.0104**
Monotherapy n (%)	30 (86%)	23 (66%)	0.0510
Dual therapy n (%)	5 (14%)	11 (31%)	0.1634
Triple therapy n (%)	0 (0%)	1 (3%)	0.3138

Results expressed as mean ± standard deviation and median interquartile range. A *p*-value <0.05 was considered statically significant

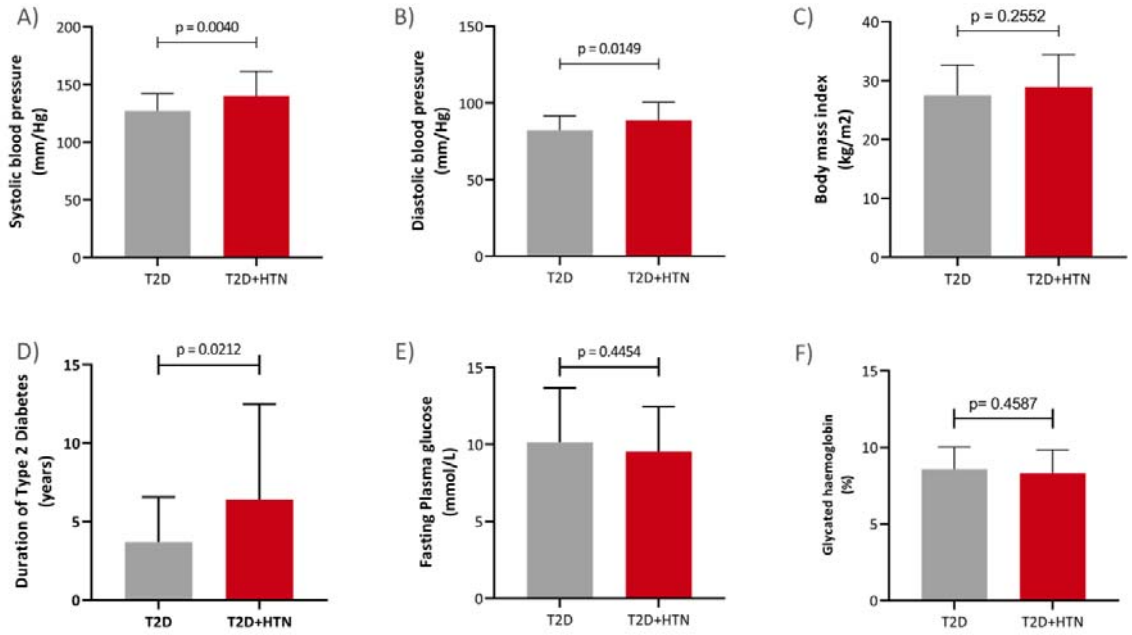


Figure 4.1 A comparison of the clinical parameters and glucose profiles between patients with type 2 diabetes (T2D) and those with T2D and hypertension (HTN) comorbidity (T2D+HTN). The levels of systolic blood pressure (SBP) (A), diastolic blood pressure (DBP) (B), and duration of T2D (D) were significantly higher in patients with T2D+HTN compared to those with only T2D. However, comparable levels were observed among the groups for BMI (C), FPG (G), and HbA1c (F). All results were expressed as mean ± standard deviation.

4.3 Hypertension comorbidity in T2D is associated with exacerbated inflammation

Although chronic inflammation prevails in T2D, the effect of HTN as comorbidity on the levels of inflammation remains elusive. In order to assess the levels of inflammation in this present study, the levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), globulins, white cell count (WCC), neutrophil-to-lymphocyte ratio (NLR), and systemic immune-inflammation index (SII) were measured. The levels of CRP were significantly increased in the T2D+HTN group (5.71 ± 3.97) when compared to the T2D group (2.48 ± 1.17), p<0.0001 (Table 4.2, Figure 4.2A). Similarly, the levels of ESR were higher in the T2D+HTN group (30.29 ± 19.78) in comparison to the T2D group (19.66 ± 14.18), p=0.0119 (Table 4.2, Figure 4.2G). The levels of globulins, a group of proteins synthesised by the liver and immune system were elevated in the T2D+HTN (38.14 ± 4.01) when compared to the T2D group (35.46 ± 3.53), p=0.0040 (Table 4.2, Figure 4.2C). The NLR was also higher in the T2D+HTN group (1.43 [1.13 – 1.94]) than in the T2D group (1.26 [1.03 – 1.51]), p=0.0494. However, the albumin/ globulin ratio (p=0.2699) and WCC (p=0.0807) were comparable between the groups (Table 4.2, Figure 4.2D and E). The SII, a novel reflector of the immune and inflammatory state, was significantly elevated in the T2D+HTN group (439.30 ± 122.7) in comparison to the T2D (377.50 ± 110.20), p=0.0298 (Table 4.2, Figure 4. F).

Table 4.2: Inflammatory profiles of included patients (n=70)

Parameter	T2D (n=35)	T2D+HTN (n=35)	*p*-value
Inflammatory profiles			
C-reactive protein (mg/l)	2.48 ± 1.17	5.71 ± 3.97	**<0.0001**
Erythrocyte sedimentation rate (mm/hr)	19.66 ± 14.18	30.29 ± 19.78	**0.0119**
Serum globulins (g/l)	35.46 ± 3.53	38.14 ± 4.01	**0.0040**
Albumin-to-globulin ratio	1.19 ± 0.17	1.14 ± 0.16	0.2699
White cell count (10^9/L)	6.10 ± 1.43	6.70 ± 1.41	0.0807
Neutrophil/Lymphocyte Ratio	1.26 [1.03 – 1.51]	1.43 [1.13 – 1.94]	**0.0494**
Systemic immune-inflammation index	377.50 ± 110.20	439.30 ± 122.7	**0.0298**

Results expressed as mean ± standard deviation and median interquartile range. A p-value <0.05 was considered statically significant

