doi.org/10.36721/PJPS.2021.34.3.SP.1217-1225.1

Influence of hs-CRP, IL-6 and TNF-α and it's role in dyslipidemia and type 2 diabetes in population of Karachi, Pakistan

Muhammad Nisar and Mehir-un-Nisa Iqbal
Department of Physiology, University of Karachi, Karachi, Pakistan

Abstract: The present study was conducted to estimate the prevalence of pro-inflammatory cytokine interleukin-6, highly sensitive C-reactive protein, and tumor necrotic factor alpha and evaluate the association and role of these inflammatory markers in the pathogenesis of type 2 diabetes mellitus. A retrospective case-control study was conducted in Karachi. 400 individuals participated in the study having 200 diabetic patients and 200 controls. The subjects' profile and anthropometric indices were recorded and the levels of FPG, fasting insulin, lipid profile, IL-6, and hs-CRP were determined. Insulin resistance, beta-cell function and sensitivity were calculated by HOMA analysis using the HOMA calculator. Using independent t-test BMI, percent body fats, HbA1c, FPG, and fasting insulin were found significant ($p<0.05$). HOMA-IR, percent beta cell, total cholesterol, triglyceride, and HDL showed significant ($p<0.05$) results among cases and controls. Similarly, TNF-α and hs-CRP were also found significant ($p<0.05$) in cases than controls. Multiple linear regression was performed to predict the values of FPG, fasting plasma insulin, and IL-6. All models were statistically significant ($p<0.05$). The current study reveals that inflammation is the fundamental mechanism in obesity-induced insulin resistance, and T2DM, expanded fat stores in the body, and sedentary lifestyle are involved in the alteration of metabolic processes.

Keywords: Highly sensitive C reactive protein, homeostasis model assessments for insulin resistance, tumor necrotic factor-alpha, interleukin 6, type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus is an abundantly implemented term to signify a complex group of syndromes with a mutual disorder in oxidation and utilization of glucose, which is secondary to the malfunction of pancreatic beta cells that are tasked to produce and discharge insulin. It is typically a protracted ailment with ten times less life expectancy and long-term blood glucose complications. Ancient Egyptians identified diabetes as a rare illness characterized by excessive urination and weight loss (Lee *et al*., 2009, Anari *et al*., 2017). Some of the features are similar to the metabolic syndrome, which have provoked the interest of the world, involving metabolism of the body, diet, inappropriate lifestyle, genetic illusion, age factor, and fat consumption (Singh *et al*., 2004, Chan, 2017). Incidence of the development of diabetes has increased markedly since 1960 in parallel with obesity (Pickup, 2004, Pickup, 2006, Catalina *et al*., 2019). In 2000, 5.2 million people were diagnosed with diabetes in Pakistan and this ratio is likely to increase up to 13.9 million by the year 2030, leading Pakistan to the fourth highest rank of diabetes in the world (Nadeem *et al*., 2013). Type 2 diabetes is influenced by obesity, which is also becoming predominant and has continually led to augment the prevalence of diabetes. Low-grade inflammation is deliberated to be linked with T2DM and obesity that may further be linked with cardiovascular complications, possibly including a cytokine-facilitated acute phase response to infections and inflammatory events. Pro-inflammatory cytokines such as IL-6 and TNF-α stimulate the liver for the production of acute-phase C-reactive protein (Andrews Guzman and Arredondo Olguin, 2014, Cheng *et al*., 2018). The etiology of CRP in the development of T2DM is still controversial, suggesting that other factors are associated in the onset of disease (Lee *et al*., 2009, Phosat *et al*., 2017).

Obesity is also diagnosed in 80% to 90% of the population diagnosed as diabetic. Therefore, obesity accounts for susceptibility to the expansion of T2DM (Akbarzadeh *et al*., 2013, Pickup, 2006, Basit *et al*., 2021). Research study shows that pro-inflammatory cytokines are released due to expanded abdominal fats, decreasing the sensitivity of insulin-responding cells towards insulin (Milas *et al*., 2020). This reduced insulin activity is the stimulus for insulin resistance and consequent T2DM. C-reactive protein is an extremely sensitive marker for systemic inflammation produced primarily by the liver upon stimulation of adipocyte-derived pro-inflammatory cytokine (Valtierra-Alvarado *et al*., 2020, Akbarzadeh *et al*., 2013).

Excess accumulation of fats is associated with increased hypertrophy and hyperplasia of adipocytes linked with increased angiogenesis, macrophages infiltration, endothelial cell activation, production of matrix components and release of inflammatory cytokines. Pro-inflammatory markers are primarily secreted from adipocytes, but a large quantity is secreted by white

*Corresponding author: e-mail: mehirunisa@uok.edu.pk

adipose tissue-derived macrophages. Dys regulation of pro-inflammatory cytokine production and its function in obese individuals leads to prolonged inflammation, which further has its effects in metabolic disorder, cardiovascular problems, and insulin resistance (Simental-Mendia *et al*., 2012, Elimam *et al*., 2019). In obesity, the adipocyte stores fat and becomes lipolytic, which increases the chances of free fatty acids to have an effect on the non-adipose organs resulting in insulin resistance and ultimately the incidence of T2DM (Fadaei *et al*., 2020, de Marañón *et al*., 2020). Knowledge about the pathophysiological instabilities may help prevent further complications of the disease. The role of Inflammation is crucial in the emergence of type 2 diabetes mellitus (Lee *et al*., 2009, Nadeem *et al*., 2013) The complications correlated with diabetes can be reduced by preliminary diagnosis, intensive care and appropriate treatment. The world is now diverted to screen the asymptomatic individuals to estimate the ultimate risk for T2DM (Organization, 2003, Catalina *et al*., 2019).

