Cytokines: Role in Immune and Glucose Metabolic Function in Type 2 Diabetes Mellitus and Hypertension in Nigeria

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ABSTRACT

Cytokines are crucial for combating infections and for other immune responses, they can develop dysregulated and pathological in inflammation, trauma, sepsis, and hemorrhagic stroke. Cytokines are produced in response to invading pathogens to stimulate, recruit, and proliferate immune cellsincludes. They include interleukins, chemokines, interferons, tumor necrosis factors (TNF), colony-stimulating factors (CSF), lymphokines and monokines. This study is aim to determine the role of Cytokines in T2DM and hypertensive subjects among Nigeria population. This study involved 140 participants comprising 103 subjects representing the disease group, where 74 subjects (71.85%) were type 2 diabetes, 29 (28.16%) were type 2 diabetes with hypertension and 37 were control subjects. 57 (40.71%) were males and 83 (59.29%) were females of the age limit between 30 to 75 years. Enzymatic Spectrophotometry assay, and Boronate affinity method was adopted to determine FBS, lipid panels and glycated hemoglobin respectively. LDL-cholesterol and BMI was determined using calculations. This current study established that patients with type 2 diabetes, regardless of hypertension, had considerably higher LDL levels compared to the control groups. The result showed statistically significant means difference in FBS, TC and LDL. The pairwise comparison using Tukey Posthoc analysis showed that the level of the parameter was significantly higher in the DM and DM/HTN than the control group. LDL level in the control group (2.79±0.41) was considerably diminished than DM (3.36±0.55) and DM/HTN (3.39±0.72) groups. The average levels of IL-1β, IL-4, and TNFα were higher in the control group than the type 2 DM and T2DM/HTN groups, and were not statistically significant ($p>0.05$). The statistical model revealed that HbA1c and total cholesterol were independently associated with type 2 DM and T2DM/HTN groups. Indications has showed that systemic inflammatory response can stimulate atherosclerotic development. High lipid increase the risk of CVD morbidity and reduced cholesterol decreases CVD risk. Cytokines are fundamental actors in the development and progression of type 2 diabetes and cardiovascular disease especially hypertension. Their roles are unified, considering their relationship can consequently offer valuable awareness into diagnosis, prevention, treatment and prognostic strategies.

Keywords: Cytokines, Inflammation, Glycated, Cholesterol, T2DM, CVD.

INTRODUCTION

Type 2 diabetes (T2D) is one of the most common chronic metabolic disorders in adulthood worldwide, whose

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pathophysiology includes an abnormal immune response accompanied by cytokine dysregulation and inflammation. As the T2D-related inflammation and its progression were associated with the balance between pro and anti-inflammatory cytokines, anticytokine treatments might represent an additional therapeutic option for T2D patients (Chatterjee *et al.,* 2017; Velikova *et al.,* 2021).

Several proposition and hypotheses have been formulated to describe the mechanisms which are commonly implicated in the spreading of diabetes, centrally on T2D. The prevalence of the condition connected to well-recognised risk factors, like the adoption of a western lifestyle, sedentary lives, lack of physical activity and an energy-dense diet (Shaw *et al.,* 2010). Genetic predisposition, ethnicity and ageing are not modifiable risk factors for T2D, while others, such as being overweight or obese, an unhealthy diet, insufficient physical activity and smoking are modifiable through behavioural and environmental changes. However, increasing evidence has shown that inflammatory pathways are the principal, common pathogenetic mediators in the natural course of diabetes under the stimulus of the risk factors (Shoelson *et al.,* 2006).

Insulin resistance (IR) in mark organs (liver, adipose tissue, skeletal muscles) and pancreatic beta-cell pathology has a key role in the pathogenic mechanisms of T2D. IR is characterized by impaired insulin-mediated glucose uptake in target cells, which is the most common driving characteristic presenting throughout the progression from prediabetes to expressed T2D. In the pancreas, islet beta cells react to IR by increasing their mass, which results in compensatory increased insulin secretion. More than 30% of the obese people usually develop T2D based on the capability of their individual function of the islet beta cells to augument for IR. Furthermore, some T2D patients will gradually progress to overt insulin deficiency and will need insulin replacement therapy. (Donath and Shoelson 2011; Velikova *et al.,* 2021).

Cytokines

Cytokines comes from the ancient Greek language: *cyto*, (κύτος), *kytos*, meaning ***"cavity, cell"*** and *kines*, meaning (κίνησις), *kinēsis*, ***"movement"***. That is ***"cavity movement"*** or ***"cell movement".*** Cytokines are crucial for combating infections and for other immune responses. However, they can become dysregulated and pathological in inflammation, trauma, sepsis, and hemorrhagic stroke (Zhu *et al.,* 2019).

