C-REACTIVE PROTEIN (CRP) AS A BIOMARKER OF INFLAMMATION IN OBESE AND NON-OBESE TYPE 2 DIABETES MELLITUS PATIENTS WITH METABOLIC SYNDROME

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TO MY BELOVED MOTHER

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ABSTRACT

Obesity, particularly abdominal obesity when associated with end-organ resistance to insulin, raised blood sugar, deranged lipid profile and high blood pressure is collectively called “Metabolic Syndrome”. Metabolic Syndrome increases the risk of developing type 2 diabetes mellitus(T2DM). Metabolic syndrome represents a range of disorders related to blood pressure, lipid profile, blood sugar and obesity related inflammation. Screening for Metabolic Syndrome is important because obesity related manifestations are associated with the deranged values of five components of Metabolic Syndrome. C-Reactive Protein (CRP) is a known biomarker associated with many metabolic disorders with underlying acute and chronic inflammation. Its use as a potential biomarker of disease states such as diabetes, hypertension and Metabolic Syndrome need to be investigated. Therefore, association of Type 2 diabetes mellitus patients with metabolic syndrome with a proinflammatory state can be confirmed by higher levels of CRP. To investigate the association of serum CRP levels with obese and non-obese type 2 diabetes mellitus patients with metabolic syndrome. A comparative cross-sectional study was performed in 120 subjects divided into 2 groups (60 obese >30kg/m^2 and 60 non-obese <30kg/m^2) type 2 diabetes mellitus patients with Metabolic Syndrome. The participants were recruited from Medical OPD National Medical Center, Karachi for a duration of six months period. The 2005 revised NCEP-ATP III criteria were used to determine participants with Metabolic Syndrome in type 2 diabetic patients. The conditions included in diagnostic criteria for Metabolic Syndrome are high blood pressure, high blood sugar, abnormal cholesterol levels (low HDL or high triglycerides), and increased fat deposits in the abdominal area. After taking informed consent and an overnight fast, fasting venous samples were sent for Fasting Blood Glucose (FBG), Triglycerides (TG), Total cholesterol (TC), Low density Lipoprotein (LDL-C), High density Lipoprotein (HDL-C) and C-Reactive Protein (CRP). 75% of type 2 diabetes mellitus patients had metabolic syndrome. The association of Metabolic Syndrome with Marital status and Education was found statistically significant with $p<0.05$. Independent sample t test gave significant mean differences for waist circumference, systolic blood pressure, fasting blood sugar, and triglycerides between metabolic syndrome and non-metabolic syndrome samples with

$p<0.05$. The Multivariate linear regression analysis, results showed increase in WC giving 0.13-time positive impact on CRP ($p<0.01$). The difference in median CRP of obese and non-obese samples of metabolic samples was also statistically significant with $p=0.01$ using Mann Whitney U test. Pearson Chi Square test did give a significant association of metabolic syndrome with SBP, FBS, HbA1C, and TG with $p<0.05$. The laboratory parameters showed higher SBP, DBP, FBG, TG and low HDL-C levels in subjects. CRP levels were higher in obese than non-obese type 2 diabetic patients with Metabolic Syndrome.

Key words: Type 2 diabetes mellitus, Metabolic Syndrome, obesity, CRP and insulin resistance.

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LIST OF ABBREVIATIONS

APR	Acute phase reactants
KDA	Kilodaltons
nCRP	Native C-Reactive protein
m CRP	monomeric C-Reactive protein
MAC	Membrane Attack complex
PAMPs	Pathogen associated molecular patterns
DAMPs	Danger associated molecular patterns
NF-kβ	Nuclear Factor kappa beta
NLRP3	nucleotide-binding domain, leucine -rich-containing family, pyrin domain-containing 3
NLR	Intracellular "NOD" like receptor
PRR	Pattern recognition receptor
IAPP	Islet amyloid polypeptide
MHO	Metabolically healthy Obesity
MUHO	Metabolically unhealthy Obesity
hsCRP	Highly sensitive CRP
RR	Relative risk
CI	Confidence interval
JNK	c-Jun N-terminal kinases
CD8	Cytotoxic T cells
TH1	Helper T cells 1
TH17	Helper T cells 17

CD4	Helper T cells
TH2	Helper T cells 2
IDF	International Diabetes Federation
BMC	bio-med central
CDC	Center for disease control
ROS	Reactive oxygen species
TLRs	Toll like receptors
NCEP	National Cholesterol Education Program
HOMA-IR	Homeostatic Model Assessment for insulin resistance
ATP	Adult Treatment Panel III
HRT	Hormone Replacement Therapy
MODY	Maturity Onset diabetes of the Young
LADA	Latent autoimmune diabetes of adults
PCOS	Polycystic ovarian syndrome
AGE	Advanced glycation products
ROS	Reactive Oxygen species
ICAM-1	Intracellular adhesion molecule-1
VCAM-1	Vascular adhesion molecule-1
PHS	Physician Health Study
WHS	Women Health Study
ARIC	Atherosclerosis Risk in communities
AFCAPS	Air Force/Texas Coronary Atherosclerosis Prevention Study
MONICA	Monitoring Trends and Determinants in Cardiovascular disease

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Obesity, particularly abdominal obesity when associated with end-organ resistance to insulin, raised blood sugar, deranged lipid profile and high blood pressure is collectively called “Metabolic Syndrome” (Alberti KG et al.,2009). If a person suffers from Metabolic Syndrome the chance of getting type 2 diabetes mellitus(T2DM) increases. (Alberti KG et al.,2009). Type 2 Diabetes Mellitus (T2DM) is a metabolic endocrine disorder which is a silent epidemic, the most common health issue in the world (Wild S et al.,2004). It is characterized by both absolute and relative deficiency of insulin action on the peripheral tissues. There is chronic hyperglycemia due to obesity related insulin resistance. This obesity related inflammation (Fig 1.1) involves activation of innate immunity observed in visceral adiposity. (Donath MY et al., 2011, Chawla A et al.,2011 and Ouchi N et al., 2011). In modern biomedical sciences, there is a continuous search for informative biomarkers that are present in biological pathways or pathological processes which can be identified for an accurate diagnosis or treatment. C-Reactive protein is one of these groups of potential biomarkers which along with Erythrocyte Sedimentation rate (ESR) is known as acute phase reactants. It indicates underlying subclinical inflammation in the body but cannot be utilized to localize site of inflammation. (Gulhar et al., 2022) In times of tissue injury, inflammatory markers called Acute Phase reactants (APR) increase or decrease in patient’s serum. The stress placed on the body releases important inflammatory markers in the body. APR is further classified into positive or negative depending on whether they increase or decrease in the serum respectively. Erythrocyte Sedimentation Rate (ESR) and CRP are positive acute phase proteins. The negative markers of APR include albumin, pre-albumin, retinol binding protein and transferrin. (Gulhar et al., 2022) Since C-Reactive proteins are involved in intracellular signaling pathways, they have a potential for becoming key biomarkers for diagnosis. CRP belongs to pentraxin family of proteins (Pathak A et al.,2019, Trpkovic M et al.,2016) (Figure 1.2).

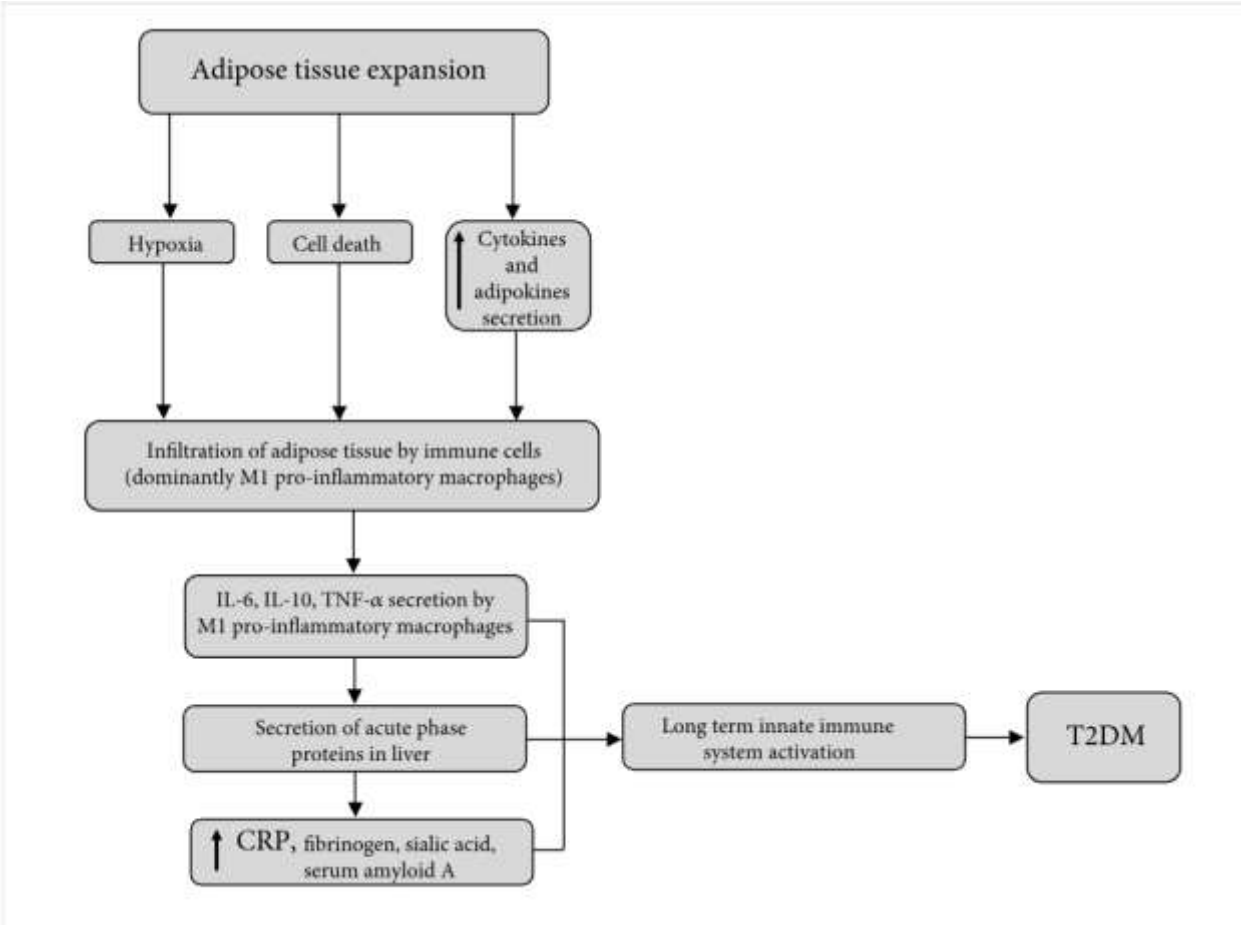


Figure 1.1: T2DM development as a result of low-grade inflammation (Julijana et al.,2022)