Inflammation has acquired universal consideration as the foremost contributor to many illnesses such as arthritis, atherosclerosis and cancer. This chronicity, the systemic low-grade inflammation which is invisible to the eyes, progresses gradually and produces deleterious outcomes (Al-Rashed *et al*., 2019). Obesity has recently been added to this group that is involved in the induction of insulin resistance and type 2 diabetes mellitus. Research is assessing the mystery of why obesity provides the basis for inflammation and induces insulin resistance (Demyanets *et al*., 2020).

Pakistan is undergoing an incredible and rapid transformation in the analytical, socio-economic, and nutritional scheme. Escalating intensities of nutritional status categorized by the shift in energy levels from higher carbohydrate with lower fats diet toward lower carbohydrate with high saturated fat intake in addition to physical inactivity, revealed the population to be at high risk of obesity and type 2 diabetes mellitus, the rapidly changing diabetic profile in Pakistan taking it in the top 5 countries list by 2030.

Although, there is sufficient research to learn the association of inflammatory cascade in obesity-induced insulin resistance. This study comprehended the role of interleukin 6, TNF-α, and high sensitive C–Reactive Protein in the pathogenesis of type 2 diabetes mellitus. This research proposed a future guideline for understanding of the disease by highlighting the role of pro-inflammatory markers in humans. This study hypothesized the prevalence of obesity and circulating intensities of hs-CRP and IL-6 increased among type 2 diabetic patients. Pro-inflammatory markers including hs-CRP, IL-6, and TNF-α are associated with the pathogenesis of type 2 diabetes mellitus. IL-6, TNF-α, and hs-CRP are the autonomous predictors for insulin resistance and consequent type 2 diabetes mellitus. This investigation aims to determine circulating levels of TNF-α, hs-CRP, and IL-6 in patients with type 2 diabetes mellitus. To outline the impact of proinflammatory markers in the variability lipid profile in patients of type 2 diabetes mellitus. To explore the effects of TNF-α, IL-6, and hs-CRP on the risk of type 2 diabetes mellitus.

MATERIALS AND METHODS

A retrospective case-control study was conducted in the Department of Physiology, University of Karachi, from January 2020 to December 2020 after taking ethical approval IBC KU-112/2020 from Institutional Bioethics Committee, University of Karachi. All participants in the current study were instructed to give their informed consent voluntarily. All the participants under study were fully aware that they reserved the right to withdraw from the study at any time, even if they had previously given consent to be part of the study. The findings of the study were treated with the highest possible degree of confidentiality. Each subject received a unique identity number. After completion of the data collection and when the subjects received their results, the data was anonymized. Names were deleted so that the data could not be traced back to any other research purposes.

The participants of the study were from both sexes (n = 400), (45-55 year of age) from Karachi with an almost similar socio-culture background. Further, those belonging to a similar group attending private diabetic clinics were also included. The study population was diagnosed with diabetic patients, as per the ADA criteria and aged, sex-matched normal healthy controls. Information about general, medical and familial aspects was gathered through a questionnaire. Routine procedures for the measurement of anthropometric profiles including BMI, W/H ratio, BMR, and percent body fats were carried out (Shiwaku *et al*., 2005). For body mass index, weight was measured using a digital portable scale with a capacity of 200kg and accuracy of 0.01kg with the participants wearing light clothing and no shoes. An elastic metric tape with a 0.5cm scale was used to measure height. On the Frankfurt plane, the participants were told to stand upright and motionless, with their hands flat on their tights and their heads adjusted. Skinfold clipper was used for estimation of percent body fats. Diagnostic approach as recommended by WHO followed by ADA was followed to screen the subjects including plasma glucose, HbA1c and occurrence of ketonuria. After FPG and ketone urea, the plasma insulin level of selected control and patients was measured using the ELISA technique. Specimens were collected at home after 12 hours of fasting. At the time of blood collection, all participants were asked to provide consent for blood tests (Trinder, 1969)

Table 1: Characteristic of study participants

Study Parameters	Minimum	Maximum	Mean & SD
Age in years	45	55	48.9±3
BMI (inches)	17	35	25.92±4.73
WHR	0.62	1.4	1.02±0.22
Body Fats (%)	16	39	26.34±5.93
Systolic BP (mm of Hg)	100	155	135.34±9.49
Diastolic BP (mm of Hg)	60	105	77.91±7.72
Pulse Rate (b/min)	30	109	63.84±24.64
Stress Score	54	250	155.89±47.11
FPG (mg/dl)	64	154	104.5±28.16
FPI (uU/ml)	2.9	61	12.46±11.01
HBA1C	4.6	9.9	6.37±1.24
HOMAIR	0.35	145	2.16±10.18
Beta Cell (%)	36.7	203.7	87.51±31.82
Beta cell Sensitivity (%)	13.5	288.9	113.88±76.9
Total Cholesterol (mg/dl)	132	446.5	203.96±53.7
Triglyceride (mg/dl)	46.79	360.12	152.38±43.05
HDL (mg/dl)	19.49	85.54	61.01±9.87
LDL (mg/dl)	13	256.46	103.36±38.5
VLDL (mg/dl)	9.35	72.02	30.19±9.12
Serum Creatinine (mg/dl)	0.6	1.8	1±0.28
eGFR (ml/min/m2)	30	91	66.07±12.33
BUN (mg/dl)	8	29	14.43±4.1
IL6 (pg/ml)	21	311	121.96±57.86
hs-CRP (mg/l)	0.2	9.1	2.39±1.87
TNF ALPHA(pg/ml)	1.1	57	14.28±14.79