Cytokine is a general term used for small secreted proteins that are key modulators of inflammation that tell immune cells where to go and what to do to keep the immune system functioning correctly. Cytokines are produced in response to invading pathogens to stimulate, recruit, and proliferate immune cells. Cytokines includes interleukins, chemokines, interferons, and tumor necrosis factors (TNF) and colony-stimulating factors (CSF). Others include lymphokines and monokines which get their names from the type of cell they produced. Cytokines are subdivided based on the nature of the immune response and the source of their production (Scarpioni, 2016). Inflammation is part of the body's defence mechanism. It is the process by which the immune system recognizes and removes harmful and foreign stimuli and begins the healing process. Inflammation can either be acute or chronic (Michels *et al.,* 2019).

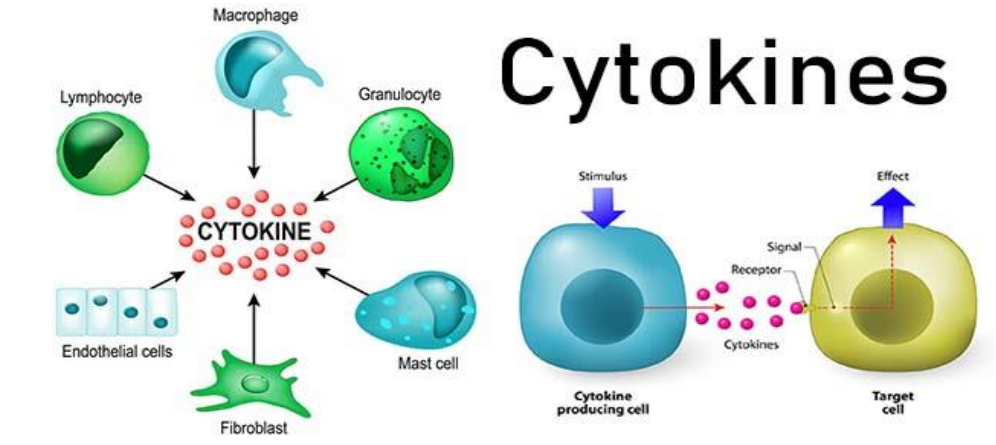

Figure 1: Signalling properties of Cytokines (Hayes *et al.,* 2021).

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The key functions of cytokines include initiate and amplify the inflammatory response (Abbas *et al.,* 2018); recruit immune cells to the site of infection or injury; increase blood flow and permeability of blood vessels and stimulate the production of other immune mediators (National Cancer Institute, 2019).

Clinical Importance of Cytokines

Proinflammatory cytokines are implicated in various diseases where inflammation plays a central role. Examples include ***autoimmune diseases*** like rheumatoid arthritis and psoriasis which mistakenly attacks healthy tissues (McInnes and Keystone 2017). ***Allergic reactions*** which activate the release of proinflammatory cytokines, leading to runny nose, itchy eyes, and swelling symptoms (Galli and Tsai 2010). ***Sepsis*** is a life-threatening condition caused by a severe infection. Uncontrolled production of proinflammatory cytokines can lead to organ damage and shock (Angus and van der Poll 2013).

Cytokines are subdivided into two groups: the anti-inflammatory and the pro-inflammatory cytokines. There are both pro-inflammatory and anti-inflammatory cytokines. The pro-inflammatory cytokines are secreted from Th1 cells, $CD4^+$ cells, macrophages, and dendritic cells. They are characterized by production of several Interleukins (IL), IL-1, IL-2, IL-12, IL-17, IL-18, IFN-γ, and TNF-α. The key pro-inflammatory cytokines are IL-1, IL-6, and TNF-α. These cytokines signal via type I cytokine receptors (CCR1) that are structurally divergent from other cytokine receptor types. They are crucial for coordinating cell mediated immune response and play a critical role in modulating the immune system. Pro-inflammatory cytokines generally regulate growth, cell activation, differentiation, and homing of the immune cells to the sites of infection with the aim to control and eradicate the intracellular pathogens, including viruses (Scarpioni, 2016).

Major anti-inflammatory cytokines are interleukin (IL)-1 receptor antagonist, IL-4, IL-6, IL-10, IL-11 and IL-13 (Opal and DePalo, 2000). Specific cytokine receptors for IL-1, tumour necrosis factor-alpha, and IL-18 also function as pro-inflammatory cytokine inhibitors. Anti-inflammatory cytokines are cytokines that ***"stop or lessen inflammation"***. The anti-inflammatory cytokines are series of immunoregulatory molecules that control the pro-inflammatory cytokine responses. The key pro-inflammatory cytokines include interleukine-1 (IL-1) which is subdivided into IL-1α and IL-1β (Scarpioni, 2016; Nelemans and Kikkert, 2019); interleukine-6 (IL-6) (Kany, Vollrath and Relja, 2019); tumor necrosis factor alpha (TNF-α) (Tay *et al.,* 2020) and chemokines, which have chemotactic activities (Clinical Microbiology Reviews, 2017). Specific cytokine receptors for IL-1, tumor necrosis factor-α, and IL-18 also function as proinflammatory cytokine inhibitors (Opal and DePalo, 2000).