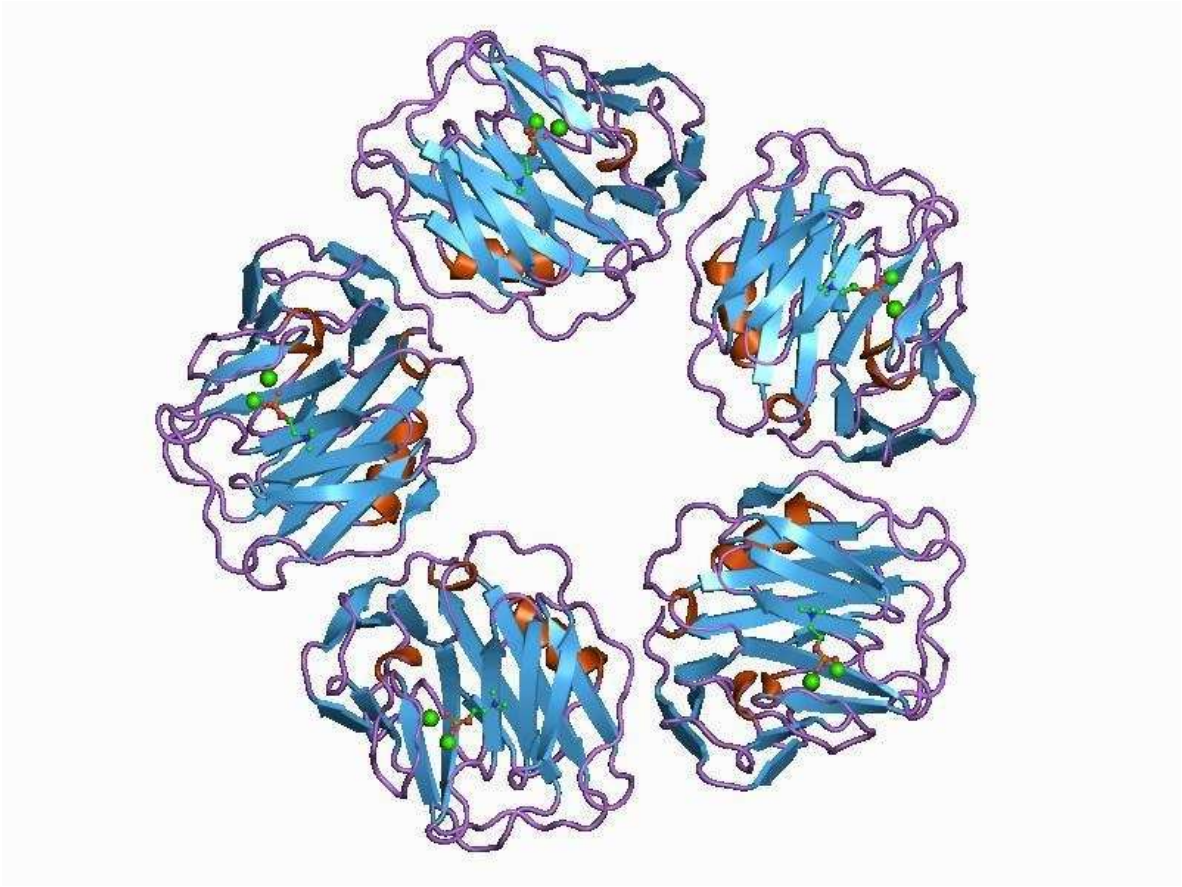


Figure 1.2: Structure of CRP belonging to the pentraxin family (Pathak A et al.,2019)

It has 206 amino acids which are arranged cyclically having five subunits attached by non-covalent bonds. It has 23 KDA molecular weight. (Shrive AK et al.,1996, WHO global report on Diabetes.,2016). CRP exits in the form of Native CRP (nCRP) which breaks down to monomeric isoforms of CRP (m CRP) across lysophosphatidylcholine in platelets and T cells Native CRP (nCRP) has more anti-inflammatory properties than m CRP. C5a and MAC (membrane attack complex) are not produced as their production is inhibited by m CRP. The alternate pathway of complement system is inhibited (Thiele JR et al.,2014). m CRP is capable of angiogenesis in both vitro and vivo and hence can cause new vessel formation in tissues where it is formed (Turu MM et al.,2008). CRP binds (PAMPs) pathogen associated molecular patterns or endogenous molecules (danger associated molecular patterns (DAMPs). Therefore, injured tissue and necrotic cells induce immunity mediated by CRP (Shrivastava AK et al.,2015, Shim K et al.,2020). CRP also activates C1q molecule in classic complement pathway activating C3 and MAC (C5-9).C3 leads to opsonization of pathogenic bacteria and viruses (Volanakis JE et al.,1982, Mold C et al.,1999). CRP also binds to Fc receptors of immunoglobulin G(IgG) which releases pro-inflammatory markers (Sportson NR et al.,2018, Nehring SM et al.,2020).

T2DM patients are mostly obese and obesity down-regulates insulin receptors in the target tissues of insulin. This insulin insensitivity along with normal or elevated insulin levels, raised blood pressure and deranged lipid profile constitutes the pathophysiology of T2DM (Rao G et al.,2001).IR is also characterized by chronic subclinical low-grade inflammation (Trypkovic A et al.,2014, Obradovic MM et al.,2015). There is activation of innate immunity which contributes to the disease progression in T2DM.Apart from CRP, other acute phase proteins such as sialic acid, fibrinogen, serum amyloid A and low albumin and transferrin are also linked to T2DM pathogenesis . White adipose tissue (WAT) in the visceral areas has a role in the pathogenesis of T2DM. Macrophages and immune cells that migrate to adipose tissue produce pro-inflammatory cytokines which result in production of CRP. Adipose tissue, because of increasing size in obesity also cannot develop simultaneous blood supply (Trayhurn P et al.,2005) in the form of neovascularization, hence hypoxia, adipocyte apoptosis and raised levels of chemokines all contribute to adipose tissue inflammation (Wellen K E et al.,2003). M1 macrophages which are pro-inflammatory in nature increases while anti-inflammatory M2 macrophages decrease in the

adipose tissue (Lontchi-Yimagou E et al.,2013). TNF-α (pro-inflammatory), IL-1β, IL-6(pro-inflammatory) are produced in high numbers in the inflamed adipose tissue (Burhans MS et al.,2018). TNF-α decreases AKT substrate and stimulate IL-18 expression in skeletal muscle contributing to IR. It also causes decreased glucose uptake in insulin sensitive tissues and vasodilation in vessels (Rask-Madsen C et al.,2003). TNF-α activates NF-κβ pathway and disturbs insulin signaling pathways. IL-6 on the other hand modulates insulinase activity in both liver and skeletal muscle contributing to T2DM and obesity. IL-6 shifts the balance of M1and M2 macrophages to inflammatory M1 cells, by increasing their response to IL-4 (Mauer J et al.,2014). (Figure 1.3). IL-1β and NLRP3 inflammasome contributes to pathogenesis of T2DM. IL-1β activates NF-kβ pathways leading to TNF-α and its own production (Dinarello CA et al.,2009). There are two signals that control the release of IL-1β. One is the pro-inflammatory signal that induces gene transcription of IL-1β with its storage in its precursor form in the cell. In active forms of IL-1β is converted to active forms by caspase 1. A large multiprotein structure is formed after cleavage and is called inflammasome (Schroder K et al.,2010). Inflammasomes belong to innate immunity molecular mechanism and recognize (PAMPs) and (DAMPs) by pattern recognition receptors (PRRs). Inflammasomes are formed when they interact with PRR which belongs to a nucleotide-binding oligomerization domain like receptor (NLR) family (Schroder K et al.,2010). NLRP3-pyrin domain containing 3 inflammasome has a major role to play in progression from obesity to T2DM (Figure 1.4). NLRP3 inflammasome activation and further IL-1β release is the first step in pancreatic β cell dysfunction. When β cells are exposed to chronic hyperglycemia: there is exhaustion of these cells leading to apoptosis and death along with altered and defective insulin release from the β cells (Maedler K et al.,2002, Zhou R et al.,2010, Boni-Schnetzler M et al.,2009). (IAPP) islet amyloid polypeptide is a protein which forms amyloid deposits in pancreas, help in IL-1β production in islets of Langerhans by activating NLRP3 expression, the caspase 1 activity and increased levels of IL-1β in adipose tissue of obese mice and humans are directly associated with insulin resistance leading to metabolic syndrome and disease progression in T2DM (Esser N et al., 2013, Vandanmagsar B et al.,2011). NLRP3 sense increase levels of metabolic fuels namely glucose (Zhou R et al.,2010), saturated fatty acid (Wen H et al.,2011, L'Homme L et al.,2013)

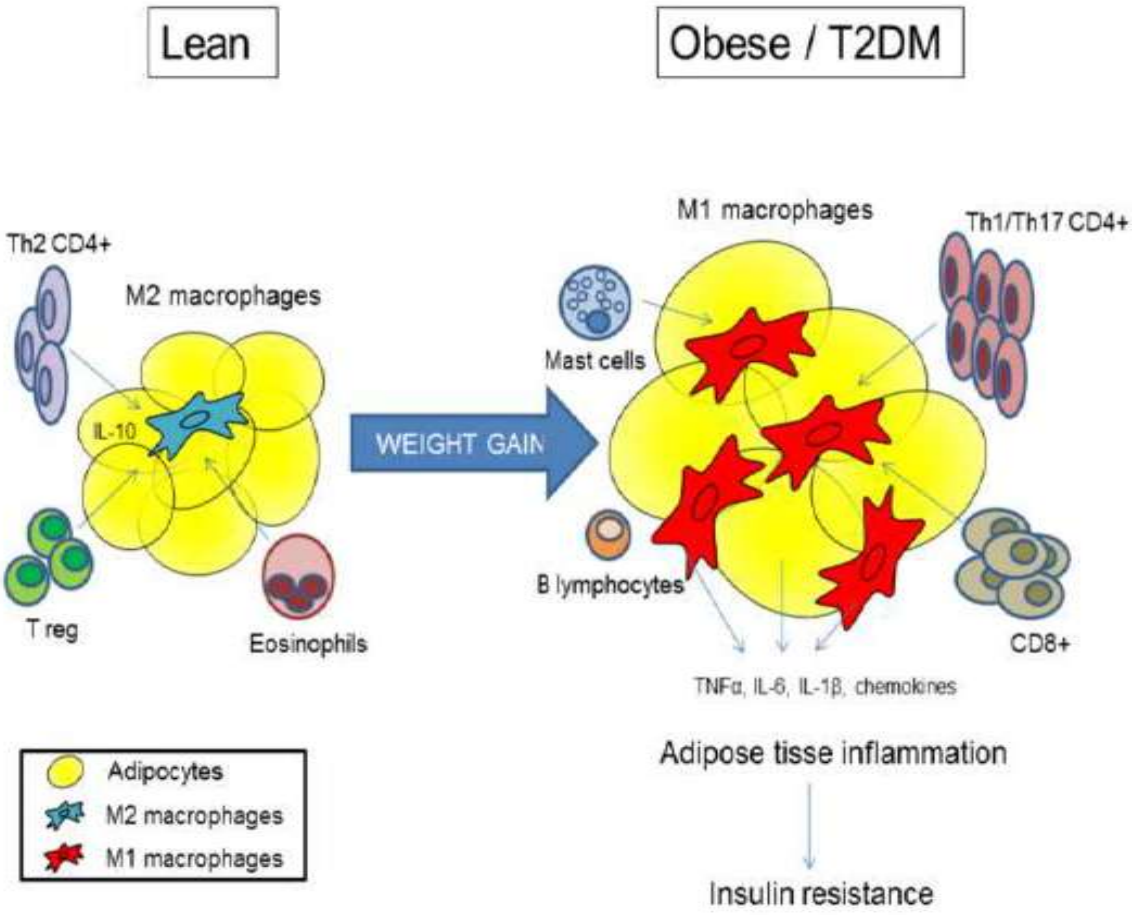


Figure 1.3: Adipose tissue inflammation in pathogenesis of metabolic syndrome and type 2 diabetes mellitus (Mauer J et al.,2014).

ceramides and uric acid (Schroder K et al.,2010). NLRP3 activation leads to IL-1β production and recruitment of many chemokines and cytokines. When NLRP3 levels are decreased, there is less insulin resistance in target tissues, which also increases insulin secretion by the pancreas (Stienstra R et al.,2011, Vandanmagsar B et al.,2011). Literature explains obesity in two terms as "Metabolically Healthy Obesity" (MHO) and "Metabolically Unhealthy Obesity" (MUHO) (Fig 1.5). MHO accounts for 30% of population who have greater sub-cutaneous fat than visceral white adipose tissue (Koster A et al.,2010) and low-fat deposits in liver (Stefan N et al., 2008) and low levels of inflammatory markers in the blood (Phillips CM et al.,2013, Koster A et al.,2010). MHO phenotype has lower activation of NLRP3 inflammasome in macrophages that have migrated to adipose tissue of obese individuals (Esser N et al.,2013). Fatty acid acting as triggers can activate inflammatory pathways in macrophages and fat cells by activating NLRP3 inflammasome (Wen H et al.,2011). Whereas unsaturated fatty acids have anti-inflammatory effects, improving insulin sensitivity in obese and T2DM persons (Summers LK et al.,2002) also without activating NLRP3 inflammasome (Wen H et al.,2011, Yan Y et al.,2013, L'Homme L et al.,2013). Hypertrophied adipose tissue itself produces inflammatory mediators in obese persons (Skurk T et al.,2007), leading to macrophage migration. As adipose tissue enlarges, the hypoxic areas are observed because of insufficient neovascularization and inadequate oxygen supply. Hypoxia itself induces macrophage and immune cell migration further aggravating adipose tissue inflammatory dysfunction (O'rourke RW et al.,2011). When fat cells enlarge, their nutrient demand increases, in the face of inadequate nutrient supply, the hypertrophied adipocytes die and release cellular contents into ECF inducing inflammatory response (Cancello R et al.,2005). Endoplasmic reticulum of fat cells undergoes stress when faced with increased nutrient intake and activates pro-inflammatory pathways leading to IR and inflammation (Cnop M et al.,2012). Chronic low-grade inflammation is the hallmark risk factor for T2DM development. Many inflammatory markers have been reported to be raised in T2DM such as leukocytes, chemokines (Herder C et al.,2002) and pro-inflammatory markers (Spranger J et al., 2003). Out of these markers, the measurement of CRP is cheap, standardized and widely available and acceptable. hsCRP (high sensitivity CRP) gives the status of