Table 2: Comparison of Anthropometry, FPG, Fasting Insulin, HOMA-IR, Percent Beta Cell, Lipid Profile and IL-6, hs-CRP, TNF-α Between Diabetic and Non-Diabetic

Study Parameter	Non-Diabetic	Diabetic	Sig
	N=200	N=200	
BMI	21.63±1.8	30.21±2.14	.009
WHR	.82±.09	1.22±.10	.576
BF (%)	21.3±2.72	31.38±3.4	.000
Systolic BP (mm of Hg)	115.6±5.78	128.3±8.17	.000
Diastolic BP (mm of Hg)	75.3±5.8	80.44±8.5	.000
FPG(mg/dl)	78.21±8.2	130.78±11.5	.000
FPI (uU/ml)	6.2±7.2	18.68±10.65	.000
HBA1C	5.28±.26	7.47±.77	.000
Percent Beta Cells	98.9±34.3	76.09±24.21	.001
Beta cell sensitivity	173.1±63.2	54.6±28.15	.000
HOMAIR	2.11±.14	2.20±.89	.032
Percent Beta cell	98.93±37.3	76.09±24.2	.001
Total Cholesterol (mg/dl)	168.9±15.3	239.01±55.6	.000
Triglyceride (mg/dl)	144.72±34.5	235.04±56.8	.000
HDL(mg/dl)	62.8±11.08	59.20±8.1	.024
LDL (mg/dl)	77.8±19.3	128.8±35.9	.000
VLDL (mg/dl)	28.2±8.03	32.17±9.7	.040
IL6 (pg/ml)	78.68±38.2	165.24±38.5	.191
hs-CRP (mg/l)	1.34±.75	3.43±2.06	.000
TNF ALPHA(pg/ml)	3.1±1.4	25.4±13.6	.000

< 0.05 considered as significant using independent t-test

Plane tubes were used to collect all blood samples. A total of 2.5 ml of blood was taken from each patient and control. The blood was centrifuged and the serum isolated within an hour of collection. The serum was isolated from the blood and kept in an Eppendorf tube for examination after centrifugation at 4000 rpm.

After fasting, plasma glucose and fasting plasma insulin were determined, with the help of the HOMA 2 calculator Version 2.2.3, HOMA analysis for measuring insulin resistance, beta-cell functions, and insulin sensitivity. Fasting plasma glucose and fasting plasma insulin were measured in mg/dl and U/ml, respectively.

HOMA analysis utilizes fasting glucose and insulin value to model beta-cells function, insulin sensitivity and insulin resistance (Levy *et al*., 1998).

Serum cholesterol, triglyceride and HDL were estimated by enzymatic method and fasting serum was used for the estimation of lipid profile. LDL and VLDL were calculated by the formula (Fossati and Prencipe, 1982). Interleukin 6 (MBS261259), hs-CRP (MBS2506093), and tumor necrotic factor-α (MBS015648) were measured for selected controls and patients using the ELISA technique(Hedlund, 1961, Kishimoto *et al*., 1992).

STATISTICAL ANALYSIS

SPSS was used to analyze the results (version 20). To compare various variables among different classes, standardized statistical programs such as the test of significance, regression analysis of coefficients, and correction were used.

RESULTS

A total of 400 subjects undertook the study, out of which 200 participants were diabetic patients and 200 participants were normal control. The age group was between 45 and 55 years; data were analyzed for quantitative analysis. Quantitative parameters included anthropometric indices (BMI, W/H and % body fat), stress score, blood tests include fasting plasma glucose, fasting plasma insulin, HOMA-IR, percent beta cell, the sensitivity of beta cells, lipid profile comprised of (total cholesterol, triglycerides, HDL, LDL, VLDL) and pro-inflammatory cytokines (IL-6, hs-CRP and TNF-α). The analysis was carried out between patients and control. The overall mean of the analysis is given in table 1. The mean age of overall participants was 48.9±3.0 and BMI of all subjects under study was found to be 25.90±4.73. Average W/H was 1.02±0.22. The result reveals the mean % body fat of all subjects as 26.34±5.93**.** The mean systolic and diastolic blood pressure of all participants was 135.74±9.4 and 77.9±7.72, respectively. Stress was detected as 155.8±47.1, average FPG was found as 104. mg/dl with a standard deviation of 28.1. Mean fasting plasma insulin was detected at 12.4µU/ml±11.01. HbA1c was detected in the blood sample and HOMA-IR, percent beta cell and percent sensitivity of beta-cell were calculated by HOMA calculator with mean values 6.3± 1.24, 2.16±10.1, 87.5±31.8 and 113.8±76.9 respectively. Mean total cholesterol of 203.9±53.7 was noted, Mean triglycerides of 152.3±43.05, average HDL of 61.01±9.8, mean LDL of 103.3±38.5, and mean VLDL of 30.1±9.1 were detected in overall participants. (table 1)

Proinflammatory cytokine profile of participants showed mean IL-6 and hs-CRP and TNF-α levels of 121.9± 57.86, 2.39±1.87 and 14.2±14.79, respectively. (table 1)