Excessive chronic production of inflammatory cytokines contribute to inflammatory diseases, which are linked to different diseases, such as artherosclerosis and cancer (Slocum *et al.,* 2016). Dysregulation has also been linked to depression and other neurological diseases. A balance between pro-inflammatory and anti-inflammatory cytokines is necessary to maintain health. Aging and exercise also play a role in the amount of inflammation from the release of pro-inflammatory cytokines. Therapies to treat inflammatory diseases include monoclonal antibodies that either neutralize inflammatory cytokines or their receptors (Sallam and Laher 2016; Scarpioni, 2016).

Cholesterol and Cardiovascular Disease

Hypercholesterolemia is associated with the accumulation of LDL in the bloodstream. This condition is very often the result of genetic and environmental factors, like polygenic hypercholesterolemia, but it can also be due to specific genetic disorders, like familial hypercholesterolemia (FH). Facts has established it that patients with some inflammatory diseases like rheumatoid arthritis (RA), may exhibit a fall in LDL cholesterol, but they still have an increased CVD risk (Bernardi *et al.,* 2018). Additionally, it has shown that inflammation promotes some changes in the composition and quality of lipoproteins sub-fractions, eventually stimulating atherosclerosis development. Goldstein and Brown revealed that FH was as a result of genetic deficiency of the LDL receptor (LDLR), leading to an irregularly low uptake of LDL by the liver. They also discovered that families transmitted hypercholesterolemia as an autosomal dominant trait and this was associated with an intense increase in the incidence of cardiovascular disease (CVD) (Goldstein and Brown 2015). Till date, it has been anticipated that FH is a group of related disorders, due to several genetic defects in addition to LDLR

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mutation (Berberich and Hegele 2018).

Inflammatory Cytokines in Type 2 Diabetes Mellitus with Cardiovascular Diseases

Inflammatory reaction that causes numerous pro-inflammatory cytokines can play a fundamental role in the pathogenicity of T2DM, which can enhance insulin resistance thereby leading to impaired glucose homeostasis. The expression of various pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 is accumulated in adipose tissue and its expression connected to systemic inflammation and related to insulin resistance. In type 2 diabetes, when the serum concentration of the pro-inflammatory cytokines like TNF-α, IL-6 and CRP are elevated as result of over-nutrition, the innate immune system is activated (Velikova *et al.,* 2021).

Previous studies have shown that inflammatory factors, like interleukins, C-reactive protein, and plasminogen activators, are essential risk factors for cardiovascular consequence in patients with T2DM (Patrick and Uzick 2001; Chait and Hartigh 2020). Adipokine has a definite impression on promoting the event of diabetes with cardiovascular events. Adipokines include lipase, leptin, and tumor necrosis factors (Fuster *et al.,* 2016; Chait and Den Hartigh 2020).

Leptin take part in carbohydrate energy metabolism, inhibits fat synthesis, and promotes weight loss. A disorder of leptin function is one of the important reasons for insulin resistance. Tumor necrosis factor is primarily secreted by adipose tissue and lymphocytes and take part in insulin resistance, which is closely related to the incidence of diabetes and cardiovascular events. Lipase is a protease secreted by adipocytes and is principally implicated in the regulation of energy, glucose, and lipid metabolism; it is closely related to obesity and insulin (Havel 2002; Havel 2004). Obesity in T2DM is caused by fat aggregation due to daily excess calories in patients, an indications to diabetes (Sobczak *et al.,* 2019). So, handling diabetes patients with cardiovascular events, is necessitate to transform the participation of patients' lifestyles (Wan *et al.,* 2017; Huang *et al.,* 2018).

Earlier research have presented that inflammatory factors, like interleukins, C-reactive protein (CRP), and plasminogen activators, are essential dangerous influences for cardiovascular events in patients with T2DM (Chait and Den Hartigh 2020). Reasonable exercise increases the basal metabolic rate and improves insulin sensitivity to better control blood glucose levels and reduce the occurrence of complications. (Wan *et al.,* 2017).

The aim of this study is to determine the role of Cytokines in T2DM subjects among Nigeria population. That is, to determine the relationship between fasting blood sugar, fasting lipid panels inflammatory cytokines [of interleukin 1 beta (IL-1β); interleukin 4 (IL-4); tumour necrosis factor alpha (TNF-α) and glycated haemoglobin (HbA1c)] among type 2 diabetes with/without cardiovascular diseases.

MATERIALS AND METHODS

Ethical Consideration

Ethical approval was obtained from the concern ethical committee groups in Ondo State, Nigeria. Informed consent were obtained from the study participants prior to enrolment into the study.