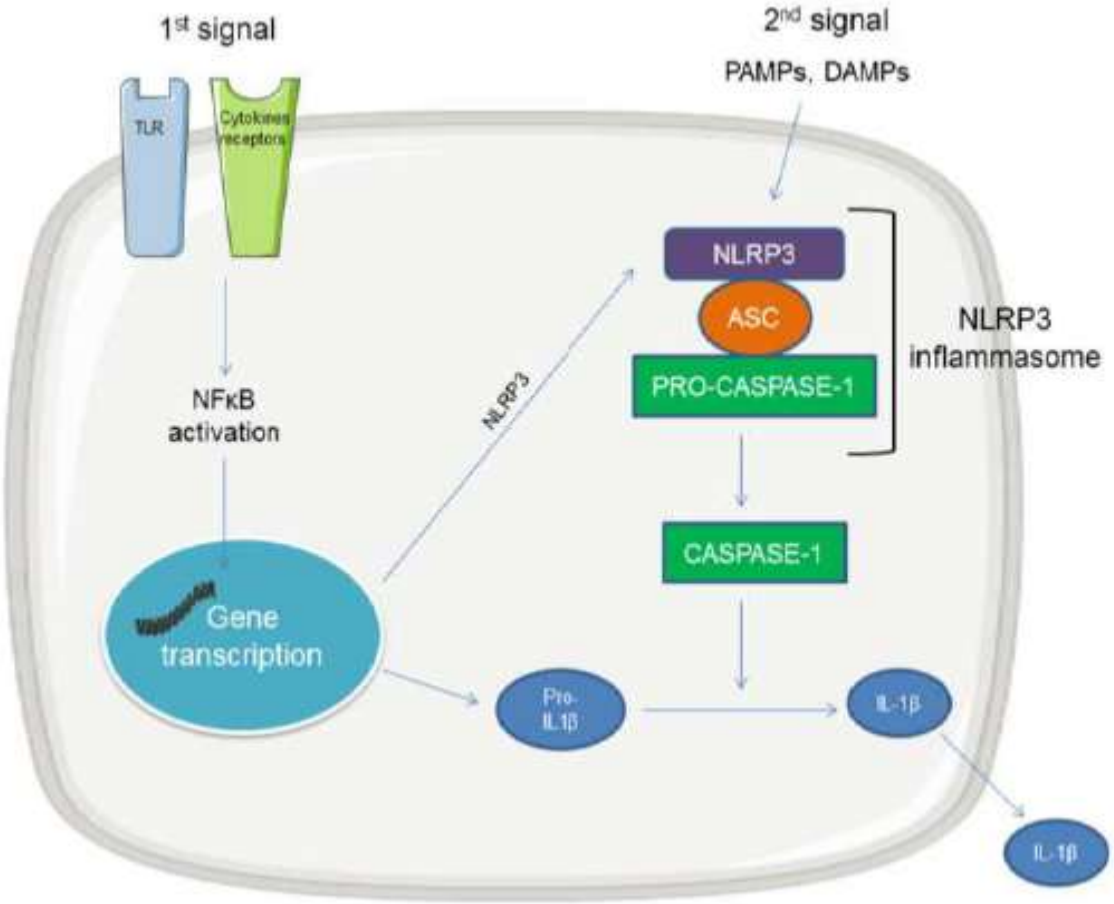


Figure 1.4: NLRP3 inflammasome (Schroder K et al,2010)

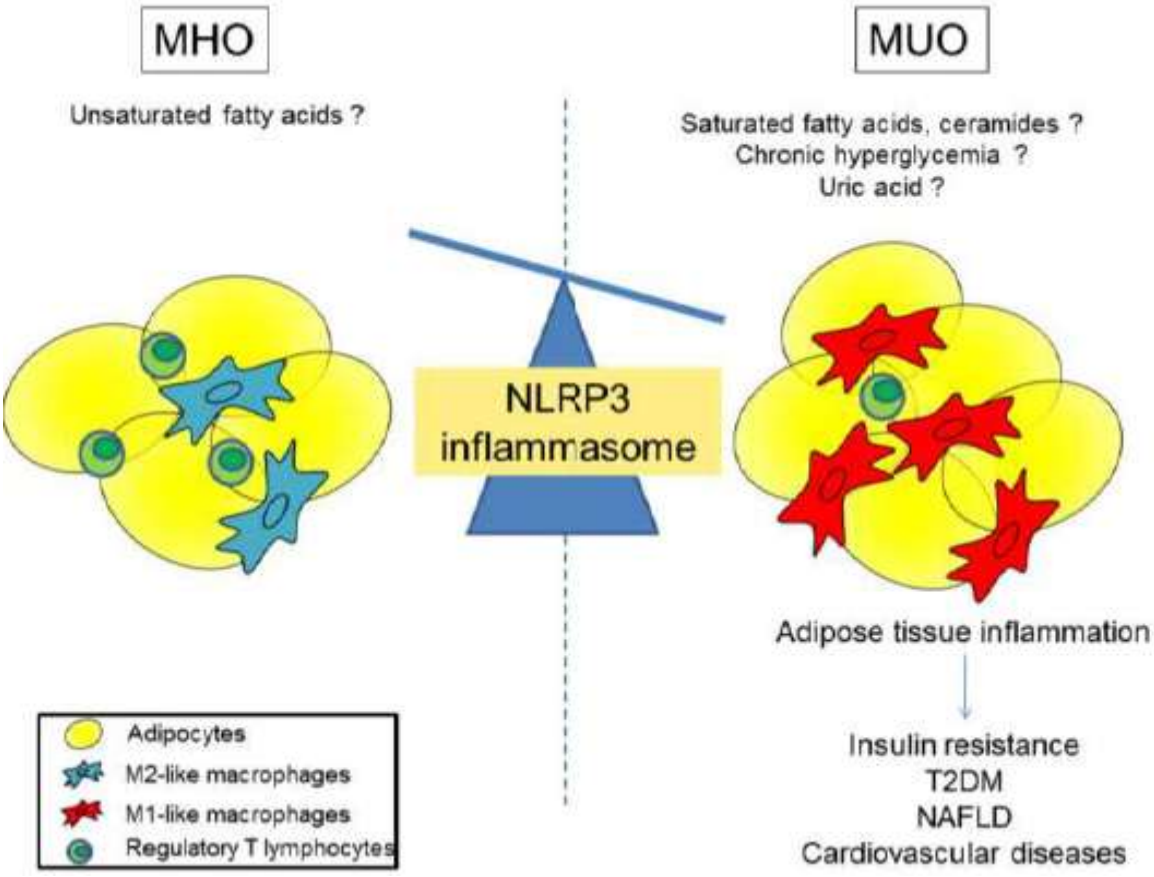


Figure 1.5: Adipose tissue inflammatory profile imbalance between metabolically healthy and unhealthy obese (Koster A et al.,2010)

prediabetes or diabetes in an early stage, when measured at low levels with great accuracy. One study showed, CRP levels to be elevated, hence predicting increased risk of T2DM (relative risk [RR] 1.26 [95% confidence interval or CI 1.16-1.37]. (Wang X et al.,2013). The prospective study (Hoorn study) spread over a period of 7 years showed that CRP was a predictor of morbidity in T2DM patients (Jager A et al.,1999) particularly coronary artery disease (Fig 1.6) (Saito I et al.,2000). In obesity and T2DM, the inflammatory sites are adipose tissue, muscle, liver and pancreas as established by animal model study of obese and T2DM animals and in obese human participants with Metabolic Syndrome or T2DM.Macrophages infiltrate these tissues and produce pro-inflammatory mediators (Chawla A et al.,2011) including TNFα, IL-6 and IL-lβ. They interfere with insulin signaling in target tissues of insulin by activation of CJUN N-terminal kinase (JNK) and nuclear factor kappa B(NF-kB) pathways (Shoelson SE et al.,2006). There is an over-expression of TNF α in adipose tissue of obese patients acc. TNF α has a direct role in obesity related inflammation leading to insulin resistance. In obesity, adipose tissue enlarges and there is up-regulation of gene encoding inflammatory markers and enhanced production of cellular cytokines (Ouchi N et al., 2011). Macrophages invade the hypertrophied adipose tissue in both animal model (mice)and in humans (Chawla A et al.,2011). Hypertrophied adipose tissue; itself produces cytokines, chemokines and inflammatory markers (Skurkt T et al.,2007). Lifestyle modifications such as exercise and healthy diet reduces the size of adipose tissue inducing under-expression of pro-inflammatory markers as the number of macrophages decreases in the adipose tissue (Bruun JM et al.,2006). Macrophages are of two types; M1 secretes IL-1β, IL-6, TNF α, are also called “Classically activated macrophages” and second population of macrophages are M2 and are called “alternatively activated macrophages that secretes IL-10. (Chawla A et al.,2011). The distinct two subtypes are in animal models(mice) but not humans (Wentworth JM et al.,2010). After, macrophage infiltration in the enlarged adipose tissue, there is a switch from M2 to M1 phenotype which is associated with insulin resistance in both rats and humans (Lumeng CN et al.,2007). M1 macrophages impair insulin signaling and adipogenesis in fat cells whereas macrophages(M2) protect against insulin resistance (Chawla et al.,2011). Apart from macrophages, lymphocytes also infiltrate the adipose tissue in obese persons and modulate the activation of macrophages (Feuerer M et al., 2009,

Nishimura S et al.,2009, Winer S et al.,2009, Deiuuis J et al,.2011). In animal models (mice) of obesity, there is expression of cytotoxic CD8 + effector cells which recruit and activate macrophages and hence increase pro-inflammatory mediators release from these cells (Nishimura S et al.,2009). The balance between pro-inflammatory cells i-e TH1 and TH17 lymphocytes and anti-inflammatory cells (TH2 and regulatory T lymphocytes) CD4 + cells subtypes is modified to release cytokines from macrophages (Winer S et al.,2009, Jagannathan Bagdan M et al.,2011, Deiuliis J et al.,2011). Regulatory T cells decreases in obese people with metabolic syndrome (Esser N et al.,2013) as these cells release IL-10 which inhibit macrophage migration and induce differentiation of M2 macrophages (Wentworth JM et al.,2010). If these regulatory T cells increase in number, insulin sensitivity will increase with decreased macrophages migration to adipose tissue (Wentworth JM et al.,2010). There is a complex interaction between innate and acquired immunity in the pathogenesis of obesity related inflammation in obese individuals, but despite everything the molecular events that initiate these processes are not fully understood.