Comparison of Anthropometry, FPG, Fasting Insulin, HOMA-IR, Percent Beta Cell, Lipid Profile And Pro-Inflammatory Markers (IL-6, hs-CRP, TNF-α) Between Diabetic and Non-Diabetic Control Group

Independent sample t-test was applied to contrast anthropometry; fasting glucose, insulin and HbA1c, HOMA analysis, lipid profile, and inflammatory cytokines were compared between patients and control. WHR and IL-6 were found non-significant (p value>0.05) among the participants under study. BMI, percent body fats, HbA1c, FPG, and fasting insulin were found significant ($p<0.05$) among patients and control. HOMA-IR, percent beta cell, total cholesterol, triglyceride, and HDL also showed significant ($p<0.05$) results among patients and control. Similarly, TNF-α and hs-CRP were found significant ($p<0.05$) in patients than in control. (table 2)

Correlations between Anthropometry, Fasting plasma glucose, Fasting insulin, HbA1c, HOMA IR, hs-CRP and TNF-α

Positive correlation exists between BMI and WHR (r= 0.927, p=0.000), BMI and Percent Body Fats (r=0.893, p =0.000) BMI and FPG (r=0.867, p=0.000) BMI and FI (r=0.535, p=0.000), BMI and hs-CRP (r=0.553, p= 0.000), BMI and TNF-α (r=0.757, p=0.000) similarly fasting plasma glucose also showed positive correlation between WHR, percent body fats, fasting insulin, hs-CRP and TNF-α (r = 0.862, p = 0.000), (r = 0.818, p=0.000), (r =0.600, p=0.000), (r=0.21, p=0.000), and (r=0.758, p= 0.000) respectively. hs-CRP is also show strong positive correlation with BMI, WHR, FPG, FI, HbA1c and TNF-α (r=0.533, p=0.000), (r=0.528, p=0.000), (r=0.460, p=0.000), (r=0.521, p=0.000), (r=0.455, p=0.000), (r= 0.572, p=0.000), and (r=0.729, p=0.000)

Multiple Regression Analysis

Multiple regression model is designed to predict the value of IL-6, using BMI, WHR, percent body fats, HbA1c, FPG, and Fasting Insulin as explanatory variables. In the diabetic group, overall model is statistically significant at $p<0.05$. The overall explained variation is .173 on IL-6.

Table 3: Model for prediction of interleukin-6

Model-1b		Beta Coefficient	P-Value	95% confidence intervel for beta		Overall significance of model using F-test
				Lower Boundary	Upper Boundary	
Diabetic	Constant		.056	-2.278	173.328	F(6.750)<0.00* R^2 = .173
	BMI	.393	.001	2.996	11.113	
	WHR	-.196	.070	-155.390	6.272	
	% Body Fats	-.250	.003	-4.557	-.975	
	HBA1C	.124	.089	-.942	13.205	
	FPG	-.057	.445	-.676	.298	
	Fasting Plasma Insulin	.353	.000	.712	1.841	
Non-Diabetic	Constant		.028	17.250	291.852	F(.921)<.481 R^2 = .028
	BMI	.091	.396	-2.537	6.388	
	WHR	-.173	.070	-142.089	5.502	
	% Body Fats	-.172	.081	-5.125	.298	
	HBA1C	.002	.981	-20.552	21.065	
	FPG	-.024	.741	-.771	.549	
	Fasting Plasma Insulin	-.065	.371	-1.109	.416	

Dependent Variable: Interleukin 6 pg/ml

Table 4: Model for prediction fasting plasma glucose

Model-1b		Beta Coefficient	P-Value	95% confidence intervel for beta		Overall significance of model using F-test
				Lower Boundary	Upper Boundary	
Diabetic	Constant		.000	68.262	115.103	F(6.849)<0.000* R^2= .150
	BMI	.220	.030	.114	2.249	
	WHR	-.005	.963	-24.650	23.520	
	IL-6	.037	.678	-.041	.063	
	Hs-CRP	-.254	.006	-2.428	-.406	
	TNF Alpha	.331	.001	.120	.441	
Non-Diabetic	Constant		.000	75.200	105.851	F(1.553)<.175 R^2 = .038
	BMI	-.186	.021	-1.575	-.132	
	WHR	.073	.382	-7.810	20.281	
	IL-6	.058	.535	-.027	.052	
	Hs-CRP	-.091	.343	-3.071	1.074	
	TNF Alpha	.073	.320	-.418	1.276	

Dependent Variable: Fasting Plasma Glucose mg/dl

Table 5: Model for prediction fasting plasma insulin

Model-1b		Beta Coefficient	P-Value	95% confidence intervel for beta		Overall significance of model using F-test
				Lower Boundary	Upper Boundary	
Diabetic	Constant		.728	-22.236	15.555	F(21.151)<0.000* R^2= .353
	BMI	-.260	.004	-2.151	-.429	
	WHR	.373	.000	19.804	58.667	
	IL-6	.131	.089	-.006	.078	
	Hs-CRP	-.144	.075	-1.556	.075	
	TNF Alpha	.469	.000	.237	.497	
Non-Diabetic	Constant		.001	8.850	35.280	F(2.19)<0.57 R^2 = .053
	BMI	-.041	.605	-.786	.459	
	WHR	-.195	.019	-26.627	-2.403	
	IL-6	-.078	.403	-.049	.020	
	Hs-CRP	.039	.681	-1.414	2.160	
	TNF Alpha	.025	.730	-.602	.859	