Data Collection

Structured questionnaire were used to collect socio-demographic data, medical history of the study participants were obtained from the medical records department. A total of 140 participants was recruited for this research study, which was determined by Leslie Fisher's formula based on the frequency of Type 2 diabetes mellitus in Nigeria (Kirkwood, 2010), 103 were subjects with T2DM (with or without cardiovascular diseases) and were randomly selected for the study. 74 were subjects with T2DM, 29 were subjects with T2DM and hypertention, while 37 subjects were recruited as control.

A 9-12hours fasting samples was used for this study. 8mL of blood sample was taken from each participant;

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5mL was dispensed into Ethylenediamine tetraacetic acid (EDTA) bottle (in ice pack) for glycated hemoglobin (HbA1c) and fasting lipid profile (FLP) assays. The remaining 3mL was dispensed into fluoride oxalate bottle for fasting blood glucose. Glycated hemoglobin (HbA1c) was analysed from EDTA bottle same day the blood samples were collected. The rest samples were prepared by separating them into plain bottles for different parameters.

Analytical Procedures

Enzymatic method was adopted for estimating fasting blood glucose, total cholesterol, HDL-cholesterol and triglyceride using Spectrophotometry assay, and Friedewald's formula was adopted for LDL-cholesterol. Boronate affinity method was adopted to determine the percentage (%) of A1c hemoglobin (HbA1c) in whole venous blood using reflection spectrometry (Clover A1c Self Analyser), fully automated and it is determined in percentage (%).Sphygmomanometer was used to measure the blood pressure, and the BMI was calculated using the weight in kilograms (kg) divided by the square of the height in meters (m^2).

Inclusion Criteria

Adult and elderly patients with age range of 30-70 years old with recent diagnosis of T2DM, based on the World Health Organization criteria, were included in this study. Therefore, patients were considered to have T2DM if they fulfilled all of the following criteria: "HbA1c ≥ 6.5%, Fasting Plasma Glucose (FPG) ≥ 117 mg/dL (6.5 mmol/L),

Exclusion Criteria

Patients who were taking lipid-lowering therapy or those with cardiovascular diseases, endocrinal conditions, liver function impairment, or renal problems were excluded from the study. Furthermore, patients with mental problems were also excluded from the study.

Data Analysis

In the present study, a total of 140 subjects comprising 103 subjects representing the disease group, where 74 subjects (71.85%) were type 2 diabetes, 29 (28.16%) were type 2 diabetes with hypertension and 37 were control subjects. 57 (40.71%) were males and 83 (59.29%) were females of the age limit between 30 to 75 years and both male and female genders was recruited.

RESULTS

Table 1: Sociodemography

Variables	Groups	Control n (%)	DM n (%)	DM/HTN (%)
Sex	Females	20 (54.1)	48 (64.9)	15 (51.7)
	Males	17 (45.9)	26 (35.1)	14 (48.3)
Age	≤40	7 (18.9)	4 (5.4)	4 (13.8)
	41-60	20 (54.1)	44 (59.5)	18 (62.1)
	61-80	10 (27.0)	26 (35.1)	7 (24.1)
	Total	**37 (100)**	**74 (100)**	**29 (100)**

The distribution table presents demographic characteristics of participants categorized by sex and age groups in both control and case groups. The mean age of participants in the case group (53.53 years) is slightly lower than that of the control group (56.17 years), but the difference is not statistically significant as seen in tables 2.

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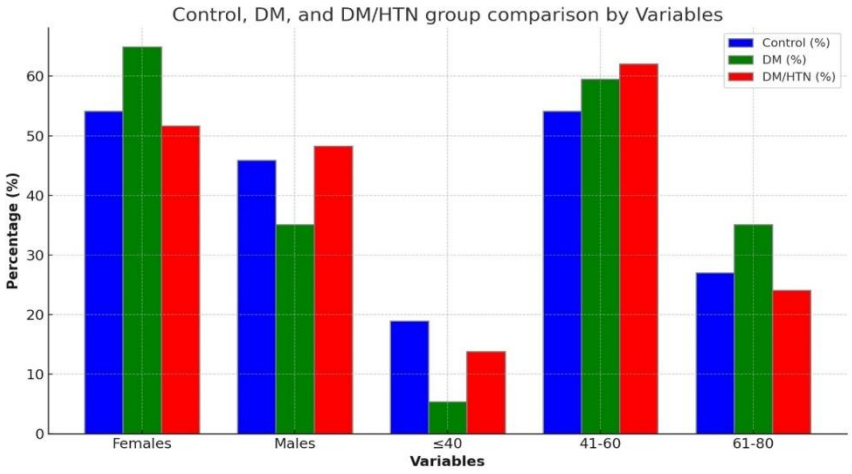
The study successfully achieved age matching, as there is no statistically significant difference in age between the control and case groups.