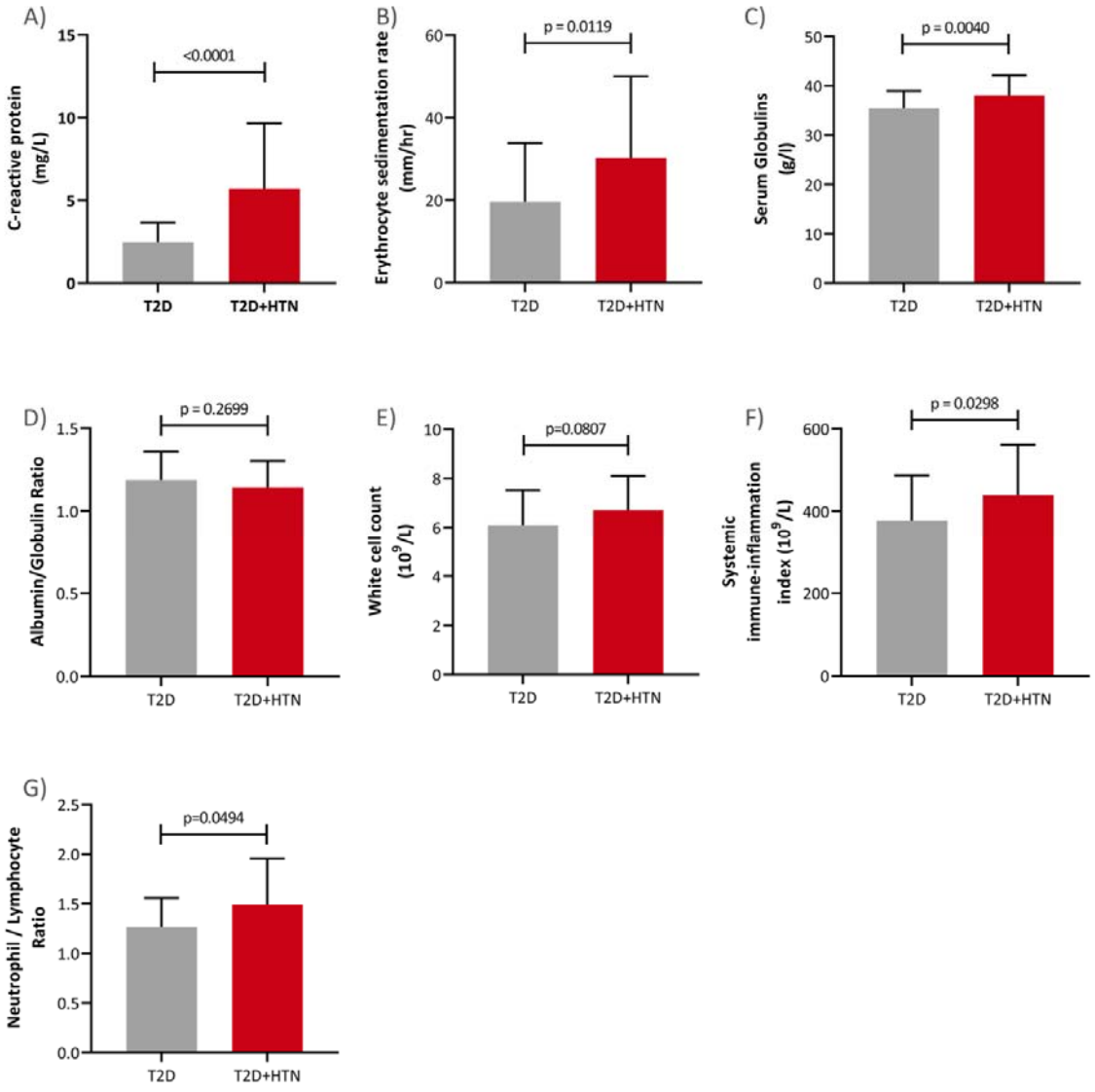


Figure 4.2 A comparison of inflammatory profiles between patients with type 2 diabetes (T2D) and those with T2D and hypertension (HTN) comorbidity (T2D+HTN). The levels of C-reactive protein (CRP) (A), erythrocyte sedimentation rate (ESR) (B), globulins (C), systemic immune-inflammation index (SII) (F), and neutrophil-to-lymphocyte ratio (NLR) (G) were significantly higher in patients with T2D and HTN in comparison with patients with only T2D, while the albumin/globulins ratio (D) and white cell count (E) showed no significant difference. All results are expressed as mean ± standard deviation.

4.4 The impact of HTN on haematological indices of patients with T2D

A complete blood count was performed to determine the effect of HTN on haematological indices. All platelet and white cell indices were comparable between the groups ($p>0.05$), except for absolute eosinophil count, which was lower in the T2D+HTN group (0.11 ± 0.06) when compared to the T2D group (0.47 ± 0.18), $p<0.0001$ (Table 4.3, Figure 4.3K). The assessment of the red cell parameters showed a lower red cell count ($p = 0.0364$), haematocrit ($p = 0.0272$) and red cell distribution width ($p=0.0211$) in the T2D+HTN group when compared to the T2D group (Table 4.2, Figure 4.4A, C, and G).

Table 4.3: Haematological parameters of included patients (n=70)

Parameter	T2D (n=35)	T2D+HTN (n=35)	*p*-value
White cell indices			
White cell count (10^9/L)	6.10 ± 1.43	6.70 ± 1.41	0.0807
Neutrophils (%)	52.78 ± 8.95	53.44 ± 8.99	0.7612
Lymphocytes (%)	37.65 ± 8.44	36.09 ± 9.36	0.4665
Monocytes (%)	6.94 ± 1.37	7.25 ± 1.97	0.4498
Basophils (%)	0.46 ± 0.26	0.38 ± 0.16	0.1429
Eosinophils (%)	1.50 [0.60 – 2.20]	1.60 [0.80 – 3.10]	0.5808
Neutrophils (10^9/L)	3.22 ± 0.87	3.50 ± 1.01	0.2180
Lymphocytes (10^9/L)	2.40 [1.64 – 2.89]	2.40 [2.14 – 2.70]	0.8450
Monocytes (10^9/L)	0.43 ± 0.12	0.48 ± 0.14	0.1206
Basophils (10^9/L)	0.03 ± 0.02	0.03 ± 0.01	0.2707
Eosinophils (10^9/L)	0.47 ± 0.18	0.11 ± 0.06	**<0.0001**
Red cell indices			
Red cell count (10^{12}/L)	4.97 ± 0.45	4.74 ± 0.45	**0.0364**
Haemoglobin (g/dL)	14.19 ± 1.01	13.68 ± 1.34	0.0729
Haematocrit (%)	43.85 ± 3.35	41.74 ± 4.40	**0.0272**
Mean cell volume (fL)	87.71 ± 6.04	89.43 ± 4.97	0.1962
Mean corpuscular haemoglobin (pg)	29.06 ± 2.01	29.32 ± 1.71	0.5701
Mean corpuscular haemoglobin concentration (g/dL)	32.64 ± 1.60	32.40 ± 1.90	0.5647
Red cell distribution width (%)	12.93 ± 0.90	13.48 ± 1.04	**0.0211**
Platelet indices			
Platelets (10^9/L)	293.30 ± 73.52	302.70 ± 31.48	0.5637
Mean platelet volume (fL)	10.80 ± 0.99	10.65 ± 0.84	0.4791

Results expressed as mean ± standard deviation and median interquartile range. A p-value <0.05 was considered statically significant

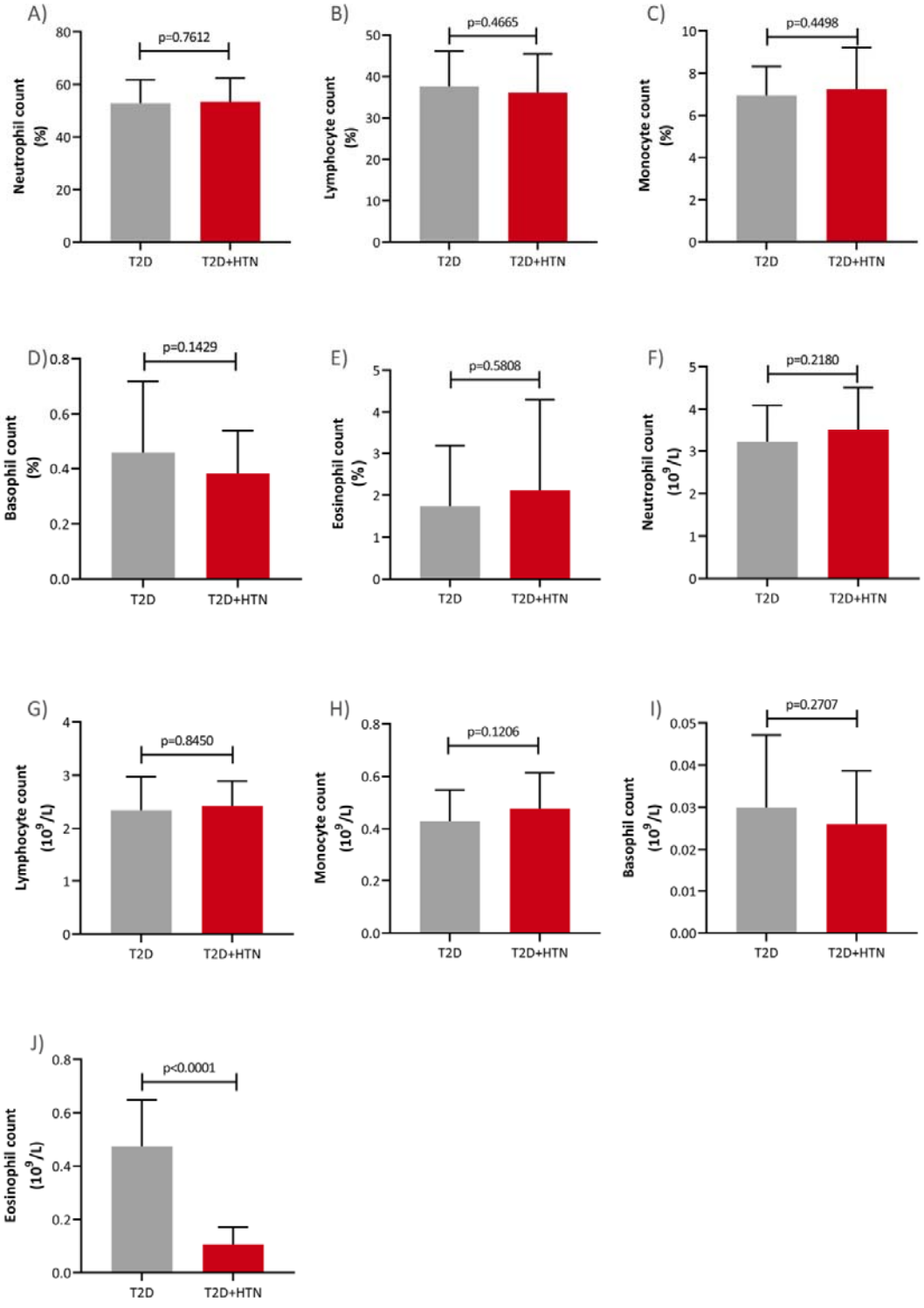


Figure 4.3: A comparison of the white cell indices between patients with type 2 diabetes (T2D) and those with T2D and hypertension (HTN) comorbidity (T2D+HTN). All indices were comparable among the groups with the exception of eosinophil count (J) indicating higher levels in patients with only T2D compared to patients with T2D+HTN. All results are expressed as mean ± standard deviation.