Dependent Variable: Fasting Plasma Insulin (Uu/ml)

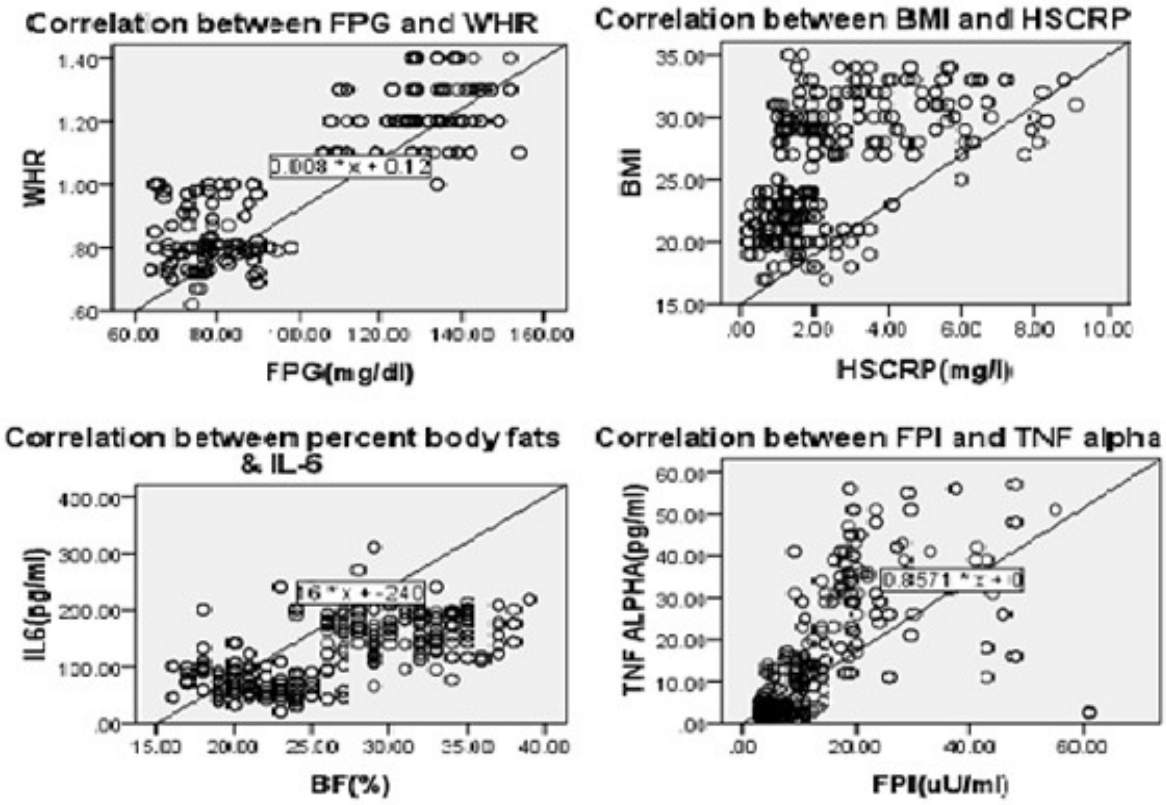

Fig. 1: Correlations between FPG and WHR, BMI and hs-CRP,BF% and IL-6 and FPI and TNF-α

BMI, HbA1c and Fasting insulin are positively associated with IL-6, whereas WHR, percent body fats, and fasting plasma glucose are negatively associated with IL-6. In the non-diabetic control group, the model is statistically insignificant at the p-value of 0.481 with a variation of .028.

Multiple regression model is designed to predict the value of fasting plasma glucose, using BMI, WHR, hs-CRP, IL-6, and TNF-α as explanatory variables. In the diabetic group, overall model is statistically significant at $p<0.05$. The overall explained variation is 6.8 on FPG.

BMI, IL-6, and TNF-α are positively associated with fasting plasma glucose, whereas WHR and hs-CRP are negatively associated with FPG. In the non-diabetic control group, the model is statistically insignificant at a p-value of 0.175 with a variation of .038.

Multiple regression model is designed to predict the value of fasting insulin, taking BMI, WHRIL-6, hs-CRP, and TNF-α as explanatory variables. In diabetic patients, the model is statistically significant at $p<0.05$). The overall variation in diabetic patients is .353 on fasting insulin. WHR, IL-6, and TNF-α are positively associated with fasting insulin, whereas, BMI and hsCRP are negatively associated with fasting insulin.

DISCUSSION

The rising obesity and metabolic syndrome are thought as the global burden for the modern era age. Obesity is a long-lasting condition associated with the overproduction of pro-inflammatory cytokines by expanded adipose tissues. Inflammation is the key component for obesity-induced insulin resistance and plays a fundamental role in the incidence of T2DM.

Anthropometric investigation of diabetic patients shows an increased level of BMI, and percent body fats, BMI and W/H ratio positively correlate with the hs-CRP and TNF-α. Results strongly suggest that being overweight and obese is the primarily observed incidence in diabetic patients.

Previous research provides the basis that anthropometry, primarily the BMI and W/H, is the well-known practice for assessment of obesity. High incidence of obesity and metabolic disturbances such as poor glycemic control are linearly associated with rising BMI and W/H. Although the peripheral tissue sensitivity to insulin action is affected, proper utilization of glucose for energy need is the compromised process. Lack of physical activity generally programs obesity that is the independent risk factor for T2DM (Anjana *et al*., 2004, Basit *et al*., 2021).