Figure 2:. Sociodemographic Characteristics of the Study Participants and the Age-Matched comparison



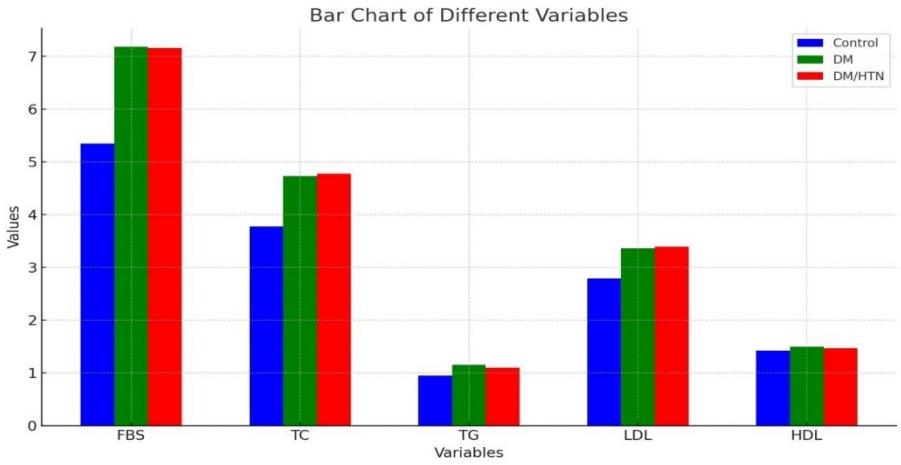
The above bar chart compares the Control, DM, and DM/HTN groups across the categories of Sex and Age. The bars represent the percentages for each group within the specified categories.

Table 2: Mean Fasting Blood Sugar and Fasting Lipid Panel of the groups

Variable	Control	DM	DM/HTN	F-value	p-value
FBS	5.35±0.6[a]	7.18±2.3[b]	7.16±2.82[b]	9.99	<0.001
TC	3.78±0.62[a]	4.73±0.73[b]	4.78±0.8[b]	24.72	<0.001
TG	0.95±0.33	1.15±0.52	1.1±0.36	2.42	0.09
LDL	2.79±0.41[a]	3.36±0.55[b]	3.39±0.72[b]	14.24	<0.001
HDL	1.42±0.26	1.5±0.27	1.47±0.3	1.007	0.37

a, b, value with same superscript were not statistically significantly difference at $p<0.05$.

Figure 3: The Analysis of Variance (ANOVA) Comparing the Groups



The analysis of variance (ANOVA) revealed significant differences in fasting blood sugar (FBS), total cholesterol (TC), and low-density lipoprotein (LDL) levels among participants grouped by disease conditions: Control, DM (Diabetes Mellitus), and DM/HTN (Diabetes Mellitus with Hypertension). The result showed

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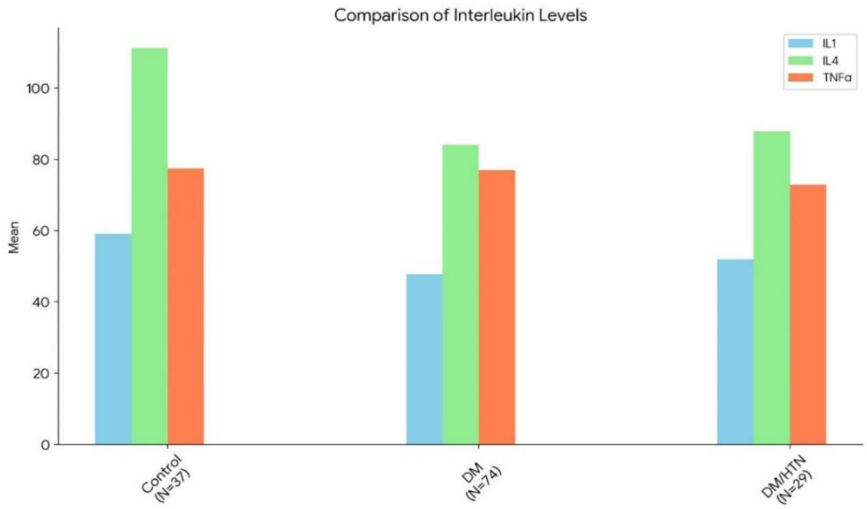
statistically significant means difference in FBS, TC and LDL. The pairwise comparison using tukey posthoc analysis showed that the level of the parameter was significantly higher in the DM and DM/HTN than the control group. The level of LDL of the control group (2.79±0.41) was significantly lower than that of DM (3.36±0.55) and DM/HTN (3.39±0.72). The mean difference between the DM and DM/HTN was statistically not significant (P>0.05)

Table 3: The Association between Cytokines and Disease Condition with/without Cardiovascular

Variable	Groups	N	Mean±SD	F-value	p-value
IL-1β	Control	37	59.09±27.78	2.54	0.08
	DM	74	47.78±24.32		
	DM/HTN	29	52.05±22.64		
IL-4	Control	37	111.31±88.15	2.83	0.06
	DM	74	84.07±40.44		
	DM/HTN	29	87.82±46.2		
TNF-α	Control	37	77.44±35.81	0.16	0.85
	DM	74	77.04±36.25		
	DM/HTN	29	72.92±34.85		

Figure 4: Association between Cytokines and the Groups



The table and bar chat above shows the level of the inflammatory cytokines across the groups. The level of interlukin-1β, interlukin-4 and TNF-α was higher in control groups than the type 2 DM with/without HTN groups. The measure of mean difference was statistically not significant (p>0.05).