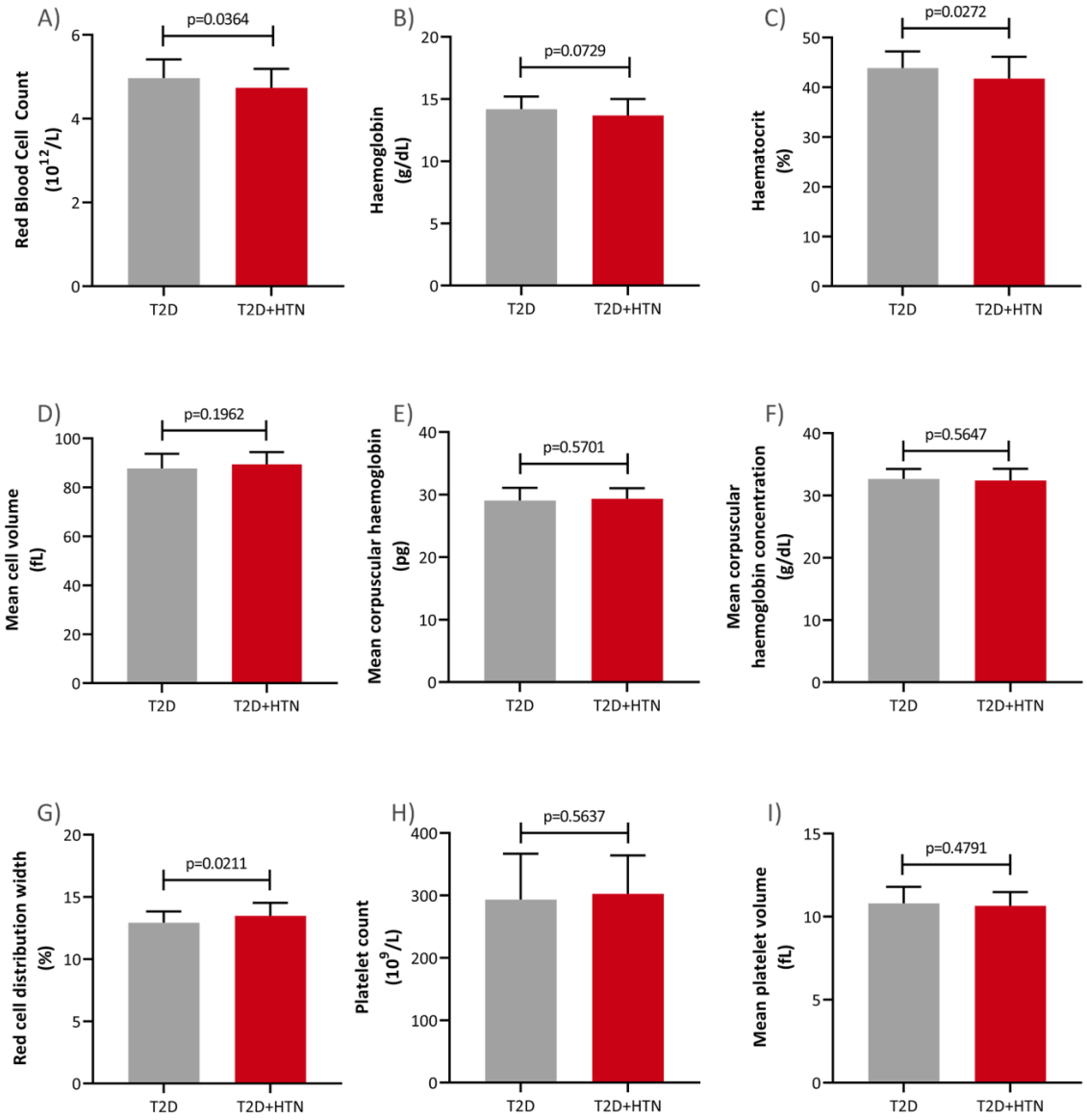


Figure 4.4: A comparison of the red cell and platelet indices between patients with type 2 diabetes (T2D) and those with T2D and hypertension (HTN) comorbidity (T2D+HTN). While haemoglobin (B), mean cell volume (MCV) (D), mean corpuscular haemoglobin (MCH) (E), mean corpuscular haemoglobin concentration (MCHC) (F), platelet count (H), and mean platelet volume (MPV) (I) showed no statistically significant differences among the groups, red blood cell (RBC) count (A) and haematocrit (HCT) (C) were significantly higher in the T2D group in comparison to the T2D+HTN group. The red cell distribution width (RDW) (G) was lower in the T2D group compared to the T2D+HTN group. All results are expressed as mean ± standard deviation.

4.5 Assessment of lipid profiles and cardiovascular risk in T2D+HTN

Altered lipid metabolism is strongly associated with an increased risk of developing accelerated atherogenesis in T2D due to dyslipidaemia (Summerhill et al., 2019). Since chronic inflammation promotes lipid dysmetabolism and atherosclerosis, lipid profiles and atherogenic index of plasma (AIP) were determined (Bernardi et al., 2018). The levels of triglycerides were significantly elevated in the T2D+HTN group (1.48 ± 0.54) compared to the T2D group (1.21 ± 0.37), p = 0.0177 (Table 4.4, Figure

4.5A). In the same manner, the levels of low-density lipoprotein cholesterol (LDL-C) were elevated in the T2D+HTN group (3.13 ± 0.89) when compared to the T2D group (2.64 ± 0.84), p=0.0189 (Table 4.4, Figure 4.5C). However, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and the HDL-C/cholesterol ratio were comparable between the two groups (p>0.05) (Table 4.4, Figure 4.5B, D and E).

Atherosclerosis is the hallmark of many CVD events such as stroke, myocardial infarction, coronary artery disease, and peripheral artery disease. The atherogenic index of plasma (AIP) is a novel index that is an optimal indicator of dyslipidaemia (Zhu et al., 2018). The AIP score was stratified into 3 categories based on the level of CVD risk (Bo et al., 2018). There were no differences in the AIP scores between the two groups (p>0.05), however, 59% of this entire cohort were overweight (BMI >25 kg/m^2) (Table 4.5, Figure 4.5 F, G, and H).

Table 4.4: Lipid profiles of included patients (n=70)

Parameter	**T2D (n=35)**	**T2D+HTN (n=35)**	***p*-value**
Lipid profiles			
Triglycerides (mmol/L)	1.21 ± 0.37	1.48 ± 0.54	**0.0177**
Total cholesterol (mmol/L)	4.60 ± 1.04	4.64 ± 0.76	0.8629
LDL-cholesterol (mmol/L)	2.64 ± 0.84	3.13 ± 0.89	**0.0189**
HDL-cholesterol (mmol/L)	1.05 ± 0.26	1.04 ± 0.26	0.9042
HDL/Cholesterol ratio	0.24 ± 0.07	0.22 ± 0.05	0.2705
Cardiovascular Risk			
Atherogenic Index of plasma (Overall)	0.11 ± 0.25	0.16 ± 0.23	0.4113
AIP low risk (<0.11) n (%)	16 (46%)	13 (37%)	0.4667
AIP intermediate risk (≥0.11, ≤0.24) n (%)	4 (11%)	10 (29%)	0.0730
AIP high risk (>0.24) n (%)	15 (43%)	12 (34%)	0.4613

Results expressed as mean ± standard deviation and median interquartile range. A p-value <0.05 was considered statically significant