Fat accumulates in humans at both subcutaneous sites and abdomen. Excess fat accumulation around waist is called abdominal adiposity and is measured by waist circumference clinically. Visceral adiposity is characterized as important component of Metabolic Syndrome (Koster A et al.,2010). Fat also accumulates in liver and skeletal muscle participating in the development of insulin resistance and subsequently metabolic syndrome (Tchernof A et al.,2013). There is more infiltration of macrophages and T lymphocytes and inflammatory markers release in visceral adiposity than in sub-cutaneous fat of obese persons (Cancello R et al.,2005, Esser N et al.,2013). Although, inflammatory cells migrate and activate in the enlarged adipose tissue of obese individuals but the number of regulatory T cells decrease in visceral adipose tissue of obese people with Metabolic Syndrome (Esser N et al.,2013). As in adipose tissue, M1 macrophages also invade the skeletal muscle of obese mice in animal studies (Nguyen MT et al.,2007). This contributes to decreased insulin sensitivity by over-expression of pro-inflammatory mediators (Weisberg SP et al.,2003, Nguyen MT et al.,2007). The number of macrophages is less in skeletal muscle as compared to liver and adipose tissue and further research is needed to see if it is the primary target for

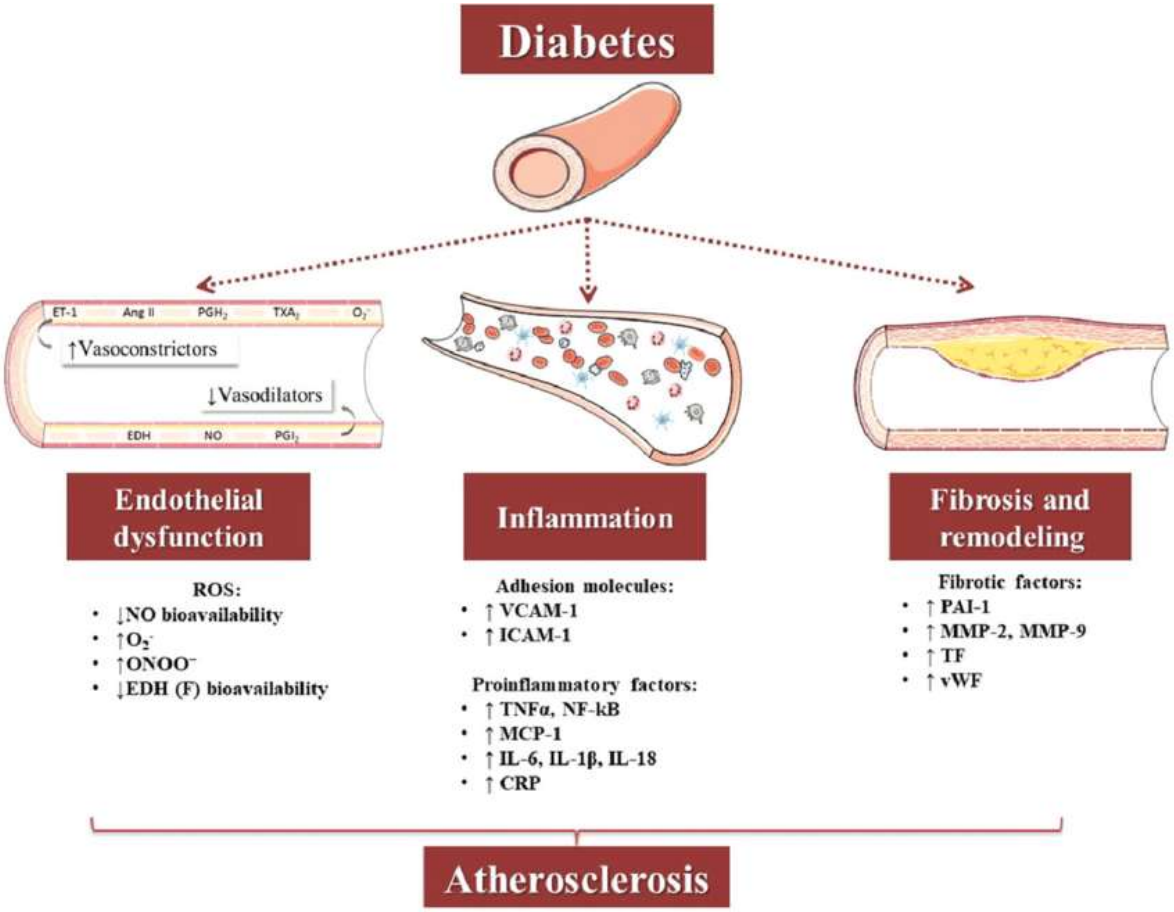


Figure 1.6: Changes encompassing microvascular and macrovascular complications associated with diabetes (Camila A et al.,2018)

obesity related inflammation in obese T2DM patients. In obese T2DM patients, the underlying chronic low-grade inflammation also involves the endocrine pancreas which is confirmed by the deposition of amyloid, fibrosis and increased beta cell death with macrophage migration to the islets of Langerhans of pancreas and release of inflammatory markers. (Donath MY et al.,2011). IL-1β is the cytokine which seems to be the regulator of islet cell inflammation in T2DM by increasing gene expression of inflammatory mediators (Dinarello CA et al.,2009) which also contributes in migration of immune cells in islet of Langerhans of pancreas. All this contributes to β cell dysfunction, reduce insulin secretion with possible β cell death and overall poor progression of disease. A chronic metabolic disorder characterized by hyperglycemia because of insulin insensitivity at tissue level with relative or absolute insulin deficiency is commonly known as type 2 diabetes mellitus (Shah et al., 2019). It has both genetic and environmental factors contributing to its disease process such as marked obesity, sedentary lifestyle and poor dietary habits (Feng et al., 2018). T2DM as a disease progresses to involve cardiovascular system, neurological system and eyes (Kumar et al., 2019). This disease is treated by preventive measures such a healthy diet and lifestyle changes along with drugs which are usually biguanides and insulin (Bhargava et al., 2018). With proper treatment T2DM can be controlled and its complications can be prevented or delayed.

EPIDEMIOLOGY AND PREVALENCE OF TYPE 2 DIABETES MELLITUS:

The prevalence of Type 2 Diabetes Mellitus is increasing globally (Fig 1.7) as depicted by recent research articles. Yoon et al. (2018) conducted a study and found out that, the prevalence of T2DM in South Korea was 11.3% in 2015, which was an increase of 2.3% from the previous study in 2011. Similarly, a study conducted by Al-Nozha et al. (2017) in Saudi Arabia reported a prevalence of 16.3%, which was an increase of 5.6% from the previous study in 2011. Additionally, a study conducted by Zou et al. (2016) in China reported a prevalence of 11.6%, which was an increase of 3.2% from the previous study in 2010(Table 1.1). Thus, proving that there is global increase in the percentage of people suffering from diabetes mellitus. Diabetes mellitus is a growing health concern in Pakistan. According to World Health Organization, the prevalence of Diabetes in Pakistan is estimated to be 8.6%, with majority of cases being type 2 diabetes. The global prevalence

of 6.2% of T2DM is less than the estimated prevalence of this disease in Pakistan. This number would increase in the years to come because of nation's rapidly growing population and changing lifestyle habits. There are many factors that can contribute to development of diabetes, such as genetics, lifestyle and environmental factors (Khera A.V. et al., 2018). The most likely risk factors for development of diabetes are middle age, having diabetes in the family, hypertension, obesity and impaired lipid profile (Wang Y et al., 2016). Majority of diabetes cases in Pakistan are found in urban areas, where risk factors such as poor diet, physical inactivity and obesity are more common. Diabetes is more common in females than males, with nearly 10% of women in Pakistan suffering from the disease.

The national prevalence of diabetes in Pakistan is 26.7%. according to IDF atlas ,2021, in the 10^{th} Edition. (Basit A et al.,2018) (Table 1.1). A study published in the journal BMC Endocrine Disorders; prevalence of T2DM in Pakistan was estimated to be 8.9% in 2018. This is an increase from the estimated prevalence of 6.3% in 2011. Urban areas had a higher prevalence of 10.8% than rural areas (7.3%) (Ahmed M et al., 2018). A separate study published in the journal Diabetes Research and Clinical Practice in 2019 reported a prevalence of T2DM in Pakistan of 11.6%. Men had a lower prevalence of diabetes i-e (10.2%) than women (13.2%). Those people who are >50 years old were more likely to be diabetic (17.9%) than those who were <50 years old (7.8%) (Khan M.A. et al., 2019). Overall, prevalence of T2DM in Pakistan is increasing and is higher in urban areas, women, and people aged ≥ 50 years old.

PATHOPHYSIOLOGY OF TYPE 2 DIABETES MELLITUS: There has been extensive research on the subject of pathophysiology of T2DM, in recent years. The disease is characterized by complex interaction of genetic, environmental and lifestyle factors. Recent research has identified a number of mechanisms for the disease progression and development of T2DM. Impaired insulin secretion is amongst the foremost reasons of development of T2DM. In T2DM, the pancreas fails to secrete sufficient amounts of insulin to maintain normal glucose levels, resulting in hyperglycemia. This is due to combination of beta cell dysfunction and inadequate beta cell mass (Liu et al.,2020). Beta cell dysfunction is caused by a number of factors, including inflammation, oxidative stress and

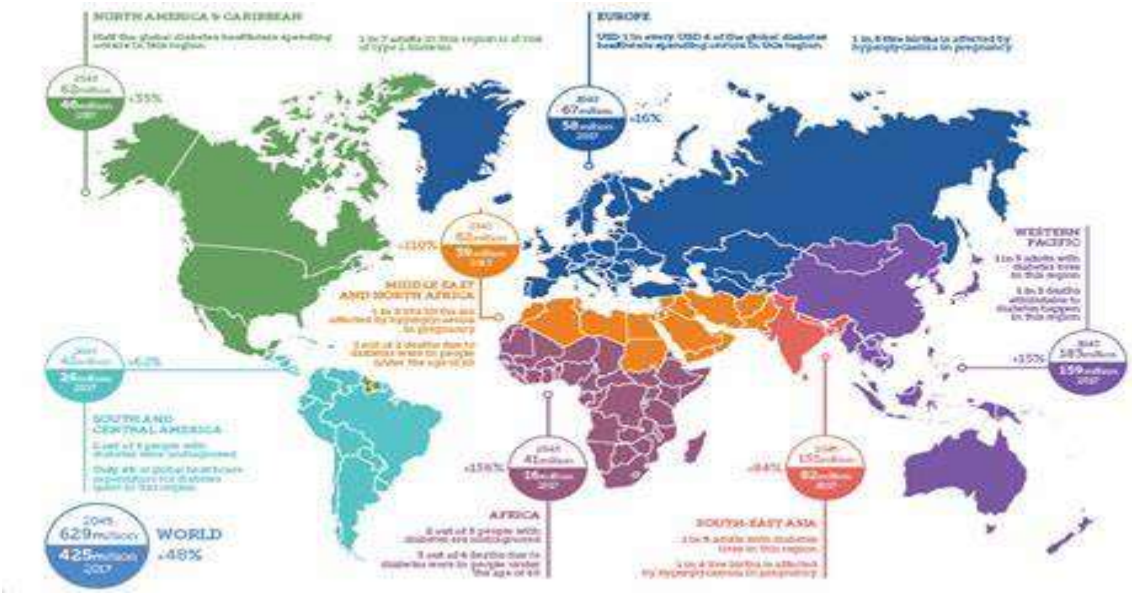


Figure 1.7: The prevalence of Type 2 diabetes mellitus worldwide (IDF Atlas., 2021)

Table 1.1: Frequency of males and females aged (20-79 years) with diabetes in 2019,2030 and 2045.

2019	Frequency of people with diabetes (millions)	Prevalence (%)
Men	240.1	9.6
Women	222.9	9.0
2030		
Men	296.7	10.4
Women	281.8	10.0
2045		
Men	357.7	11.1
Women	342.5	10.8

Source: IDF Atlas: 10th Edition.

advanced glycation end products. Inadequate beta cell mass is caused by apoptosis, which is triggered by the same factors cause beta cell dysfunction. Insulin resistance is another mechanism which leads to T2DM. It occurs when the cells fail to respond to insulin at the tissue level resulting in elevated levels of glucose in the blood. Insulin resistance occurs because of many factors both genetic and environmental including obesity, sedentary lifestyle and a diet rich in fat. Furthermore, insulin resistance is exacerbated by inflammation and oxidative stress.