Insulin resistance rises with the expansion of stores body fat with a consequent increase in inflammatory markers. Fat is the harmonically active tissue responsible for insulin response. Increased percent body fat were found among the participant patients under study and it showed positive correlation with fasting plasma insulin, further increased adipocyte were found to be associated with increase in the fasting plasma glucose value as well as rise in the HbA1c. Increased adipocytes causes insulin resistance due to which tissue glucose uptake particularly in liver and muscle is compromised resulting in increase blood glucose.Increase percent body fats and W/H ratio represents central obesity which create metabolic disturbance further its deterioration induces inflammation the release of inflammatory markers. The result shows that percent body fat is increased in diabetic subjects as compared to non-diabetic, representing less physical activity existence in diabetic subjects, taking the subjects toward auxiliary complication of diabetes.

Visceral fats increase in proportion with increased body mass index and obesity is deliberated to be the main factor for T2DM. Furthermore, visceral adipocytes secrete a disproportionate volume of free fatty acids that are resilient to the antilipolytic effects of insulin. Several studies supported that free fatty acids are the major supervisory body of glucose and elevated levels are associated with insulin resistance at the level of liver and muscles. Risks for T2DM become apparent by increased visceral fat accumulation (Gastaldelli, 2008, Anjana *et al*., 2004, Esser *et al*., 2014, Anari *et al*., 2017).

The high degree of dyslipidemia in terms of triglycerides, cholesterol, and VLDL was detected in participant patients, as compared to the control group. Dyslipidemic characteristics are associated with the risk of CVD and increased mortality rate with T2DM. Although, insulin is antilipolytic in adipose tissues and promotes lipogenesis in the liver, but decreased sensitivity of this hormone in adipose tissues leads to excess lipolysis and increased free fatty acid release in the bloodstream.

Research studies demonstrated that dyslipidemia is the consequence of T2DM, characterized by greater efflux and reduced clearance of lipoproteins. Poor glycemic control imparts defected lipid metabolism, besides hypertension, hyperinsulinemia, and central obesity associated with diabetes, and propels the victim toward CV complication. Insulin resistance causes an alteration in lipid metabolism (Phosat *et al*., 2017, Pickup, 2006). Pieces of evidence supported the view that enhanced cholesterol and TG production is mediated by hyperinsulinemia (Songa *et al*., 2013, Marques-Vidal *et al*., 2013, Chan, 2017).

Results revealed that high levels of FPG, fasting plasma insulin, HbA1c, HOMA-IR, IL-6, and hs-CRP are present in diabetic patients showing that dyslipidemia causes an increase in insulin resistance and subsequent increase in the inflammatory markers. Inflammatory markers disrupt glucose homeostasis and interfere with metabolism causing persistent increase blood glucose and insulin resistance which further produces the complication.

Multiple linear regression was performed to predict the risk of increasing FPG. In the diabetic group BMI, IL-6, and TNF-α are positively associated with fasting plasma glucose, whereas WHR and hs-CRP are negatively associated with FPG. This is the main point as previous researchers showed direct relation of IL-6 and hs-CRP with fasting plasma glucose. Our study reveal the relationship of IL-6, and TNF-α. Another model was designed to predict the value of fasting insulin. In diabetic patients, the model was statistically significant. WHR, IL-6, and TNF-α are positively associated with fasting insulin, whereas BMI and hs-CRP are negatively associated with fasting insulin.

The study reveals that obesity-induced chronic inflammation is associated with high glucose levels. IL-6 is produced by the expanded adipose tissues which pass via the bloodstream into the portal circulation and stimulates the secretion of hs-CRP (Mtintsilana *et al*., 2019). This acute-phase protein enhances insulin resistance and produces a cyclic cascade for chronic low-grade inflammation. Studies imparted that positive correlation of insulin resistance with IL-6 and hs-CRP strongly shows that obesity-induced pro-inflammatory cytokine production and induced hs-CRP released play a pivotal role in the incidence of T2DM. (Tangvarasittichai *et al*., 2016, Phosat *et al*., 2017, Lainampetch *et al*., 2019)

It is evident from research that inflammation is the consequence of obesity. Normally, white adipose tissues are gathered by matrix, stroma, macrophages, blood vessels, and neuroendocrine cells. In obesity, increase in fats increases the generation of new adipocytes along with macrophages infiltration and angiogenesis, increase in the number of adipocytes and macrophages leading to elevated levels of pro-inflammatory cytokines such IL-6, and programs the inflammation in the body by subsequent activation of neuroendocrine cells (Elimam *et al*., 2019). Most of the adipokines are secreted by macrophages, therefore, an imbalance in the secretion and action of cytokine including IL-6 is produced. Rising levels of IL-6 perform its action via the trans-signaling pathway through sIL-6R, initiating a pathway that produces pathological effects of IL-6 with resultant insulin resistance and increased production of hsCRP from the liver; hs-CRP mediates its role to initiates a cascade that promotes IL-6 production and insulin resistance, reduced sensitivity, decline in the percent beta cell and increase in insulin secretion. Recent studies indicated that in obesity, expanded fat stores promote inflammatory cytokines induced insulin resistance and ultimately lead to

metabolic disorder the type 2 diabetes mellitus (Lainampetch *et al*., 2019, Marques-Vidal *et al*., 2013).