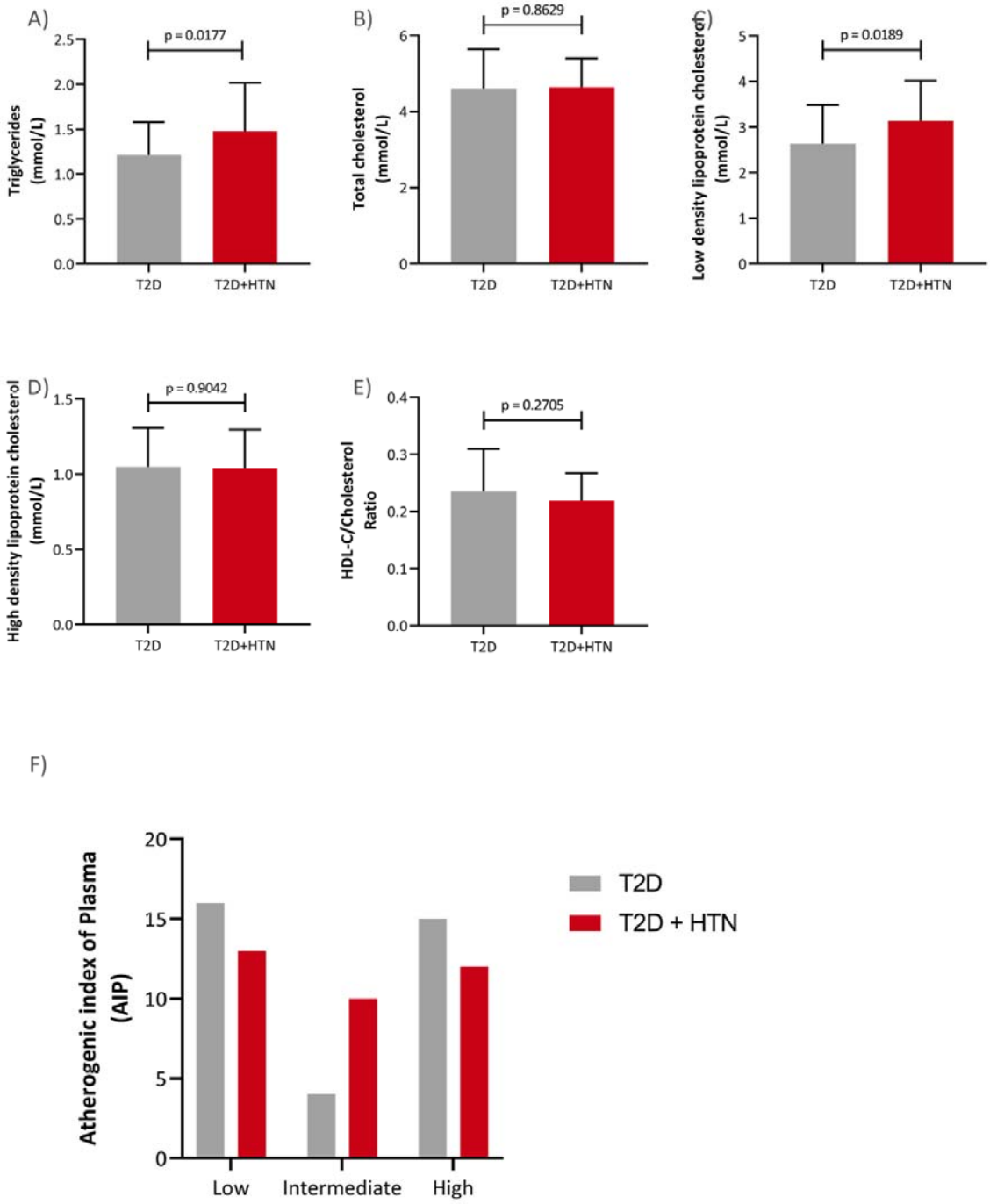


Figure 4.5: A comparison of the lipid profiles and atherogenic index of plasma between patients with type 2 diabetes (T2D) and those with T2D and hypertension (HTN) comorbidity (T2D+HTN). The levels of triglycerides (A) and low-density lipoprotein cholesterol (LDL-C) (C) were significantly higher in the T2D+HTN group compared to the T2D group while no significant differences could be observed in total cholesterol (B), high-density lipoprotein cholesterol (HDL-C) (D) and HDL-C/cholesterol ratio (E) among the groups. Furthermore, the levels of the atherogenic index of plasma (AIP) (F) were comparable among the groups. All results are expressed as mean ± standard deviation.

4.6 Liver function in patients with T2D and HTN

The liver functions in the metabolism of glucose and energy, making its dysfunction in T2D apparent (Jensen-Cody & Potthoff, 2021). In order to assess the effect of comorbid HTN in patients with T2D, the levels of various liver enzymes were measured. These included total protein (TP), albumin (ALB), total bilirubin (T-Bil), direct bilirubin (D-Bil), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). Where the levels of TP were elevated in the T2D+HTN group (80.52 ± 5.85) in contrast to the T2D group (77.13 ± 5.10), p=0.0121, the ALB levels were similar among both groups, p>0.05 (Table 4.4, Figure 4.6A and B). The levels of T-Bil were significantly higher in the T2D group (6.81 ± 3.28) compared to the T2D+HTN group (4.97 ± 1.42) (p=0.0080) (Table 4.4, Figure 4.6C). As expected, D-Bil was also significantly higher in the T2D group (2.56 ± 0.67) than in the T2D+HTN group (1.94 ± 0.56) (p=0.0133) (Table 4.4, Figure 4.6D). While no significant differences could be seen in the levels of GGT and LDH among the groups, ALP levels were significantly higher in the T2D+HTN group (81.58 ± 19.98) in comparison to the T2D group (71.48 ± 12.69) (p=0.0176) (Table 4.4, Figure 4.6E, F and I). When considering the levels of the two aminotransferases, a significant difference could only be seen in AST levels (p=0.0002) with the T2D+HTN group (22.58 ± 7.08) at a higher mean than the T2D group (16.91 ± 3.55) (Table 4.4, Figure 4.6G and H).

Table 4.5: Liver function profiles of included patients (n=70)

Parameter	T2D (n=35)	T2D+HTN (n=35)	*p*-value
Liver function profiles			
Total Protein	77.13 ± 5.10	80.52 ± 5.85	**0.0121**
Albumin	41.72 ± 3.47	42.98 ± 3.78	0.1491
Total Bilirubin (µmol\l)	6.81 ± 3.28	4.97 ± 1.42	**0.0080**
Direct Bilirubin (µmol\l)	2.56 ± 0.67	1.94 ± 0.56	**0.0133**
Alkaline Phosphatase (IU\L)	71.48 ± 12.69	81.58 ± 19.98	**0.0176**
Gamma-glutamyl Transferase (IU\L)	30.17 ± 9.99	32.04 ± 11.87	0.5665
Alanine Aminotransferase (IU\L)	18.56 ± 6.42	19.98 ± 4.51	0.3003
Aspartate Aminotransferase (IU\L)	16.91 ± 3.55	22.58 ± 7.08	**0.0002**
Lactate Dehydrogenase (IU\L)	207.9 ± 35.74	219.60 ± 25.44	0.1501

Results expressed as mean ± standard deviation and median interquartile range. A p-value <0.05 was considered statically significant.