Thus, recent research has identified a number of mechanisms underlying development and progression of T2DM (Liu et al., 2020). These include impaired insulin secretion due to beta cell dysfunction and inadequate beta cell mass, along with insulin insensitivity at tissue level due to factors which are both genetic and environmental (Liu et al., 2020). Decreased in beta cell mass is associated with an increase in pro-inflammatory cytokines that leads to apoptosis of beta cells. Additionally increased hepatic glucose production is caused by an increase in gluconeogenesis which is driven by the hormone glucagon (Liu et al., 2020). Finally, increased peripheral resistance to insulin action is caused by an increase in inflammatory cytokines, which can lead to increase in resistance to insulin action at tissue level in both skeletal muscle and fat cells (Liu et al., 2020). Taken together, these pathophysiological processes contribute to the development of T2DM.

T2DM stems from obesity related insulin resistance. When fat accumulates in an excess amount in the body, it causes physical impairment in terms of poor health, the condition is called obesity (Golley et al., 2018; Sarmiento et al., 2017). It is influenced by many factors which are genetic, environmental, biological and behavioral (Golley et al.,2018). Obesity is defined as a body mass index of $\geq$ 30 kg/m2 according to the chronicles of World Health Organization (WHO) (Sarmiento et al.,2017). The body mass index is obtained by dividing a person's weight in kg by the square of their height in meters (Golley et al.,2018). Additionally, a person of BMI between 25 and 29.9 kg/m2 is overweight, according to the same classification as given by WHO (Sarmiento et al.,2017) (Table 1.2). The general population is either obese or overweight (Figure 1.8), which is a growing health concern, in recent years.

Table 1.2: BMI of Adults Ages 20 or older (Weir CR et al.,2023)

BMI	CLASSIFICATION
<18.5	**Under weight**
18.5-24.9	**Normal or Healthy Weight**
25-29.9	**Overweight**
30+	**Obesity**
40+	**Severe Obesity**

PREVALENCE OF OBESITY: According to recent research, the number of people affected by obesity is increasing at an alarming rate as proven by a study done by WHO in 2018, which revealed that > 1.9 billion people are overweight and 650 million are obese in which 39% of these persons were more than 18 years of age i-e overweight and 13% of them were obese (WHO.,2018). (CDC) Centers for Disease Control and Prevention in 2019, reported that prevalence of obesity was highest among adults aged 40-59 years, with 42.8% of men and 44.8% of women in this age group classified as obese (CDC.,2019). Non-Hispanics black adults aged 40-59 years with 49.6% of men and 57.9 % of women in this group classified as obese (CDC.,2019) (Figure 1.10). Clearly, this is a major public health concern that needs to be addressed. The top eight most obese countries in the world lie in the South Pacific region with an average BMI, namely Cook Island (32.9), Nauru (32.5), Niu (32.4), Samoa and Tonga (32.2) followed by Tuvalu (30.8), Kirabati(30.1) and Saint Lucia (30.6)(World Health Organization.,2023).Ethiopia and Madagascar are among the least obese countries in the world with an average BMI of 21.1. Japan, China, India and Bangladesh having a BMI in the range of 21.8-22 are also amongst the least obese countries in the world. In Pakistan, 8.6 % of adults are obese. (Fig 1.9). Obesity has become a growing public health concern in Pakistan. Recent researches have shown that the prevalence of obesity in Pakistan is increasing, with estimates ranging from 10-30%. The Journal of Pakistan Medical Association in 2019, published a study which reported that the prevalence of obesity among adults aged 20-50 years was estimated to be 17.3%. Additionally, the study found that females (19.3%) were more obese than males (15.3%) (Khan et al., 2019). The Pakistan Journal of Medical Sciences in 2020 showed that the prevalence of obesity among adults aged 20-60 years was 24.8% with the prevalence being higher among females (26.2%) than males (23.3%) (Ahmed et al.,2020). Urban areas had a higher prevalence of (26.2%) than in rural areas (21.2%). Overall, the number of people affected by obesity in Pakistan are increasing, and is higher among females than males, as well as higher in urban areas than in rural areas.

PATHOPHYSIOLOGY OF OBESITY: Recent research has revealed that there is low grade chronic inflammation in obesity as there is production of pro-inflammatory cytokines such as IL-6 and C reactive protein (CRP) (Kumar and Sharma., 2016). This inflammatory state is believed to be caused by accumulation of macrophages in adipose tissue, which

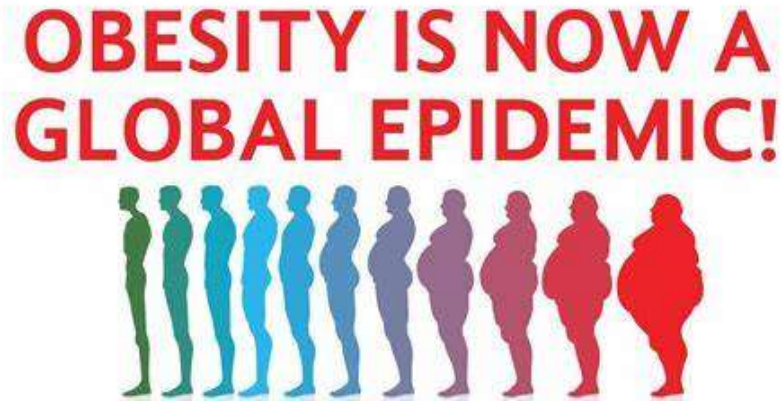


Figure 1.8: Obesity & Overweight - (iator.net)

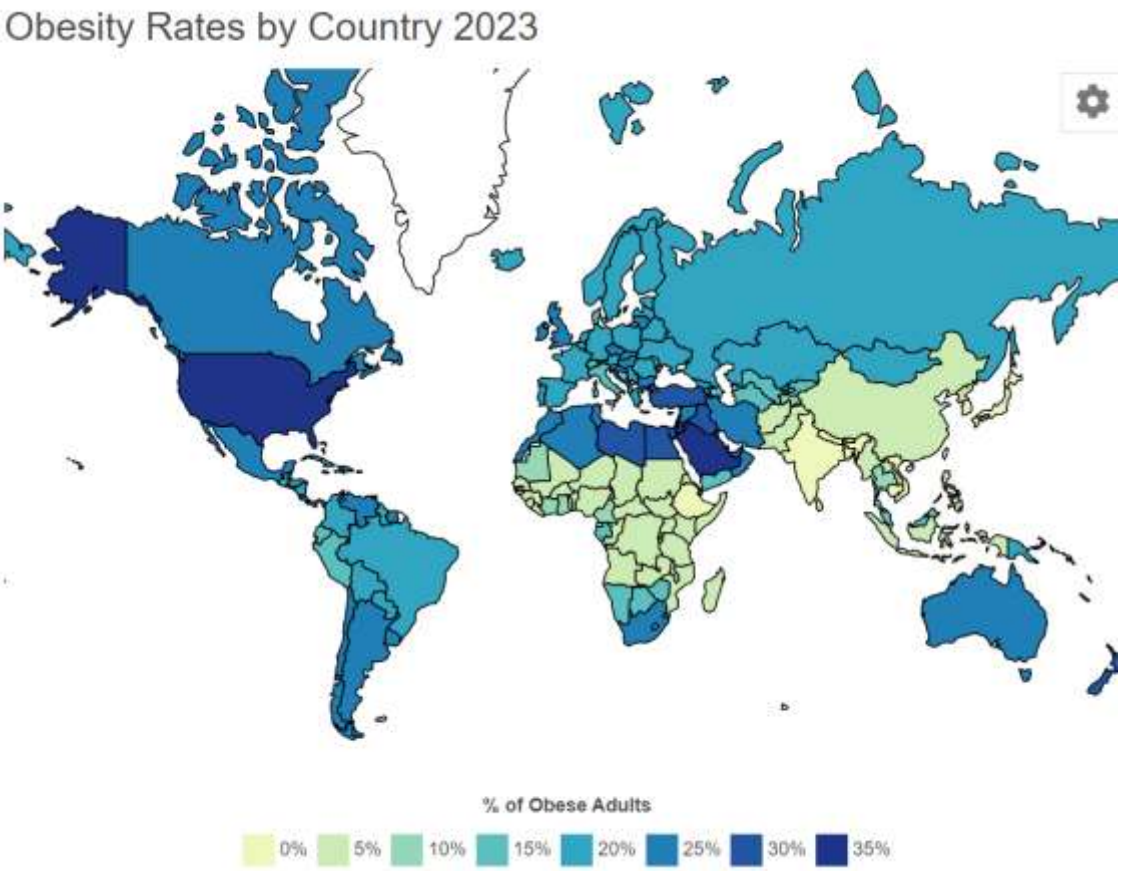


Figure 1.9: Prevalence of obesity among adults, BMI≥30-age standardized by country- World Health Organization (2023)

releases inflammatory mediators, such as chemokines, reactive oxygen species (ROS), and Toll-like receptors (TLRs) (Friedman and Halaas,1998). A, regulatory transcription factor released in inflammation is activated (NF-kB) because of increased production of pro-inflammatory cytokines (Kumar and Sharma.,2016). When NF-kB is activated, there is gene expression leading to production of pro-inflammatory cytokines, exacerbating the inflammatory state (Friedman and Halaas.,1998). Thus, chronic inflammation leads to obesity dependent diseases, such as T2DM, Fatty Liver disease and diseases of Cardiovascular system (Kumar and Sharma.,2016). Obesity is an accumulation of excess fat stores in the body. Fat cells known as adipocytes are very active cells capable for producing different adipokines that act at different locations increasing the risk of diabetes and cardiovascular diseases. The excess macronutrient in the adipose tissue stimulates the release of cytokines in the body namely IL-6, TNF-α and reduced production of adiponectin which predisposes to inflammatory state and oxidative stress. IL-6 causes the liver to release CRP which is sensitive to detect underlying sub-clinical inflammation in the body. Obesity states has been categorized into obese and non-obese according to BMI into Obese (>25kg/m2) and non-obese (<25Kg/m2) groups. This has been defined by the Asia Pacific Body Mass Index criteria based on World Health Organization Western Pacific Region. (*Obesity and Overweight*, n.d.), which has not yet been standardized.

PREVALENCE OF METABOLIC SYNDROME:

Metabolic syndrome is becoming one of the prevalent conditions in the world with a prevalence rate of 20-25% in 2021.(Ranasinghe et al., 2017). Recent research has shown that the prevalence of metabolic syndrome is increasing worldwide. The International Journal of Environmental Research and Public Health published a study in 2020, showing that Metabolic Syndrome in adults aged 18-64 years was 21.5%, which is higher than the global prevalence of 17.9%. Women were more susceptible to develop Metabolic Syndrome than men. It was more common in the lower socioeconomic group of people. A study published in the journal BMC public health in 2020 found that the prevalence of Metabolic Syndrome in adults aged 18-64 years in United Kingdom was 17.7%. This figure is lower than the global prevalence and the prevalence of Metabolic Syndrome in USA.

Metabolic Syndrome has a prevalence of 30% in Pakistan. A study conducted in 2019 by the Department of Biochemistry at the University of Karachi, Pakistan investigated in a sample of 5000 adults aged 18-65 years; the prevalence of Metabolic Syndrome, which came to be 28.4% with highest numbers found in adults aged 45-54 years. (Ahmed, S., et al.2019). In contrast to other studies conducted worldwide, the prevalence was more in men (33.3%) in men than in women (22.3%). A study by the Medicine Department of Punjab University, Pakistan in 2018 found the prevalence to be 29.2% in adults aged 18-65 years. But here the prevalence was higher in males (34.2%) than in females (24.2%) (Ahmed. S., 2018).

CRITERIA FOR DIAGNOSING METABOLIC SYNDROME:

Metabolic syndrome (Figure 1.10) is the medical term for a group of conditions associated with high blood pressure, high blood sugar, abnormal cholesterol levels (low HDL or high triglycerides), and increased fat deposits in the abdominal area (typically measured by waist circumference as well as BMI) (Wang et al., 2020). The Selection of patients with Metabolic syndrome is based on 2005 revised criteria of National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III).