Experimental and clinical data have established that the liver, pancreas, and adipose tissues are the primary site of inflammation in presence of obesity. Adipose tissues in obesity typically produce cytokines that perform their function in an autocrine and paracrine manner, contributing to insulin resistance. Furthermore, hs-CRP mediated pro-inflammatory pathway initiation leads to a worse prognosis of T2DM. Obesity induces infiltration of macrophages. They are crucial for excess production of proinflammatory cytokine. Their recruitment is linked with obesity, systemic inflammation, insulin resistance, and metabolic syndrome (Esser *et al*., 2014, de Marañón *et al*., 2020).

CONCLUSION

The emerging concepts of inflammatory mechanisms in obesity-induced type 2 diabetes mellitus have turned the research focus towards cytokines. Cellular changes occur in the adipose tissues in response to overnutrition and lack of physical activity, which primes towards the induction of inflammatory mechanism and production of pro-inflammatory cytokines, with excessive resilient free fatty acid that functions in the reduction of insulin action on muscles, adipose tissues and liver, leading to the development of insulin resistance and eventual T2DM.

The present study suggests the clinical involvement of inflammatory cascade in the pathogenesis of T2DM. Our study revealed that BMI and percent body fat were increased, with elevated levels of FPG, fasting plasma insulin, insulin resistance, and pro-inflammatory cytokines IL-6 and hs-CRP, whereas decreased percent beta cell and its sensitivity were observed. Inflammatory markers interfere with metabolism and alter the homeostasis of glucose. The occurrence of inflammation-induced type 2 diabetes mellitus in obese individuals is not an overnight process, instead, people spend various stages without being known. Our study postulated that pro-inflammatory cytokines IL-6 and hs-CRP are higher in patients of T2DM. It became evident that elevated levels of pro-inflammatory cytokines are the key supervisory bodies for the certainty of obesity-induced insulin resistance.

REFERENCES

Akbarzadeh M, Eftekhari MH, Dabbaghmanesh MH, Hasanzadeh J and Bakhshayeshkaram M (2013). Serum Il-18 and hs-CRP correlate with insulin resistance without effect of calcitriol treatment on type 2 diabetes. *Iran J. Immunol.,* **10**(3): 167-176.

Al-Rashed F, Ahmad Z, Iskandar MA, Tuomilehto J, Al-Mulla F and Ahmad R (2019). TNF-alpha induces A pro-inflammatory phenotypic shift in monocytes through Acsl1: Relevance to metabolic inflammation. *Cell. Physiol. Biochem.,* **52**: 397-407.

Anari R, Amani R, Latifi SM, Veissi M and Shahbazian H (2017). Association of obesity with hypertension and dyslipidemia in type 2 diabetes mellitus subjects. *Diabetes Metab Syndr:***11**(1): 37-41.

Andrews Guzman M and Arredondo Olguin M (2014). Association between ferritin, high sensitivity C-reactive protein (hs-CRP) and relative abundance of hepcidin mrna with the risk of type 2 diabetes in obese subjects. *Nutr. Hosp.,* **30**(3): 577-584.

Anjana M, Sandeep S, Deepa R, Vimaleswaran KS, Farooq S and Mohan V (2004). Visceral and central abdominal fat and anthropometry in relation to diabetes in Asian Indians. *Diabetes Care,* **27**(12): 2948-2953.

Basit A, Askari S, Zafar J, Riaz M, Fawwad A and Members N (2021). NDSP 06: Prevalence and risk factors for obesity in urban and rural areas of Pakistan: A study from Second National Diabetes Survey of Pakistan (NDSP), 2016-2017. *Obes. Res. Clin. Pract.,* **15**(1): 19-25.

Catalina MOS, Redondo PC, Granados MP, Cantonero C, Sanchez-Collado J, Albarran L and Lopez JJ (2019). New insights into adipokines as potential biomarkers for type-2 diabetes mellitus. *Curr. Med. Chem.,* **26**(22): 4119-4144.

Chan M (2017). Obesity and diabetes: The slow-motion disaster. *Milbank Q,* **95**(1): 11.

Cheng L, Zhuang H, Yang S, Jiang H, Wang S and Zhang J (2018). Exposing the causal effect of c-reactive protein on the risk of type 2 diabetes mellitus: A mendelian randomization study. *Frontiers in Genetics,* **9**(1): 657.

De Maranon AM, Iannantuoni F, Abad-Jimenez Z, Canet F, Díaz-Pozo P, Lopez-Domenech S, Roldan-Torres I, Morillas C, Rocha M and Víctor VM (2020). Association between proinflammatory markers, leukocyte-endothelium interactions, and carotid intima–media thickness in type 2 diabetes: Role of glycemic control. *Clin. Med.* (Lond), **9**(8): 2522.

Demyanets S, Kaun C, Kaider A, Speidl W, Prager M, Oravec S, Hohensinner P, Wojta J and Rega-Kaun G (2020). The pro-inflammatory marker soluble suppression of tumorigenicity-2 (St2) is reduced especially in diabetic morbidly obese patients undergoing bariatric surgery. *Cardiovasc Diabetol.,* **19**(1): 26.

Elimam H, Abdulla AM and Taha IM (2019). Inflammatory markers and control of type 2 diabetes mellitus. *Diabetes Metab. Syndr:***13**(1): 800-804.

Esser N, Legrand-Poels S, Piette J, Scheen AJ and Paquot N (2014). Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res. Clin. Prac.,* **105**(2): 141-150.

Fadaei R, Bagheri N, Heidarian E, Nouri A, Hesari Z, Moradi N, Ahmadi A and Ahmadi R (2020). Serum

levels of Il-32 in patients with type 2 diabetes mellitus and its relationship with tnf-alpha and I1-6. *Cytokine,* **125**(1): 154832.