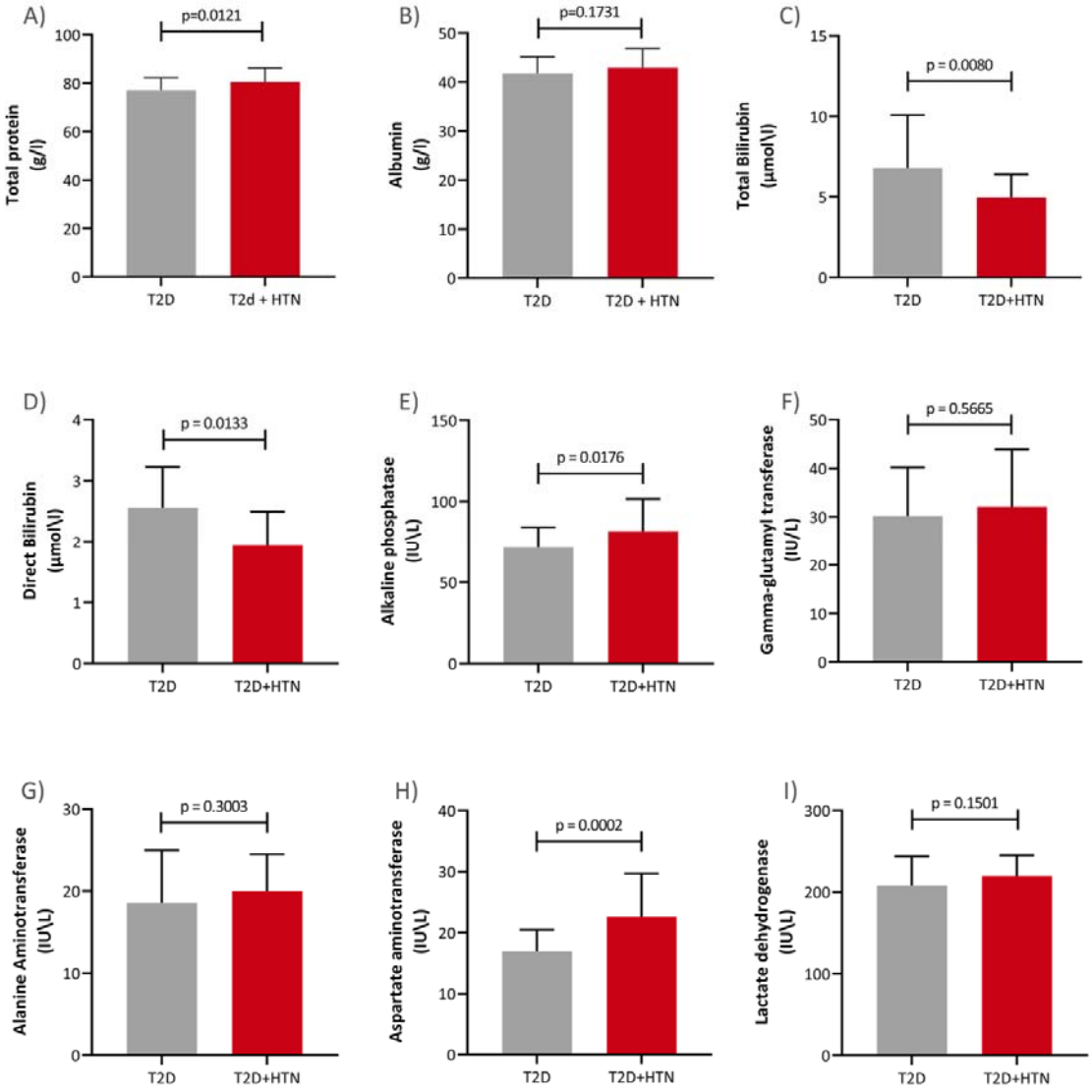


Figure 4.6: A comparison of the liver function between patients with type 2 diabetes (T2D) and those with T2D and hypertension (HTN) comorbidity (T2D+HTN). Significantly higher levels were observed in the T2D+HTN group compared to the T2D group for total protein (TP) (A), total bilirubin (T-Bil) (C), direct bilirubin (D-Bil) (D), alkaline phosphatase (ALP) (E), and aspartate aminotransferase AST (H), while the levels of ALB (B), GGT (F), alanine aminotransferase (ALT) (G), and LDH (I) were comparable among the groups. All results are expressed as mean ± standard deviation.

4.7 Renal Function

Decreased renal function has been linked to T2D and HTN independently, therefore the levels of renal function biomarkers such as potassium, sodium, urea, and creatinine were assessed in conjunction with the estimated glomerular filtration rate (eGFR). In this study, no significant differences were found in the levels of potassium, sodium, and creatinine ($p>0.05$), however, the levels of urea were significantly higher in the T2D+HTN group (4.20 ± 1.10) compared to the T2D group (3.52 ± 0.76) ($p=0.0056$) (Table 4.5, Figure 4.7A, B, C and D). The main function of the kidneys lies in their ability to filter the blood, therefore the eGFR was calculated. The T2D+HTN group (98.95 ± 21.59) had a

significantly lower eGFR when compared to the T2D group (111.6 ± 21.40) (p=0.0161) (Table 4.5, Figure 4.7E).

Table 4.6 Renal function of included patients (n=70)

Parameter	T2D (n=35)	T2D+HTN (n=35)	*p*-value
Renal Function			
Sodium (mmol\l)	135.20 ± 3.19	136.40 ± 5.54	0.2947
Potassium (mmol\l)	4.61 ± 0.59	4.48 ± 0.45	0.2943
Urea (mmol\l)	3.52 ± 0.76	4.20 ± 1.10	**0.0056**
Creatinine (µmol\l)	72.42 ± 13.67	75.96 ± 16.57	0.3623
eGFR CKD-EPI (ml/min/1.73m^2)	111.6 ± 21.40	98.95 ± 21.59	**0.0161**

Results expressed as mean ± standard deviation and median interquartile range. A p-value <0.05 was considered statically significant.

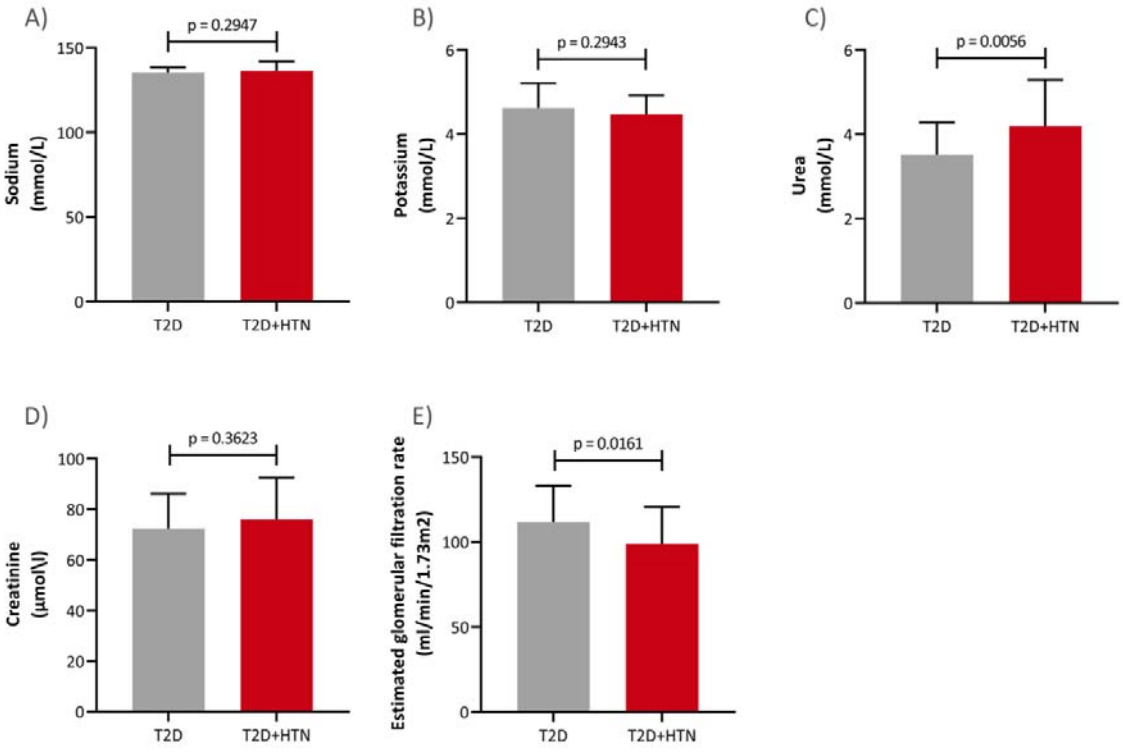


Figure 4.7 A comparison of the renal function parameters between patients with type 2 diabetes (T2D) and those with T2D and hypertension (HTN) comorbidity (T2D+HTN). Levels of urea (C) were significantly higher among patients with T2D+HTN compared to those with T2D only, whilst significantly lower levels of estimated glomerular filtration rate (eGFR) (E) could be found in the T2D+HTN group. There were no significant differences between the groups for sodium (A), potassium (B), and creatinine (D). All results are expressed as mean ± standard deviation.

4.8 Correlation analysis between inflammation, blood pressure, haematological indices, lipid profiles, renal and liver function

A multiple bivariate analysis was performed to determine whether there are any associations between the measured parameters. All significant correlations are summarised in Table 4.6. As expected, the duration of T2D was positively associated with the patients' age (r=0.40, p=0.0177), while the eosinophil count showed a negative association (r=-0.34, p=0.0442). The levels of CRP were negatively associated systolic blood pressure (r= -0.59, p = 0.0014), diastolic blood pressure (r=-0.45, p=0.0224), and eGFR (r=-0.45, p= 0.0203). Moreover, the levels of CRP positively correlated with RBC count (r=0.52, p= 0.0066), urea (r=0.51, p= 0.0078), T-Bil (r=0.58, p= 0.0020), AST (r=0.50, p= 0.0101), and ALP (r=0.58, p= 0.0021). No associations were found between CRP and lipid profiles (p>0.05). The NLR were positively correlated to ESR (r=-0.39, p= 0.0192) and SII (r=-0.65, p<0.0001).