Table 4: The association between the Cytokines, FBS, BMI, systole and Diastole across the groups

		FBS		Systole		Diastole		BMI	
Status	Variable	R	R^2	R	R^2	R	R^2	R	R^2
Control	IL-1β	0.07	0.005	0.136	0.018	0.242	0.059	0.069	0.005

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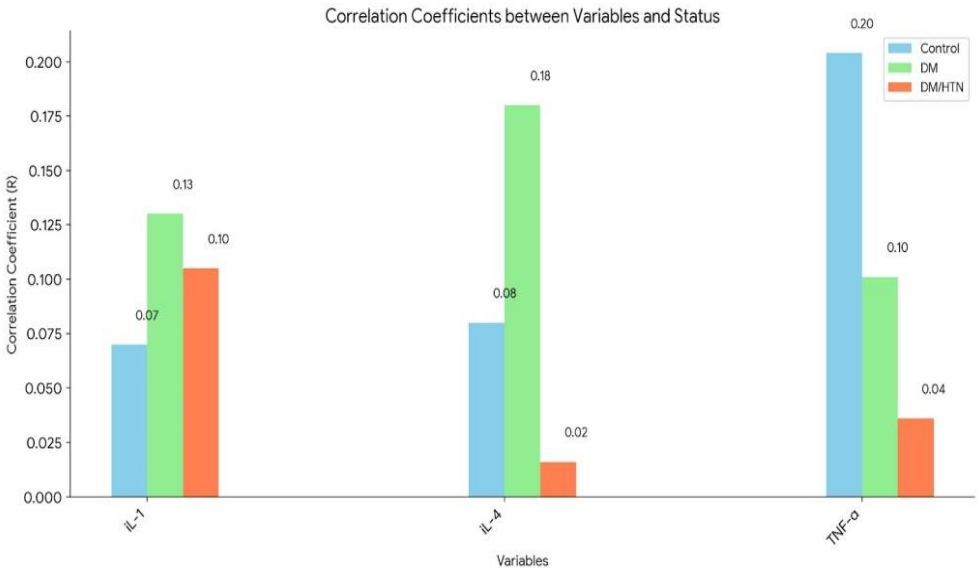
	IL-4	0.08	0.006	0.106	0.011	0.013	0.000	0.280	0.079
	TNF-α	0.204	0.042	0.064	0.004	0.005	0.000	0.094	0.009
DM	IL-1β	0.130	0.017	0.178	0.032	0.141	0.020	0.018	0.000
	IL-4	0.180	0.032	0.112	0.013	0.003	0.000	0.043	0.002
	TNF-α	0.318*	0.101	0.176	0.031	0.091	0.008	0.034	0.001
DM/HTN	IL-1β	0.105	0.011	0.144	0.021	0.156	0.024	0.005	0.000
	IL-4	0.016	0.000	0.063	0.004	0.022	0.000	0.205	0.042
	TNF-α	0.036	0.001	0.152	0.023	0.201	0.040	0.338	0.114

Figure 5: Bar Chat Showing the association between the Cytokines and the Groups



The table showed the correlation (R) and coefficient of determination (R^2) between DM, DM/HTN and other associated variables. The result showed statistically significant positively low (**0.318**) correlation between TNF-α and FBS, while TNF-α account for 10.1% (**R^2=0.101**) changes in FBS levels among type 2 diabetic mellitus patients without cardiovascular disease. The relationship between other variables was not statistically significant, with poor correlation except association of TNF-α with BMI which showed non-statistically significant positively low correlation and account for 11.4% variances in BMI of the type 2 diabetes with cardiovascular disease.

Table 5: The Multinomial regression analysis

Status	Variable	B	p-value	OR (95%CI)
DM	**Age**	0.00	0.92	1.00 (0.96 - 1.05)
	HbAic (%)	0.91	0.01	2.47 (1.23 - 4.98)
	TC	1.50	0.01	4.64 (1.5 - 14.41)
	LDL	1.32	0.05	3.71 (0.99 - 14.07)
	IL4	-0.01	0.06	0.99 (0.98 - 1.00)