1. Waist-circumference (WC) ≥ 80 cm in women and ≥ 90 cm in men (Abdominal Obesity).
2. Increased Triglycerides ≥150 mg/dl.
3. Low High-Density Lipoprotein -cholesterol (HDL-C) <50 mg/dl in women and < 40 mg/dl in men.
4. Increased BP≥130/85 mm of Hg or the use of antihypertensive medication.
5. High Fasting Plasma Glucose (FPG) ≥100 mg/dl or the use of oral hypoglycemic agents. / 2-hour oral glucose > 140mg% IGT or HOMAIR.

The greater number of these conditions affect an individual, the higher the risk for one of the critical diseases listed above. Metabolic syndrome affects a range of organ systems including cardiovascular, endocrine, and central nervous system. WHO, NCEP-ATP III and IDF recognizes Metabolic Syndrome as a major risk factor for both T2DM and Cardiovascular Diseases (CVD) (Figure 1.11) It is associated with obesity and insulin resistance, which along with the conditions lead fatty buildups in artery walls

(atherosclerosis) (Wang et al., 2020). Chronic low-grade inflammation is associated as an important factor that determines metabolic syndrome as proven by previous studies. The inflammatory condition associated with Metabolic Syndrome leads to its abnormal biochemical and clinical status.(Maleki et al., 2014) There are contradictory conclusions about Metabolic Syndrome and some Inflammatory biomarkers in recent studies.

C-REACTIVE PROTEIN(CRP):C-Reactive Protein (CRP) -biomarker of sub-clinical inflammation has been found to be elevated in glucose intolerant condition and in other deranged components of Metabolic Syndrome. (Figure 1.12). C-reactive protein is an important biomarker for inflammation and is widely used in medical research. CRP is of hepatic origin and is protein in structure. CRP levels are a measure of body's response to inflammation (Friedman et al.,2019). Recent research has revealed that CRP is raised in diseases of cardiovascular system, T2DM and rheumatological disorders. CRP has been studied extensively in recent years, and its role in the diagnosis and management of various diseases is becoming increasingly well understood (Khera et al.,2018). A, recent study found that in Chinese adults, high CRP was associated with increased risk of cardiovascular diseases (Zhang et al.,2020). This study also suggests that CRP levels could be used as a predictor of future cardiovascular events. In addition, another study found raised levels of CRP is associated with increased risk of developing T2DM in Chinese adults (Chen et al.,2019). The authors concluded that CRP can be used as a biomarker for early detection and management of T2DM.

C-reactive protein is a ring-shaped protein which has pentameric structure (Pagana KD.,2019).It is found in blood and it increases in the blood due to inflammation(Burris CA.,2006).Hepatocytes synthesize CRP and it is among the acute phase proteins.IL-6 released from macrophages and T cells are responsible for its secretion(McPherson RA.,2011).It belongs to pentraxin family of proteins(Devaraj S.,2009).It mechanism of action is to bind lysophosphatidylcholine expressed on dead and dying cell surfaces so that it can activate complement(Williamson MA.,2011).

The normal range of C-reactive protein (CRP) is <1.0mg/dl in healthy adults (Pagana KD.,2019). The risk of developing cardiovascular diseases with the levels of CRP are; Low risk at <1.0 mg/dl, Moderate risk at 1-3mg/dl and high risk at >3mg/dl. CRP levels are

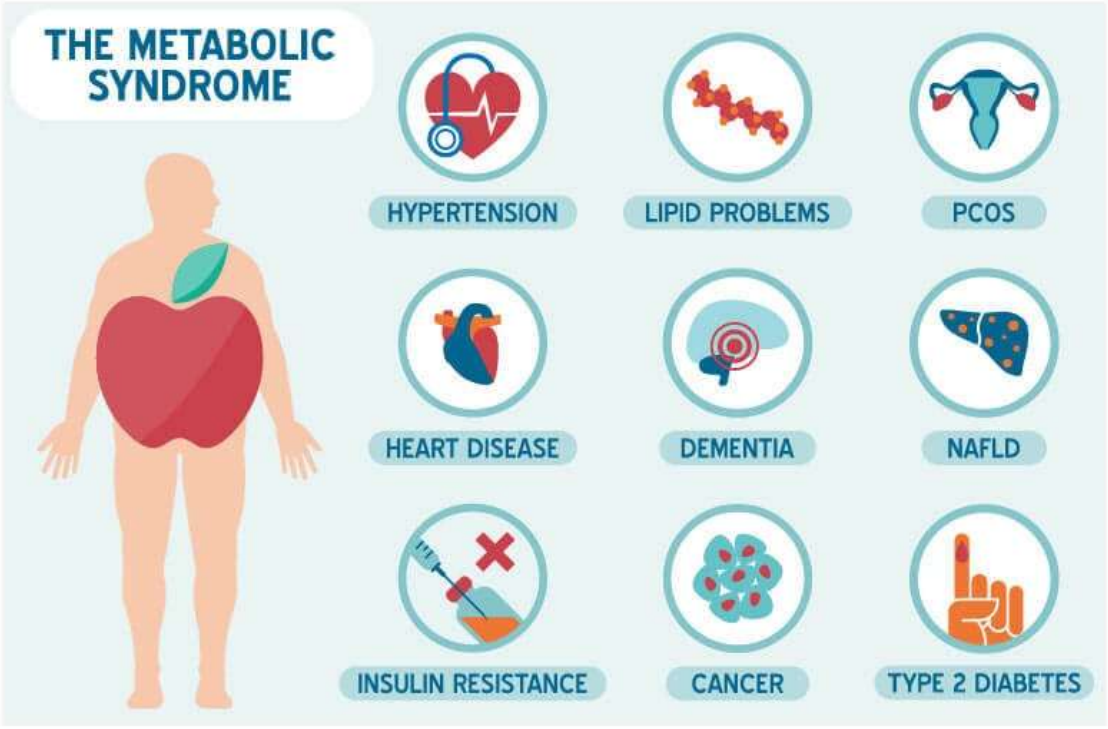


Figure:1.10: The Metabolic Syndrome (https://fortmyerschirostudio.com/conditions-treated/blood-sugar-imbalance

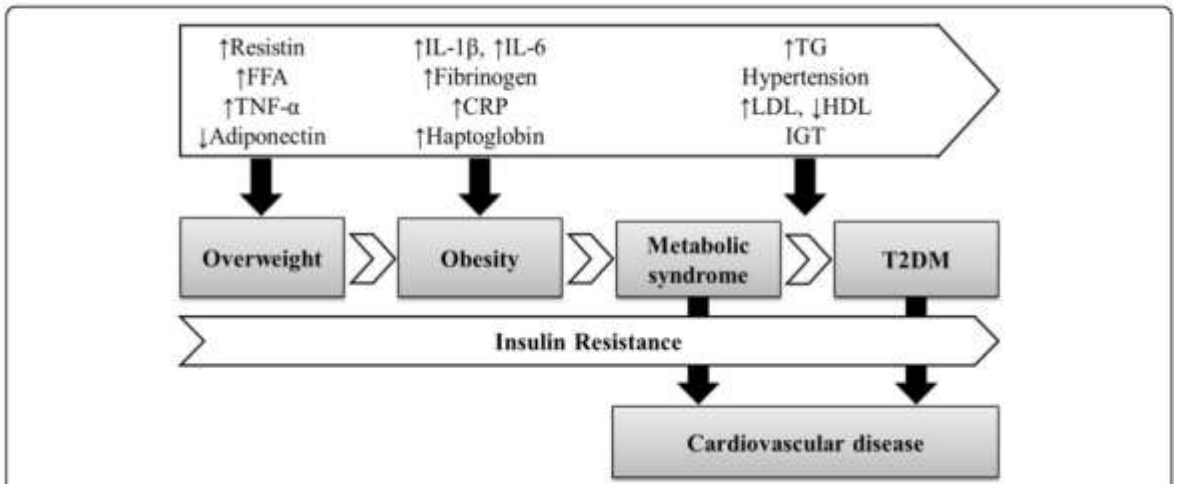


Figure 1.11: Relationship between inflammation, metabolically unhealthy obesity and development of type 2 diabetes and cardiovascular diseases (Ellulu.,2017)

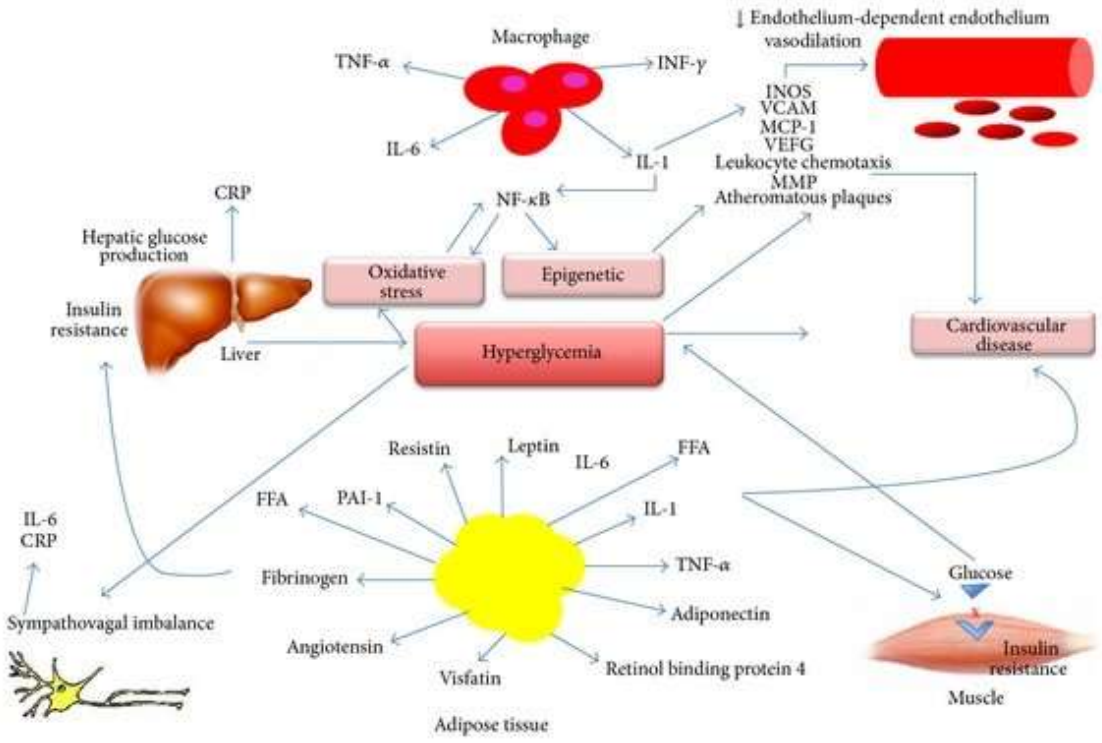


Figure 1.12: Pathogenesis of cardiovascular disease in diabetes (Alessandra Saldanha de Mattos Matheus et al.,2013)

increased in any tissue injury that leads to inflammation such as Infection, Metabolic Syndrome, Acute pancreatitis, Post-surgery, Burns, Leukemia, Tobacco smoking, HRT and Obesity. CRP levels are decreased in Exercise, Weight loss, Alcohol consumption and Drugs such as statins, Niacin and Fibrates (Burris CA.,2006). Gender and ethnic background affect CRP levels. African Americans have higher levels than Caucasians and females have higher levels than males (Burris CA.,2006). CRP is among the acute phase proteins as they increase in blood for short time only. Other proteins that exhibit acute phase reaction are serum amyloid A alpha 1, acid glycoproteins, haptoglobin and fibrinogen. This response is referred as low-grade inflammation (Devaraj S et al.,2009). CRP is also produced by vascular endothelial cells, smooth cells and fat cells (Burris CA.,2006) (McPherson RA et al.,2011). Tillet and Francis found this protein in the blood in 1930(Williamson MA.,2011). The gene for CRP is located on chromosome 1. It has a molecular weight of 25106 Da with a make-up of 224 amino acids (Barr WC RJ.,2005). The letter "C" in CRP denotes the ability of this protein to react with carbohydrate antigen of streptococcus pneumonia capsule (Cases JP et al.,2008). It bears no relationship with either protein C or C-peptide. CRP binds to Fc receptors and activates complement (McPherson RA.,2011). When CRP levels are high in blood, it indicates underlying inflammation while absence of high CRP excludes inflammation (McPherson RA.,2011). CRP levels changes with body mass index (BMI) and requires careful interpretation. Heart disease is known to have an element of chronic inflammation to it; therefore, CRP can be used as a marker of atherosclerosis associated inflammation (Cases JP.,2008).