A positive association between RBC and TP (r=0.37, p= 0.0310), HCT (r=0.57, p= 0.0003), UREA (r=0.95, p<0.0001), T-Bil (r=0.93, p<0.0001), D-Bil (r=0.95, p<0.0001), AST (r=0.71, p<0.0001), and ALP (r=0.86, p<0.0001) were found as well, while RBC correlated negatively with eGFR (r=-0.53, p= 0.0010). Assessment of associations between renal and h function tests revealed a negative correlation between eGFR and ALP (r=-0.59, p= 0.0003), AST (r=-0.54, p= 0.0012), T-Bil (r=-0.61, p= 0.0006), and D-Bil (r=-0.73, p= 0.0030), while urea levels showed a strong positive correlation to levels of ALP (r=0.89, p<0.0001), AST (r=0.78, p<0.0001), T-Bil (r=0.94, p<0.0001) and D-Bil (r=0.94, p<0.0001).

Table 4.7 The significant correlations between CRP and blood pressure, red blood cell count, and metabolic parameters

Parameter 1	Parameter 2	Pearson's coefficient *r*	*p*-value
T2D Duration	AGE	0.40	**0.0177**
	Eosinophils	-0.34	**0.0442**
CRP	SBP	-0.59	**0.0014**
	DBP	-0.45	**0.0224**
	RBC	0.52	**0.0066**
	Urea	0.51	**0.0078**
	eGFR	-0.45	**0.0203**
	T-Bil	0.58	**0.0020**
	AST	0.50	**0.0101**
	ALP	0.58	**0.0021**
NLR	ESR	0.39	**0.0192**
	SII	0.65	**<0.0001**
RBC	TP	0.37	**0.0310**
	HCT	0.57	**0.0003**
	UREA	0.95	**<0.0001**
	eGFR	-0.53	**0.0010**
	T-Bil	0.93	**<0.0001**
	D-Bil	0.95	**<0.0001**
	AST	0.71	**<0.0001**
	ALP	0.86	**<0.0001**
Urea	ALP	0.89	**<0.0001**
	AST	0.78	**<0.0001**
	T-Bil	0.94	**<0.0001**
	D-Bil	0.94	**<0.0001**
eGFR	ALP	-0.59	**0.0003**
	AST	-0.54	**0.0012**
	T-Bil	-0.61	**0.0006**
	D-Bil	-0.73	**0.0030**

A p-value <0.05 was considered statically significant. A negative *r* value indicates an inverse association while a positive *r* value indicates a direct association.

5 CHAPTER FIVE: DISCUSSION

The aim of the study was to investigate the impact of hypertension (HTN) comorbidity in type 2 diabetes mellitus (T2D) on inflammation, cardiovascular risk, and hepatorenal function. Notably, patients with T2D+HTN had exacerbated levels of inflammation and cardiovascular risk. In addition, these patients were associated with impaired hepatorenal functions. This increased inflammatory state in patients with T2D+HTN was inversely associated with blood pressure and renal function while a positive association was observed with increased hepatic enzymes.

The increase in hepatic enzymes indicates of hepatic damage with a direct impact on lipid metabolism (Islam et al., 2020). This study further found that lipid metabolism is altered in patients with T2D+HTN with higher levels of triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) in such patients. Moreover, TG levels are predictive of cardiovascular disease (CVD) risk and the atherogenic index of plasma (AIP) score results revealed that approximately two-thirds of all included patients are at intermediate to high risk of atherosclerotic complications (Ye et al., 2019). These findings highlight the impact of inflammation on the hepatorenal function. Indeed, a strong positive association was found between serum urea levels and hepatic enzymes while a moderate inverse association was found between estimated glomerular filtration rate (eGFR) and hepatic enzymes. Thus, a decline in renal function translates into an increase in hepatic damage and vice versa. Furthermore, hepatic damage is associated with increased levels of red blood cells (RBC) (Lang et al., 2015). This supports the findings of this study in that a positive association was found between the levels of RBC and the levels of hepatic enzymes. Notably, the levels of C-reactive protein (CRP) were also positively associated with the various red cell indices with lower levels of red blood cells (RBC) and haematocrit (HCT) found in patients with T2D+HTN whilst red cell distribution width (RDW) was elevated in these same patients. Significant changes in red cell indices have been found in patients with T2D relating to the increased occurrence of diabetic complications such as diabetic kidney disease (DKD) and liver disease. In fact, in this study, RBC levels were positively associated with worsening renal function. These results suggest that chronic inflammation is central to a destructive cycle between liver function, lipid metabolism, renal function, and CVD risk, whether directly or indirectly.

Cardiovascular disease risk develops in a milieu of altered metabolism and dysregulated inflammatory responses (Koziarska-Rościszewska et al., 2021). This puts patients with T2D at an increased risk of CVD which is further aggravated by the presence of HTN comorbidity (Ye et al., 2019). Although the underlying mechanisms may be multifactorial, chronic inflammation is implicated as the key player in the pathophysiology of cardiovascular complications in T2D (Maio et al., 2021). In fact, the mediators

of chronic inflammation such as CRP, TNF-α, and IL-6 are associated with the development of secondary HTN (Savoia & Schiffrin, 2007). Blood pressure (BP) is strongly regulated by the renin-angiotensin-aldosterone system (RAAS) which is in turn overstimulated by CRP, an acute phase reactant (Zhao et al., 2013). Upon the activation of RAAS, several neurohormonal responses are initiated and lead to vasoconstriction, increased BP and activation of the endothelium (Takahashi et al., 2011). Furthermore, inflammation is exacerbated by the release of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-6 which facilitates the recruitment of macrophages in endothelial cells that have the potential of initiating atherosclerotic plaque formation (Satirapoj et al., 2018). The current study revealed that HTN comorbidity in T2D is associated with increased levels of chronic inflammation, thus inadvertently increasing the atherogenic and CVD risk. The increased inflammation in patients with HTN comorbidity in this study was denoted by increased levels of erythrocyte sedimentation rate (ESR) and neutrophil-to-lymphocyte ratio (NLR), which are both associated with increased CVD risk (Chuang Li et al., 2020; Saylik & Sarıkaya, 2021). In fact, elevated levels of NLR, even at normal leukocyte levels, are predictive of atherosclerotic events and CVD mortality. This is due to the affinity of neutrophils to infiltrate atherosclerotic plaques and initiate their dislodgement (Balta et al., 2016). This study reports on elevated NLR in patients with T2D+HTN when compared to those with only T2D. Although the ratio was within the normal range, this is suggestive of a trajectory towards atherosclerotic disease and cardiovascular events.

A novel marker of systemic inflammation, systemic immune-inflammation index (SII), was recently described (Jie Wang et al., 2021). It combines the NLR with the platelet count and was initially used as a prognostic marker for hepatocellular carcinoma (HCC) (M. Xu et al., 2021). Its use has since been extended to assessing the impact of BP variability, morning BP surges, target organ damage and overall CVD risk (Saylik & Sarıkaya, 2021). Moreover, SII is an independent predictor of carotid intima-media thickness in patients with HTN (Çırakoğlu & Yılmaz, 2021). The assessment of the SII in the current T2D cohort showed higher levels in patients with HTN comorbidity, thus suggesting an increased CVD risk. In fact, SII and ESR are good predictors of future cardiovascular events that are also mediated by altered lipid metabolism (Charles-Schoeman et al., 2019; Guo et al., 2020).

Lipid metabolism in T2D is influenced by chronic inflammation induced by the exacerbated release of pro-inflammatory cytokines that downregulate the activity of lipoprotein lipase (LPL) and very-low-density lipoprotein (VLDL) receptors (Makoveichuk et al., 2017; Musso et al., 2015). Consequently, lipolysis within adipose tissue (AT) is increased and circulating triglyceride-rich lipoproteins (TRLs) are neither broken down nor stored in AT resulting in increased concentration of circulatory free fatty

acids (FFA) and TRLs (Musso et al., 2015). This study found that TG and LDL-C levels are increased in patients with HTN+T2D. Dyslipidaemia, characterised by elevated TG and LDL-C as well as reduced levels of high-density lipoprotein cholesterol (HDL-C), is associated with increased atherogenic risk hence this study investigated the AIP in the included patients. Although the AIP scores were comparable between the two groups, over 50% of the included patients had an intermediate to high risk of developing atherosclerotic complications. This suggests that atherogenesis may play a role in the development of secondary HTN, a hypothesis that remains to be elucidated in the population of interest by conducting longitudinal studies.