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	[Sex=Female]	0.28	0.61	1.31 (0.45 - 3.84)
	[Sex=Male]	0b		1
DM/HTN	**Age**	-0.03	0.29	0.97 (0.92 - 1.02)
	HbAic (%)	0.59	0.02	2.55 (1.21 - 5.33)
	TC	1.71	0.01	5.10 (1.50 - 18.54)
	LDL	1.34	0.09	3.68 (0.83 - 16.31)
	IL4	-0.01	0.16	0.99 (0.98 - 1.00)
	[Sex=Female]	-0.20	0.75	0.82 (0.24 - 2.77)
	[Sex=Male]	0b		1

The table showed the multinomial regression analysis. The multinomial regression analysis was conducted to investigate the predictor variables that independently associated with the conditions with other variables held constant. The independent variables that were statistically significant at univariate analysis were entered into the model. Age and sex were entered into the model being important biological factor. Variable that showed non-normality and multicollinearity were remove from the model. The test of model fitting statistics showed that variable entered into the model was fit (χ^2=67.80, d=12, p<0.001). Among all the independent variables entered into the model HbA1c and Total cholesterol were statistically significantly (p<0.05) associated with the type DM with or without HTN independently with other variable held constant.

DISCUSION

This current finding show that the control group has a significantly lower LDL level compared to both DM (Diabetes Mellitus) and DM/HTN (Diabetes Mellitus with Hypertension) groups. This aligns with previous study of Rahman et al (2022) which was established that patients with type 2 diabetes, regardless of hypertension, had considerably higher LDL levels in relation to healthy or control groups. The result showed statistically significant means difference in FBS, TC and LDL. The pairwise comparison using Tukey Posthoc analysis showed that the level of the parameter was significantly higher in the DM and DM/HTN than the control group. The level of LDL of the control group (2.79±0.41) was considerably diminished than that of DM (3.36±0.55) and DM/HTN (3.39±0.72). The mean difference between DM and DM/HTN was statistically not significant (P>0.05). Again, Serum lipid profile in hypertensive and normotensive type 2 diabetes mellitus patients (a comparative study) also conformed with the work of Unlucelik et al (2003) that demonstrated that both hypertensive and normotensive type 2 diabetics had elevated LDL levels compared to a healthy control group. These studies, along with the current findings, suggest a strong link between diabetes and elevated LDL cholesterol.

Again, findings from this study also show that the control group has a significantly lower TG level compared to both DM (Diabetes Mellitus) and DM/HTN (Diabetes Mellitus with Hypertension) groups (p-value = 0.09). This is disagreeing with previous study of Son, (2019) where the TG values of patients with T2DM were significantly higher than those with non-diabetes patients (Son, 2019).

In this present study, the average levels of IL-1β, IL-4, and TNF-α were higher in the control group than in the type 2 DM groups, the difference was not statistically significant (p>0.05), this is in accordance with the study of Smith and Doe, (2023) which might be due to variability within groups, small sample size, or the effect of other factors (Smith and Doe 2023). This is contradictory to the studies of Botha-Scheepers et al (2008) and Everett et al (2018) which indicated that elevated pro-inflammatory cytokines, such as IL-6 and TNF-α, have been observed to accompany anti-inflammatory IL-10 production in certain inflammatory conditions to compensate for inflammation (Botha-Scheepers *et al.*, 2008 and Everett *et al.*, 2018). Predominantly, pro-inflammatory mediators have been implicated in the pathogenesis of both type 2 diabetes and cardiovascular

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disease (CVD). Their roles in these conditions are complex and interconnected. Cytokines, like interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), can contribute to insulin resistance, which is a key feature of type 2 diabetes. These cytokines can impair insulin signalling in peripheral tissues, leading to reduced glucose uptake and elevated blood sugar levels. Chronic inflammation, mediated by cytokines, can damage pancreatic beta cells, which produce insulin. This can lead to a decrease in insulin secretion, further exacerbating hyperglycemia.

Cytokines play a pivotal role in the development of atherosclerosis, a key risk factor for CVD. They contribute to the formation of atherosclerotic plaques by promoting inflammation, oxidative stress, and the migration of immune cells to the arterial wall. Cytokines can impair the function of endothelial cells, which line the blood vessels. This can lead to increased vascular permeability, thrombosis, and a pro-inflammatory environment that favours atherogenesis. Type 2 diabetes and CVD share numerous risk factors, like obesity, physical inactivity, and a high-fat diet. These factors can also contribute to increased cytokine production, and further worsening the risk of both conditions. The presence of type 2 diabetes can amplify the risk of CVD, and vice versa. This is partially due to the collective pathophysiological mechanisms involving cytokines and inflammation (Smith and Doe, 2023).