The indications and applications of getting a CRP done are:

1. CRP is raised in inflammatory conditions such as infection and autoimmune disorders.
2. It is ordered along with Erythrocyte sedimentation rate (ESR).
3. When suspected signs of infection are present in a patient, then CRP is ordered.
4. CRP can be used to monitor conditions of rheumatoid arthritis and Systemic Lupus Erythematosus (SLE) to determine efficacy of the treatment given.
5. It is used to monitor the progression of heart diseases.

CRP has a half-life of 19 hours (Pepys MB et al., 2003). Age and BMI increases CRP. CRP levels are measured in the blood and can be used to diagnose and monitor inflammation in the body. It is also used to help predict the risk of cardiovascular disease and other inflammatory conditions. CRP has proven to be an independent risk factor for cardiovascular diseases. (Friedman et al.,2019; Khera et al.,2018). CRP was first discovered in 1930 by Tillet and Francis, who identified it as a protein in the blood that was reactive to the presence of the C-polysaccharide of pneumococcus (Tillet WS et al.,1930). Subsequent research in 1940s and 1950s focused on its role in inflammation and it has since been found to be a useful marker for various diseases. During an acute phase reaction, CRP rises rapidly within 2 days and decreases rapidly with the infection being resolved. It is not used for diagnostic purposes as many diseases have increased CRP. It can be used as supportive evidence for suspected inflammatory conditions such as giant cell vasculitis, where other features of the disease are absent. CRP>10 mg/dl indicate significant inflammation therefore monitoring CRP levels may give useful information of disease such as flare-ups in RA (McPherson RA et al.,2007). Recent research has shown a strong correlation between C-reactive protein (CRP) and T2DM. Wang et al (2018) showed in their study that T2DM was associated with higher levels of CRP. Similarly, a study conducted by Zhu et al. (2020) found that higher levels of CRP signified underlying disease of T2DM. The study concluded that CRP may be a useful biomarker for predicting the risk of type 2 diabetes mellitus.

A study conducted by the National Institute of Health (NIH) in 2019 found that individuals with higher levels of CRP were prone to T2DM. This study had over 4000 participants in which those with higher levels of CRP, had more chances to develop T2DM than those with lower levels of CRP. Another study conducted by University of Cambridge in 2020 also found a direct association between CRP and T2DM. This study had over 3000 participants in which T2DM was associated with raised levels of CRP. Hence CRP can be an eminent factor in determining the likelihood of developing T2DM. It is also linked to development of cardiovascular diseases, certain neurological conditions such as stroke and other inflammatory diseases (Chang et al.,2020). In addition to its use in assessing risk, CRP can be used to monitor the progression of disease and to guide treatment decisions. Research has shown that higher levels of CRP are associated with poorer outcomes and that

reducing CRP levels can improve patient outcomes. For example, studies have shown that reducing CRP levels through lifestyle changes, such as exercise and a healthy diet, can reduce the risk of cardiovascular disease and other inflammatory conditions.

CRP has been linked to a variety of health conditions, including obesity. It was found by recent research that obese individual had higher levels of CRP than those with BMI of normal range. This suggests, obesity may be associated with increased inflammation in the body. A study published in the journal Obesity in 2019, found obesity was associated with higher levels of CRP. This study included over 1000 participants and found that obese individuals had higher CRP. Those obese individuals with higher CRP were also prone to develop metabolic syndrome, which itself causes heart diseases, neurological conditions and T2DM (Kawai, N. et al., 2019). A study published in the journal Nutrition in 2019 found that obesity was associated with higher levels of CRP in children. This study included over 1000 children and found that those obese children had higher CRP levels and also had metabolic syndrome (Lemos, J. et al., 2019).

A study published in the journal Diabetes Care in 2017 assessed the association of obesity with CRP in individuals with metabolic Syndrome. The study included 1722 participants with Metabolic Syndrome and found that those with higher body mass index (BMI) had raised CRP than those with lower BMI (Kang Y et al.,2017). Metabolic Syndrome patients have elevated levels of CRP as shown by recent research. In a study published in the journal Atherosclerosis, researches revealed that in a sample of 8945 adults with Metabolic Syndrome, there was increased levels of CRP (Kaur, Y et al.,2018). Another study done at University of California, San Francisco which was published in the journal Diabetes Care, found that in a sample of 2912 adults with Metabolic Syndrome, CRP was raised (Kang, Y et al.,2017). These studies suggest that there is a greater risk of Metabolic Syndrome with raised levels of CRP. Elevated CRP may be an indicator of underlying low-grade inflammation. Additionally, elevated CRP levels are associated as signs of poor diet and lifestyle choices which are risk factors for developing metabolic syndrome. A study conducted by Kaur et al (2018) found that CRP levels were significantly higher in obese diabetic individuals compared to non-diabetic individuals (Kaur, A et al.,2018). According

to recent research, C-reactive protein (CRP) levels are significantly higher in obese diabetic individuals compared to non-diabetic individuals (Cakir, S et al.,2019).

1.2 RATIONALE OF THE STUDY:

Chronic low-grade inflammation is the underlying pathophysiology of obesity related inflammation in Metabolic Syndrome in patients suffering from type 2 diabetes mellitus. C-Reactive Protein (CRP) is the best studied biomarker of inflammatory status in both Metabolic Syndrome and Type 2 diabetes mellitus. Therefore, CRP can be used as an indicator for the prediction of risk factor for developing CHD and stroke in type 2 diabetic patients with Metabolic Syndrome.

1.2.1 THEORETICAL GAP:

Persistent chronic hyperglycemia occurs in Type 2 diabetes mellitus (T2DM). A variety of metabolic abnormalities constitute the Metabolic Syndrome which increases the risk of developing T2DM. CRP, the inflammatory biomarker increases in both Metabolic Syndrome and T2DM. The theoretical gap in this case is the lack of understanding of the association between CRP and T2DM in patients with Metabolic Syndrome. Many studies have showed an association between elevated CRP and T2DM. However, these studies did not look for the presence of Metabolic Syndrome in the population studied. This is important because Metabolic Syndrome is known to increase the risk of T2DM and act as confounding factor in the association between CRP and T2DM. Therefore, there is a need for studies that investigate the association between CRP and T2DM in patients with Metabolic Syndrome.

1.2.2: CONTEXUTUAL GAP:

We do not understand the mechanisms by which CRP is found to be elevated more in obese individuals in type II diabetes mellitus with Metabolic Syndrome. It is already investigated that CRP is a marker of inflammation and chronic low-grade inflammation is the underlying pathophysiology of Metabolic Syndrome and T2DM. However, the specific pathways are not known and need to be investigated to determine the clinical implications of raised CRP in this population.

1.2.3: METHODOLOGICAL GAP:

Type 2 diabetes mellitus patients with metabolic syndrome have been found to have elevated levels of C-reactive protein (CRP) in their blood. However, there is a methodological gap in the association of CRP with metabolic syndrome in these patients. The gap lies in the fact that many studies have not accounted for confounding factors such as age, sex, and body mass index (BMI) when analyzing the association between CRP and metabolic syndrome. This has led to inconsistent results and a lack of consensus on the exact relationship between CRP and metabolic syndrome in type 2 diabetes mellitus patients.

In order to fill this gap, future studies should take into account these confounding factors and use standardized methods for measuring CRP levels. This will allow for more accurate and reliable conclusions to be drawn about the association between CRP and metabolic syndrome in type 2 diabetes mellitus patients.

1.3: PROBLEM STATEMENT:

To diagnose a case of Metabolic Syndrome in type 2 diabetes mellitus patients requires assessment of its five components namely high blood pressure, high lipid profile, high blood sugar and increased abdominal circumference Metabolic syndrome is related to obesity and obesity is related to acute and chronic inflammation. An inflammatory marker such as C-Reactive Protein can serve the purpose as it's an indicator of underlying subclinical inflammation, which can be used as a predictor for risk factor of CHD and stroke in type II diabetic patients with Metabolic Syndrome.

1.4: RESEARCH QUESTION:

Can C- Reactive Protein (CRP) be used as a biomarker for obesity related inflammation in type 2 diabetes mellitus patients with Metabolic Syndrome?

HYPOTHESIS:

(A) NULL HYPOTHESIS:

There is no difference in CRP levels between obese and non-obese type II diabetes mellitus patients with Metabolic Syndrome.

(B) ALTERNATE HYPOTHESIS:

There is a difference in CRP levels between obese and non-obese type II diabetes mellitus patients with Metabolic Syndrome.

1.5: OBJECTIVE(S) OF THE STUDY:

1. To determine the association of serum CRP levels and metabolic syndrome in obese and non-obese type 2 diabetes mellitus patients.
2. To study the association of CRP levels with T2DM and its related variables.
3. To determine the CRP levels in type 2 diabetes mellitus patients and relate it to cardiovascular risk.
4. To estimate CRP levels in subjects with and without Metabolic Syndrome in T2DM patients.
5. To assess the association of C-reactive protein (CRP) with type 2 diabetes mellitus (T2DM) and to determine the joint effect of obesity and metabolic syndrome on them in a local population of Karachi (Pakistan).
6. To assess the correlation of individual components of Metabolic Syndrome and CRP among T2DM patients.
7. To determine the relation between glycemic indicators, lipid profile and CRP in individuals with type 2 diabetes mellitus patients with or without Metabolic Syndrome.

1.6 SIGNIFICANCE OF THE STUDY:

Metabolic Syndrome is a constellation of disorders comprising of abnormal lipid profile, hypertension, visceral adiposity and glucose intolerance. All four disorders are depicted by abnormal values of high BP, high Lipid profile, high FBG and high Waist circumference measurements. The prevalence of Metabolic syndrome in the world especially those of developing countries is on the rise (20-25% in 2021). Obesity has been classified by the World Health Organization into Obese (>30kg/m^2) and non-Obese(<30kg/m^2) based on BMI. Obesity related inflammation and its corresponding markers for diagnosing Metabolic Syndrome have been reviewed in the literature. Metabolic Syndrome presents as a high-risk profile for the development of diseases related to heart, kidney, liver, pancreas, adipose tissue and brain. These disorders include type 2 diabetes mellitus and cardiovascular

diseases and cerebrovascular accidents. CRP is an inflammatory biomarker released from liver cells. Because of its secretion in obesity related inflammation, it can be used as a sixth indicator to detect inflammatory state in Metabolic Syndrome subjects, which can predict the risk of developing CHD and stroke in these patients.