Increased lipid concentrations in systemic circulation due to adiposity and chronic inflammation is highly prevalent in T2D and HTN (Athyros et al., 2018; Toshikuni et al., 2017). This increase in lipid concentrations puts these patients at risk of liver diseases such as non-alcoholic liver disease (NAFLD) (Meng Yang et al., 2021). The mechanisms involved include hepatic insulin resistance (IR) aggravated by proinflammatory cytokines that stimulate the increased uptake of FFAs from circulation as well as decreased FFA oxidation within hepatocytes (Meng Yang et al., 2021). The elevated levels of TNF-α in circulation upregulates the production of TG within hepatic tissues, whilst hepatic IR decreases their subsequent export in VLDLs (Shi et al., 2019). The fat build-up within hepatocytes resulting in the formation of fat droplets is called steatosis. These fat droplets are highly susceptible to oxidation by reactive oxygen species (ROS) resulting in oxidative damage and exacerbation of inflammation (Ipsen et al., 2018). When steatosis is combined with inflammation, it becomes steatohepatitis or more commonly known as non-alcoholic steatohepatitis (NASH). The progression of NASH leads to fibrosis, cirrhosis, liver failure, and in some cases, HCC (Lonardo et al., 2018; Toshikuni et al., 2017). Furthermore, hepatic fibrosis is characterised by increased resistance to blood flow into the liver via the hepatic portal vein causing portal HTN (Baffy, 2018).

The current study revealed elevated levels of total protein (TP), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in patients with T2D+HTN in comparison to those with T2D albeit the levels were within the normal range. A recent study reported elevated levels of AST and ALP in patients with NAFLD when compared to those without NAFLD (Cao et al., 2021). Therefore, the included patients with T2D+HTN may be at risk of developing NAFLD. This is further supported by a higher SII in patients with T2D+HTN. Further investigation is required in these patients in order to accurately assess such a risk. In continuation with risk assessment, decrease levels of total bilirubin (T-Bil) are indicative of increased levels of ROS and oxidative stress in the vasculature of patients with HTN (Jing Wang et al., 2017). This study found lower levels of T-Bil and direct bilirubin (D-Bil) in patients with T2D+HTN

suggesting increased vascular damage, atherogenesis and increased CVD risk. This seems plausible since the leading physiological cause of persistent elevated BP is vascular damage.

Blood pressure is regulated in part by the kidneys, where the micro variations in BP are detected within the microcirculation of the renal tissue and the subsequent secretion of renin from the adrenal glands to the liver activates the RAAS (Takahashi et al., 2011). Physiologically, this links hepatic and renal function translating to the dysfunction in one disrupts the functionality of the other. The current study supported this by revealing an association between the hepatic enzymes and the biomarkers of renal function, specifically, urea and eGFR. With increased levels of inflammation, a fatty liver, in response to increased intracellular accumulation of TG, upregulates its secretion of TG packaged in VLDLs into circulation (Musso et al., 2015). Consequently, atherogenic dyslipidaemia is induced whereby TG and oxidized LDL particles promote glomerular injury and mesangial cell proliferation (Gyebi et al., 2012; H. Wang et al., 2021). Moreover, an inflammatory response is triggered within the renal tissue by microvascular damage and the spill over of systemic pro-inflammatory mediators such as CRP, TNF-α, and IL-6. These proinflammatory cytokines infiltrate renal cells and tissues where they facilitate the recruitment of leukocytes via the up-regulation of monocyte chemoattractant protein (MCP) -1 (Satirapoj et al., 2018). This results in increased ROS production, oxidative damage, and endothelial dysfunction that promote atherosclerotic microvascular remodelling (Iwai et al., 2019). This remodelling limits the blood flow in the kidneys, reducing oxygen delivery and inducing hypoxia (J. Li et al., 2019). Therefore, as systemic inflammation increases, renal function is compromised. In this study, a decline in renal function was associated with increased levels of CRP. Furthermore, HTN has also been associated with a decline in renal function and this study showed that patients with T2D+HTN had elevated levels of blood urea levels and lower eGFR levels (Yu et al., 2019). Interestingly, this study also showed an association between renal function and RBC count with a lower RBC count found in patients with T2D+HTN. This may be explained by the alteration of erythropoiesis in HTN.

Erythropoiesis is the process by which RBCs are produced, usually triggered by tissue hypoxia (J. Li et al., 2019). During hypoxia, the expression of hypoxia-inducible factor 1 alpha (HIF-1α) is upregulated and its consequent interaction with the erythropoietin (EPO) gene stimulates the increased production of EPO (Persson & Palm, 2017). Erythropoietin is subsequently transported to the bone marrow to activate erythropoiesis (Portolés et al., 2021). In chronic diseases, such as T2D and HTN, two mechanistic explanations that contribute to a decrease in RBC levels exist whereby chronic inflammation is a key player in both (Kim et al., 2021; Pagani et al., 2019). The first is that chronic inflammation suppresses the expression of erythropoietin in the kidneys, thus decreasing

erythropoiesis and in turn RBC count (Pagani et al., 2019). The second is that chronic inflammation upregulates the expression of hepcidin, an important regulator of iron metabolism (Kim et al., 2021). Increased levels of hepcidin inhibit intestinal iron absorption as well as the release of iron from macrophages (Vela, 2018). Iron is essential in the final stages of erythropoiesis whereby iron is critical in the synthesis of haemoglobin, a protein that provides RBCs the ability to transport and deliver oxygen to cells and tissues (Pagani et al., 2015). A deficiency of iron disrupts erythropoiesis, either by halting the process entirely or resulting in abnormal RBCs in circulation (Camaschella & Nai, 2016). In the current study, red cell distribution width (RDW) was higher in patients with T2D+HTN which is indicative of increased variation in RBC size in these patients. Red cell distribution width is associated with decreased RBC deformability, which impairs the ability of RBCs to deform and navigate easily within the microvasculature to deliver oxygen to cells and tissues (K. V. Patel et al., 2013). This result in tissue hypoxia, explaining the association between RDW and increased CVD risk (Isik et al., 2012). Red blood cell deformability has also been linked to the levels of nitric oxide (NO), a potent vasodilator (Yaqi Wang et al., 2021). In fact, reduced RBC deformability is associated with reduced concentrations of NO metabolites, therefore supporting RDW as an independent predictor of incident HTN (Tsuda, 2020). Lastly, RDW has previously been associated with carotid intima-media thickness, reflecting its use in assessing atherosclerotic disease risk as well as CVD risk (Nam et al., 2018).

The current study had a few limitations. Firstly, is its cross-sectional nature, which lacked follow-up examinations of the patients. Therefore, a causal effect could not be established between HTN and the various findings of this study. A longitudinal study will be imperative in stratifying the cardiovascular risk over time. In the absence of waist measurements, the cardiometabolic index could not be calculated. This would have been instrumental in linking fat distribution with the various cardiovascular risk factors as well as determining the CVD risk of patients included in this study. Nonetheless, this study finds its significance in being the first to use the SII in patients with T2D+HTN. Moreover, it showed that the CVD risk of patients with T2D+HTN is heavily influenced by the inflammatory state in these patients. This study reported the impact of HTN in a T2D cohort on metabolism, inflammation, and organ function.

6 CHAPTER SIX: CONCLUSION

This study described the effects of hypertension (HTN) comorbidity in T2D on inflammation, cardiovascular risk, and hepatorenal function. In this cohort, patients with T2D and HTN comorbidity (T2D+HTN) had a higher degree of inflammation and increased cardiovascular disease (CVD) risk when compared to those with T2D. The increased inflammatory state in (T2D+HTN) was associated with altered hepatorenal function. Hypertension comorbidity in T2D is associated with exacerbated levels of inflammation and increased CVD risk. Moreover, it is congruent with altered renal and hepatic function. Finally, modern therapeutic strategies in the treatment and management of T2D and HTN have been successful in adequately controlling the glycaemic and hypertensive state in these patients. However, the continual rise in the morbidity and mortality of T2D and HTN suggest strategies beyond glycaemia and blood pressure. Perhaps a good start would be addressing the underlying chronic inflammation and in doing so, normalising the metabolic alterations as well as lowering the threat of CVD in patients with HTN comorbidity in T2D.

7 CHAPTER SEVEN: STUDY LIMITATIONS

The current study had a few limitations. Firstly, is its cross-sectional nature, which lacked follow-up examinations of the patients. Therefore, a causal effect could not be established between HTN and the various findings of this study. A longitudinal study will be imperative in stratifying the cardiovascular risk over time. In the absence of waist measurements, the cardiometabolic index could not be calculated. This would have been instrumental in linking fat distribution with the various cardiovascular risk factors as well as determining the CVD risk of patients included in this study.