This study shows a positively low correlation of 0.318 between TNF-α and FBS in type 2 diabetic mellitus patients without cardiovascular disease which suggests a weak positive correlation between these two variables. However, the R2 value of 0.101 indicates that only 10.1% of the changes in FBS can be explained by variations in TNF-α. This inconsistency can be attributed to numerous reasons. FBS levels are influenced by various factors like insulin resistance, glucose production, renal function and cardiovascular disease itself. TNF-α may be one of these factors, but it is unlikely to be the sole determinant of FBS changes. Other factors may contribute more significantly to the noticeable variations in FBS, making it difficult to isolate the individual effects of any specific variable (American Diabetes Association, 2022). The incidence of impenetrable factors can obscure the true relationship between variables for example, the association between a variable and FBS may be irritating by the presence of cardiovascular disease, making it difficult to determine whether the variable is directly influencing FBS or if the observed relationship is merely a reflection of the underlying cardiovascular disease (Hotamisligil, 1999). The relationship between TNF-α and FBS may be more multifaceted than a simple linear correlation. There might be non-linear or threshold effects, where changes in TNF-α only impact FBS levels beyond certain thresholds. Additionally, the effect of TNF-α on FBS might be mediated by other factors like insulin or adiponectin (Hotamisligil, 1999). Correlation analysis can only establish a relationship between two variables, but it cannot determine causativeness. Variations in FBS can lead to fluctuations in TNF-α, since correlation analysis does not account for impenetrable factors that might influence both TNF-α and FBS levels, this is in accordance with Grundy and Hansen, (2004) and Grundy et al (2019) which showed that elevated levels of certain cytokines like interleukin-6, tumor necrosis factor-alpha have been associated to increased body mass index (BMI), fasting blood sugar (FBS), and insulin resistance (Grundy and Hansen, 2004; Grundy *et al.,* 2019). It has also be implicated that higher BMI is frequently associated with increased systolic and diastolic blood pressure, as well as elevated FBS and lipid levels (World Health Organization, 2000); and chronic inflammation, facilitated by cytokines, plays a crucial role in the development of atherosclerosis and other cardiovascular diseases (Ross, 1999). Occasionally quantitative error can introduce noise into the data, making it difficult to detect true relationships between variables.

In this present study, the statistical model revealed that HbA1c and total cholesterol were independently associated with the presence of type 2 diabetes mellitus (DM), both with and without hypertension (HTN) which is in line with Deshmukh et al (2015) and Naqvi et al., (2017). In contrary, our results are inconsistent with another study which also reported no significant relationship between these parameters that found no relationship between HbA1c and TC or LDL-C (Alzahrani *et al.,* 2019). However, these results are inconsistent with the results of other numerous studies that have stated a significant relationship between HbA1c and TC and LDL-C (Deshmukh *et al.,* 2015; Hussain *et al.,* 2017; Kundu *et al.,* 2017). HbA1c was a significant predictor of diabetes mellitus status with or without HTN. For each unit increase in HbA1c, the odds of having diabetes increase by approximately 2.47 and 2.55 times in DM and DM/CVDs respectively compare to the control. TC was a significant predictor of diabetes mellitus status without or with HTN. For each unit increase in TC, the odds of having diabetes without or with HTN increase by approximately 4.64 and

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5.10 times respectively compare to the control. For Sex (being female) was not a statistically significant predictor of diabetes mellitus with or without HTN (p-values = 0.61 and 0.74). The odds ratio suggests that females may have slightly lower odds (0.82) of having diabetes with HTN compared to male.

This means that even when controlling for the effects of other factors in the model, changes in HbA1c and total cholesterol levels were still significantly linked to the development or progression of DM, regardless of whether HTN was present. This study also show that LDL and iL-4 showed a marginal statistically significant association with DM without CVDs which correlates with the study of Reklou et al (2018). Research have shown that the systemic inflammatory response can stimulate atherosclerosis development. Based on this concept, it has been shown that IL-1β and TNF-α promote LDL binding to various cell types in vitro (Reklou *et al.*, 2018). ***"Lipid hypothesis"*** recommends that there is a linear relationship between cholesterol and the risk of CVD morbidity, implicating that cholesterol reduction drops CVD, and considerable scientific efforts has been put into effective ways to lower blood cholesterol. Previous studies have suggested a marginal association between low-density lipoprotein (LDL) cholesterol and interleukin-4 (IL-4) levels and the development of type 2 diabetes mellitus (DM) without cardiovascular diseases (CVDs) Bernardi et al (2018) found a statistically significant positive correlation between LDL cholesterol and IL-4 levels in patients with DM but without CVDs (Bernardi *et al.*, 2018).

CONCLUSION

Lipid metabolism and the immune system are interwoven. The prevalence of obesity, lipids and interleukins symbolises the key mediators of cardio-metabolic diseases. Cytokines are fundamental actors in the development and progression of type 2 diabetes and cardiovascular disease especially hypertension. Their roles are unified, considering their relationship can consequently offer valuable awareness into diagnosis, prevention, treatment and prognostic strategies. Therefore, more research is necessary to entirely comprehend the relationship between type 2 DM, hypertension, and the levels of IL-1β, IL-4, and TNFα; and as well as innovative lipid mediators with anti-inflammatory properties, which could characterized new promising therapeutic tools.

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