Fossati P and Prencipe L (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.,* **28**(10): 2077-2080.

Gastaldelli A (2008). Abdominal fat: Does it predict the development of type 2 diabetes: Oxford University Press.

Hedlund P (1961). Clinical and experimental studies on c-reactive protein (acute phase protein). *Acta Medica Scandinavica. Supplementum,* **361**: 1-71.

Kishimoto T, Akira S and Taga T (1992). Interleukin-6 and its receptor: A paradigm for cytokines. *Science,* **258**(5082): 593-597.

Lainampetch J, Panprathip P, Phosat C, Chumpathat N, Prangthip P, Soonthornworasiri N, Puduang S, Wechjakwen N and Kwanbunjan K (2019). Association of tumor necrosis factor alpha, interleukin 6, and c-reactive protein with the risk of developing type 2 diabetes: A retrospective cohort study of rural thais. *J. Diabetes Res.,* 9051929.

Lee C, Adler A, Sandhu M, Sharp S, Forouhi N, Erqou S, Luben R, Bingham S, Khaw K and Wareham N (2009). Association of c-reactive protein with type 2 diabetes: Prospective analysis and meta-analysis. *Diabetologia,* **52**(3): 1040-1047.

Levy JC, Matthews DR and Hermans MP (1998). Correct homeostasis model assessment (Homa) evaluation uses the computer program. *Diabetes Care,* **21**(12): 2191-2192.

Marques-Vidal P, Bastardot F, Von Kanel R, Paccaud F, Preisig M, Waeber G and Vollenweider P (2013). Association between circulating cytokine levels, diabetes and insulin resistance in a population-based sample (colaus study). *Clin Endocrinol (Oxf),* **78**(2): 232-241.

Milas O, Gadalean F, Vlad A, Dumitrascu V, Velciov S, Gluhovschi C, Bob F, Popescu R, Ursoniu S, Jianu DC, Matusz P, Pusztai AM, Secara A, Simulescu A, Stefan M, Patruica M, Petrica F, Vlad D and Petrica L (2020). Pro-Inflammatory cytokines are associated with podocyte damage and proximal tubular dysfunction in the early stage of diabetic kidney disease in type 2 diabetes mellitus patients. *J. Diabetes Complications,* **34**(2): 107479.

Mtintsilana A, Micklesfield LK, Chorell E, Olsson T, Shivappa N, Hebert JR, Kengne AP and Goedecke JH (2019). Adiposity mediates the association between the dietary inflammatory index and markers of type 2 diabetes risk in middle-aged black south african women. *Nutrients,* **11**(6): 1246.

Nadeem A, Naveed AK, Hussain MM and Raza SI (2013). Correlation of inflammatory markers with type 2 diabetes mellitus in Pakistani patients. *J. Postgrad. Med. Inst.,* 27.

Organization WH (2003). The World Health Report 2003: Shaping The Future, World Health Organization.

Phosat C, Panprathip P, Chumpathat N, Prangthip P, Chantratita N, Soonthornworasiri N, Puduang S and Kwanbunjan K (2017). Elevated C-reactive protein, interleukin 6, tumor necrosis factor alpha and glycemic load associated with type 2 diabetes mellitus in rural thais: A cross-sectional study. *Bmc Endocr Disord,* **17**(1): 44.

Pickup JC (2004). Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care,* **27**(3): 813-823.

Pickup JC (2006). Inflammatory markers and type 2 diabetes. *Diabetes Technol The.,* **8**(1): 1-6.

Shiwaku K, Anuurad E, Enkhmaa B, Nogi A, Kitajima K, Yamasaki M, Yoneyama T, Oyunsuren T and Yamane Y (2005). predictive values of anthropometric measurements for multiple metabolic disorders in asian populations. *Diabetes Res. Clin. Pract.,* **69**(1): 52-62.

Simental-Mendia LE, Lazalde B, Zambrano-Galvan G, Simental-Saucedo L, Rabago-Sanchez E, Rodriguez-Moran M and Guerrero-Romero F (2012). Relation between c-reactive protein and impaired fasting glucose in obese subjects. *Inflammation,* **35**(Issue): 1742-6.

Singh R, Shaw J and Zimmet P (2004). Epidemiology of childhood type 2 diabetes in the developing world. *Pediatric Diabetes,* **5**(3): 154-168.

Songa RM, Siddhartha K and Sudhakar K (2013). Lipidprofile in type2 diabetes mellitus with obesity. *Bopams* **1**(2): 132-137.

Tangvarasittichai S, Pongthaisong S and Tangvarasittichai O (2016). Tumor necrosis factor-alpha, interleukin-6, c-reactive protein levels and insulin resistance associated with type 2 diabetes in abdominal obesity women. *Indian J. Clin Biochem.,* **31**(1): 68-74.

Trinder P (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann.Clin. Bioch,* **6**(1): 24-27.

Valtierra-Alvarado MA, Castaneda Delgado JE, Ramirez-Talavera SI, Lugo-Villarino G, Duenas-Arteaga F, Lugo-Sanchez A, Adame-Villalpando MS, Rivas-Santiago B, Enciso-Moreno J and Serrano CJ (2020). Type 2 diabetes mellitus metabolic control correlates with the phenotype of human monocytes and monocyte-derived macrophages. *J. Diabetes Complications,* **34**(11): 107708.