8 CHAPTER EIGHT: RECOMMENDATIONS

A longitudinal study is recommended to assess the AIP in patients with T2D and HTN as well as patients with only HTN or T2D. This would clarify the link between AIP and T2D and HTN. Furthermore, further studies assessing the use of SII in a T2D population would elucidate the use of this systemic inflammatory marker in T2D as well as the CVD risk attributed to it. In assessing CVD risk, the use of hepatic enzymes needs further research with a larger cohort of patients with T2D to eliminate confounding factors such as age, comorbidities, race, and gender disparities. In addition, it is also recommended that future research be focused on assessing the use of hepatic enzymes in the diagnosis of NAFLD, since other techniques such ultrasonography and x-rays are often not accessible in developing countries, such as Namibia. Finally, a greater benefit to third-world or developing countries would be further investigation in readily available biomarkers in assessing overall CVD risk, with a clear established reference ranges as guidance.

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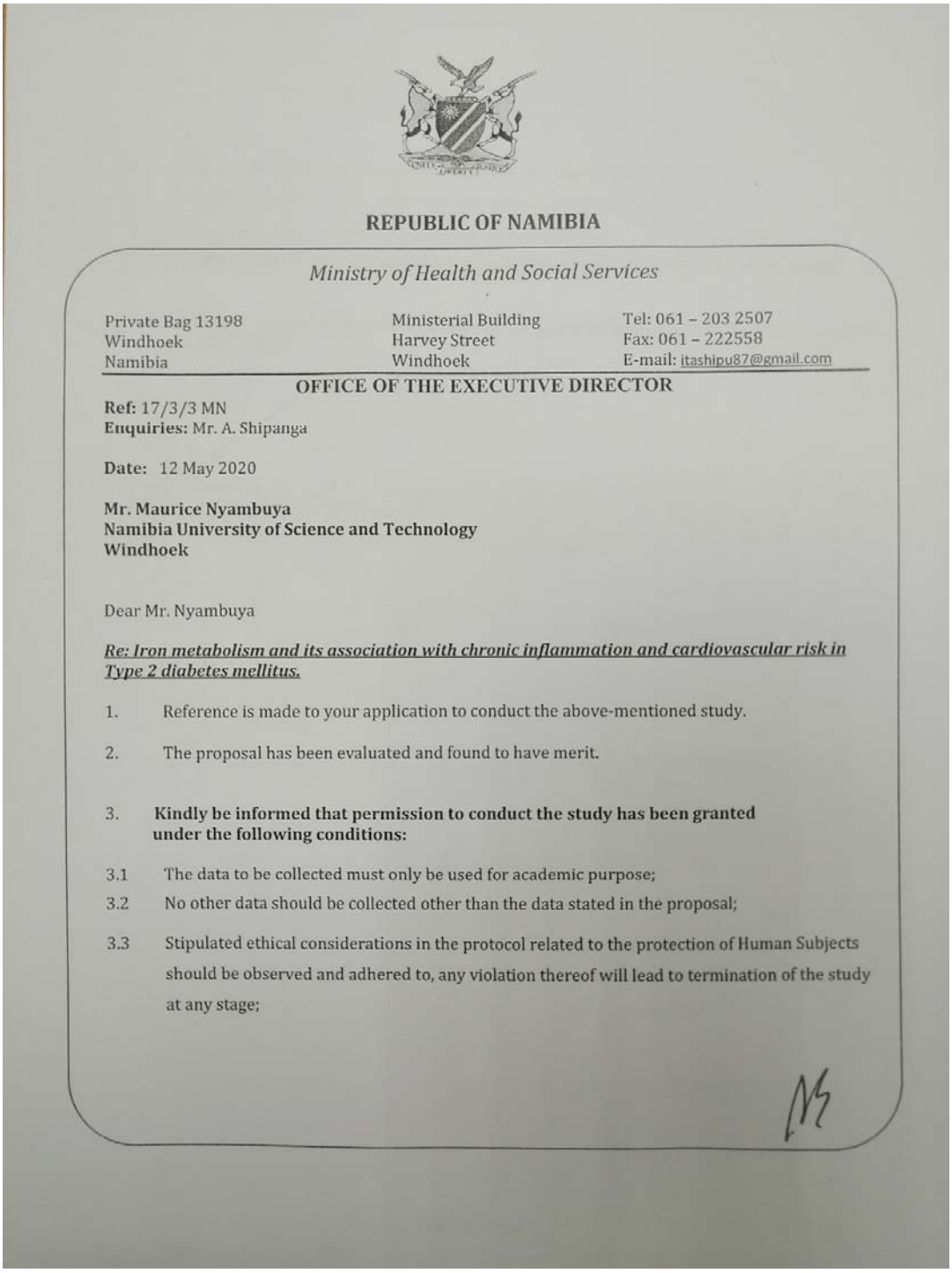
10 APPENDICES

Appendix A: Ethical clearance from the Namibian University of Science and Technology (FHAS 1/2020)



REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

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OFFICE OF THE EXECUTIVE DIRECTOR

Ref: 17/3/3 MN
Enquiries: Mr. A. Shipanga

Date: 12 May 2020

Mr. Maurice Nyambuya
Namibia University of Science and Technology
Windhoek

Dear Mr. Nyambuya

Re: Iron metabolism and its association with chronic inflammation and cardiovascular risk in Type 2 diabetes mellitus.

1. Reference is made to your application to conduct the above-mentioned study.

2. The proposal has been evaluated and found to have merit.

3. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**

3.1 The data to be collected must only be used for academic purpose;

3.2 No other data should be collected other than the data stated in the proposal;

3.3 Stipulated ethical considerations in the protocol related to the protection of Human Subjects should be observed and adhered to, any violation thereof will lead to termination of the study at any stage;

3.4 A quarterly report to be submitted to the Ministry's Research Unit;

3.5 Preliminary findings to be submitted upon completion of the study;

3.6 Final report to be submitted upon completion of the study;

3.7 Separate permission should be sought from the Ministry for the publication of the findings.

4. All the cost implications that will result from this study will be the responsibility of the applicant and **not** of the MoHSS.

Yours sincerely,

BEN NANGOMBE
EXECUTIVE DIRECTOR

"Health for All"

Appendix B: Ethical clearance from the Ministry of Health and Social Services (17/3/3 MN)

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FACULTY OF HEALTH AND APPLIED SCIENCES RESEARCH ETHICS COMMITTEE

(FHAS-REC)

DECISION/FEEDBACK ON RESEARCH PROPOSAL ETHICAL CLEARANCE

Dear: Prof/Dr/Mr/Ms/Other Mr. Maurice Nyambuya
NUST Staff Number: 1006785

Research Topic:	Iron Metabolism and its association with Chronic Inflammation and Cardiovascular Risk in Type 2 Diabetes Mellitus
Supervisor (if applicable):	Nil
Co-supervisor(s): if applicable	Nil
Qualification registered for (if applicable):	ND Biomedical Technology, B Tech Biomedical Technology, MSc Biomedical Technology.

Re: Ethical Screening Application No: FHAS 1/2020

The Faculty of Health and Applied Sciences Research Ethics Screening Committee has reviewed your application for the above-mentioned research project. Based on the recommendation of the expert reviewer, the research as set out in the application is hereby:

(Indicate with an X)

Approved: i.e. may proceed with the project, subject to Ministry of Health and Social Service Clearance.	X
Approved provisionally: i.e. may proceed but subject to compliance with recommendation(s) listed below	
Not approved: Not to proceed with the project until compliance with recommendation listed below and resubmit ethics application for consideration	

It is important to note that as a researcher, you are expected to maintain ethical integrity of your research. You are encouraged to strictly adhere to the research ethics policy of NUST. You should remain within the scope of your research proposal and support evidence as submitted to the FHAS-REC. Should any aspect of your research change from the information as presented, which could have an impact or effect on any research participants/subjects/environment, you are to report this immediately to your supervisor and to the FHAS-REC as applicable in writing. Failure to do so may result in withdrawal of approval.

Kindly consult the committee if you need further clarification in this regard. We wish you success in your research endeavour and are of the belief that it will have positive impact on your career as well as the development of NUST and the society in general.

Ethical issues that require compliance/ must be addressed : None		
No.	**Ethical issues**	**Comment/recommendation**

NB: May attach additional page as required

Sincerely Yours,

Name: Prof Sylvester R Moyo Signature: Date: 4th March 2020

Chairperson: FHAS Ethics Screening Committee.

Appendix C: Turnitin Report

Ernst Thesis

ORIGINALITY REPORT

22%	17%	16%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Fransina Ndevahoma, Bongani B. Nkambule, Phiwayinkosi V. Dludla, Munyaradzi Mukesi, Kandiwapa N. Natanael, Tawanda M. Nyambuya. "The effect of underlying inflammation on iron metabolism, cardiovascular risk and renal function in patients with type 2 diabetes", eJHaem, 2021 Publication	2%
2	worldwidescience.org Internet Source	1%
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