CHAPTER 2

LITERATURE REVIEW

As mentioned, due to the critical nature of CRP in gene expression and pathway signaling of inflammation, there is increased research in it being used as a diagnostic and prognostic biomarker due to its unique properties. Research exists on its role as biomarker for arthritis and tumors, but there is yet limited information for the potential of CRP as a biomarker for early diagnosis of subclinical inflammation in type 2 diabetes mellitus patients with metabolic syndrome (Ellulu et al., 2017). However, there are promising results from the many studies aimed at researching this objective. Acute phase reaction comprises of release of acute phase proteins in the blood which includes C-reactive protein (CRP), alpha 1 antitrypsin, ceruloplasmin, alpha 1 acid glycoprotein, haptoglobin and fibrinogen (Figure 2.1) and (Table 2.1). The conditions which give rise to inflammation are infection, post-operative states, cancer etc. where 1000-fold increase occurs in the CRP levels. All these proteins produced in the inflammatory response have some key roles to play in inflammation like CRP activates the classical component of complement pathway; α 1 antitrypsin can neutralize certain proteases released in inflammatory states. IL-1 is a protein released from macrophages, stimulates release of hepatic acute phase proteins. Gene transcription of these proteins is stimulated by IL-1 and IL-6. (Fahed et al., 2022). Acute phase reaction is a non-specific general response to tissue injury like that of fever and increased white cell count. There is a sequential release of acute phase proteins in this inflammatory response to tissue injury which involves, firstly the release of CRP and α 1 chymotrypsin within 12 hours followed by α 1 acid glycoprotein rise in the blood, finally α 1 antitrypsin, haptoglobin, C4 and fibrinogen levels rise with the final rise in C3 and ceruloplasmin levels within 2-5 days. The acute phase proteins measurements during inflammation do not give a diagnostic value but that of prognostic nature to see the progress of the disease and for looking at the response to treatment. There are some proteins which are decreased in the acute phase response to injury. Albumin, prealbumin and transferrin levels decrease. The CRP levels however rise during this response. The median concentration is 0.8 mg/L, and 3 mg/L comes in the 90^{th} percentile and 10mg/L comes in the 99^{th} centile of healthy adults. The hepatic production of CRP is influenced by

IL-6. In about 6 hours, serum CRP levels rises by >s5mg/L and peaks in 2 days. The plasma half-life of CRP is 19 hours. CRP is raised in obesity (Osborn O et al., 2008). CRP was associated with leptin (positively) and with adiponectin (negatively). Hence, Leptin increased CRP synthesis while adiponectin decreased it. Several of the recent studies showed a positive association between atherosclerotic events and CRP. The pathogenesis of being a pro-atherosclerotic agent lies in the up-regulation of angiotensin type 1 receptor expression. Meta- analysis performed by Emerging Risk Factors Collaboration showed that there is a 60% increase in vascular risk as CRP increase by 1 SD. Ethnicity and sex were not found to be confounders in obesity related rise in CRP.(Therapeutic Advances in Cardiovascular Disease, n.d.) This was confirmed by a meta-regression analysis between CRP and obesity in both children and adults. When CRP was measured along with metabolic markers among obese and normal weight post-menopausal women, higher levels of CRP along with high TG were found in the serum of these patients. (Therapeutic Advances in Cardiovascular Disease, n.d.) For each 1 unit increase in BMI in a study comprising of Indian Children, there was an increase in odds ratio of CRP by 37% (95% CI: 1.23-1.53, $p<0.001$). It was found in one study that BMI, smoking habits, age and TG were significant predictors of CRP. (Song et al., 2019)Another study done in normotensive and hypertensive subjects found out that Waist Hip Ratio (WHR) was a sensitive index of visceral obesity and was associated with chronic inflammation in obese hypertensive patients.(Sproston & Ashworth, 2018) estimated the prevalence of high CRP (≥ 3 mg/L) among non-diabetic and normotensive and normo-lipidemic adults in USA. Among Normal weight individuals, the prevalence of high CRP (≥3 mg/L) was 14.4% and 31.6% for over-weight and 36.0% for obese individuals. Additional factors such as age, gender, smoking, sedentary lifestyle is closely correlated with CRP and serum cytokine concentration.(Mandole et al., 2018). It was determined that the chronic stress in major life events (like work, family, caregiving, financial issues) worsens CRP in men and women aged > 45 years. Women were found to be more stressed than men. CRP was found to be higher in patients with pulmonary hypertension than individuals without hypertension. These biomarkers are acute phase proteins, released in inflammatory response to tissue injury. Amongst them is the C-reactive protein (CRP). It is a biochemical marker of inflammation released in the acute phase response of tissue injury

(Kumar and Sharma., 2016). It is a non-specific marker of inflammation, tissue injury and acute or chronic infections. CRP has become an independent risk factor for cardiovascular disease (Gulhar et al,2022). It is a plasma protein synthesized and released by hepatocytes. It was discovered by Tiller and Francis in 1930 in Oswald Avery's lab. The letter C in CRP comes from its ability to precipitate the polysaccharide capsule of strep pneumonia bacteria (Pepys MB et al., 2003) As CRP discloses about the underlying chronic low-grade inflammation in obese type 2 diabetes mellitus patients with Metabolic Syndrome. It has also predictive value for future cardiovascular events as obesity, diabetes mellitus and Metabolic Syndrome are all risk factors for disrupted cardiovascular health (Table 2.2). One of the key pieces of information necessary to use CRP as a biomarker for the metabolic disorders, is determining the CRP levels in both healthy and unhealthy metabolic states. A study by (Hong et al., 2020) sought to evaluate the connection between CRP levels, the metabolic state, and presence of sex dissimilarity in a population based five year follow up period. Their findings identified that CRP levels were increased in male subjects in an unhealthy metabolic state, but not in females with obesity. The authors suggest that based on their findings and supporting evidence, CRP may be a viable biomarker to characterize a metabolically unhealthy status and risk of CVD.(Sears et al., 2005) However, it is unclear if CRP indicates the underlying metabolic state or remains a mediator of metabolic risk

Diabetes mellitus is a chronic endocrine disorder fairly common in the world. It has six types. Type 1, Type 2 diabetes mellitus, gestational diabetes, latent autoimmune diabetes of adults (LADA), Maturity onset diabetes of the Young (MODY) and Neonatal diabetes (Zheng Y et al., 2017). Type 1 diabetes is less common, not familial and sporadic in nature. It has complete beta cell dysfunction due to autoimmune destruction by several environmental insults like viruses. Absolute insulin deficiency occurs secondary to complete beta cell dysfunction. Type 2 diabetes is characterized by relative insulin deficiency or end-organ resistance to insulin action i-e insulin resistance secondary to excessive weight gain in the form of raised BMI and unhealthy diet (Shah et al.,2019) There is a profound disturbance in the carbohydrate, protein and fat metabolism in diabetes due to disruptive insulin action, insulin secretion or both (WHO consultation group., 1999). Insulin resistance constitutes a series of symptoms because of inadequacy of insulin

action in reducing raised blood glucose levels as target tissues. (Li C et al.,2017). Insulin resistance at the cellular level is the downregulation of receptors in the target tissues. (Czech MP et al., 2017, Malone JI et al., 2019). Secondary diabetes has endocrine causes such as Cushing's Syndrome, glucagonoma and polycystic ovarian syndrome (PCOS). Persistent hyperglycemia of prolonged duration in the wake of polyuria, polyphagia and polydipsia are the cornerstones of diabetes symptomology. T2DM also is a leading cause of illnesses like clinical depression, neuropathy, retinopathy, blondness, renal failure, non-traumatic lower limb amputations, cardiovascular and cerebrovascular diseases (CDC.,2020). Diabetic patients die prematurely due to the complications and aggressive disease progression. The number of deaths doubles in the world for diabetic individuals from those who were non-diabetic from the year 1990-2010. (Lozano R., 2012). The development of diabetes has many contributing factors like genetic makeup of an individual, unhealthy diet, sedentary lifestyle, prenatal causes, age and obesity (Singh R et al.,2004). Thus, there are modifiable risk factors namely consumption of unhealthy food comprising of processed food, animal food, sugary foods, refined grains and foods of high glycemic index, a shift from living an aerobic lifestyle to sedentary one, smoking status, sleep problems and mental illnesses like depression (Liu et al.,2014). Among the Non-modifiable risk factors are age, genetic make-up and male gender. About, 346 million people in the world are suffering from diabetes. With persistent hyperglycemia, certain molecules are released such as protein kinase C, advanced glycation end products (AGE) and reactive species oxygen (RSS) such as polyols (Ghanem AA et al.,2011). There is a growing interest in the underlying inflammatory role in the development of diabetes . The pathogenesis of type 2 diabetes mellitus occurs secondary to activation of innate immunity which leads to development of chronic inflammation which is a pre-requisite for insulin resistance and defective beta cell function (Pickup J et al.,1998). According to study by Donath and Shoelson (2011), the activated innate immunity is triggered by many factors namely raised BMI, deranged lipid profile and hyperglycemia. All these factors cause release of proinflammatory cytokines such as (TNF alpha), tumor necrosis factor -alpha and IL-1(Interleukin-1). Both promote insulin resistance and beta cell dysfunction. It was found that, activating innate immunity is linked with intracellular accumulation of lipids in muscles and liver tissues. Both activate toll like receptors (TLR-4) which again produces

cytokines that leads to insulin resistance (Friedman and Halaas,1998). Most Type 2 Diabetes mellitus patients are obese. The two conditions are considered to have chronic low-grade inflammation as their pathophysiology and mediate also the endothelial dysfunction associated with cardiovascular diseases. Abdominal obesity is the starting point of all these abnormalities contributed by hyperglycemia. Raised blood glucose levels are related to the underlying inflammation and type 2 diabetes mellitus complications (Lobner K et al.,2004). Endothelial dysfunction is particularly mediated by proinflammatory and inflammatory cytokines such as TNF-α, CRP, and IL-6. There is also expression of chemokines such as IL-1 and adhesion molecules which are (ICAM-1 and VCAM-1 and P-selectin with the under-expression adiponectin which is an anti-atherogenic factor (Maiorino MI et al.,2018). BMI differentiates between obese and non-obese category. Those >30kg/m^2 are considered as Obese and <30kg/m^2 are considered as non-Obese. Since obesity and diabetes mellitus often occur together; a new term known as Diabesity has been introduced in recent years. IL-6 is the cytokine released in this response which triggers hepatic synthesis of CRP (Brooks GC et al.,2010). Childhood obesity was found to be a major risk predictor for CRP in young adulthood (Toprak D et al., 2011). The pathophysiology of obesity revolves around chronic low-grade inflammation and release of pro-inflammatory and inflammatory markers in obesity. Unhealthy obese individuals are prone to overexpress CRP and IL-1. Adipocytes undergo hyperplasia and hypertrophy due to various nutritive stimuli leading to an inflammatory state. Hypoxia occurs in the adipose tissue due to reduction in blood supply to fat cells because of their large size (Cinti S et al.,2005). Hypoxia leads to necrosis of fat cells and macrophage infiltration in the necrosed adipose tissue and release of pro-inflammatory biomarkers from the necrosed tissue. (Trayhum P et al.,2004). According to DICARVIA study, obesity was highly common in type 2 diabetes mellitus patients (Francisco J et al., 2018). Obesity is one of the overarching characteristics that can be both a cause and a consequence of metabolic disorders.(Devaraj et al., 2009) indicate that serum levels of CRP are increasingly higher correlating to BMI. However, it is not consistent, peaking specifically during differentiation of pre-adipocytes (3T3–L1) into adipocytes, and then falls to background levels at the mRNA level. This occurs via two distinct mechanisms associated with regulation of CRP expression, but it is observed that there is an upregulation of pro-

inflammatory cytokines. The shifting of the ratio of pro- and anti-inflammatory cytokines is the potential causal factor in the chronic inflammation seen during obesity which then results in insulin resistance and other associated conditions (Mattiotti et al., 2018.It all starts with a raised BMI of > 30kg/m2 which is categorized as obesity according to World Health Organization (WHO) (Sarmiento et al.,2017). Obesity itself is an invitation to the various endocrine and metabolic disorders such as diabetes mellitus. Hyperglycemia in the face of underlying obesity gives rise to further deranged components of body's homeostatic system such as lipid profile and blood pressure. Added to raised BMI in the form of increased waist circumference, where excessive amounts of fat accumulate in the abdominal area, the term of Metabolic Syndrome arises. Obesity, diabetes and Metabolic Syndrome are risk factors for impaired cardiovascular health and gives rise to cardiovascular events such as angina, myocardial infarction and premature deaths due to cardiac arrythmias. All of the above-mentioned conditions have an underlying low grade chronic inflammation working as their pathophysiology. There is a pyramidal display of events in an obese individual where one event leads to another. Obesity in itself is a risk factor for diabetes and both obesity and Diabetes are risk factors for Metabolic Syndrome. All three are risk factors for future cardiovascular events and these three are depicted by CRP levels (Mohammad S et al.,2022). (Table 2.2). Metabolic Syndrome is itself has many components to its existence, namely hypertension, raised sugar levels, dyslipidemia and increased waist circumference i-e abdominal obesity (Wang et al., 2020). Kylin introduced Metabolic Syndrome as a combination of high blood sugar, hypertension and gout in 1920. (Frazer et al., 2015)In 1947, Vague identified visceral obesity linked to CVD and T2DM. (Frazer et al., 2015) Avagaro and Crepaldi in 1965 considered Metabolic Syndrome to be a constellation of hypertension, high blood sugar and obesity(Frazer et al., 2015). Dr. Reavan, in 1988 called Met S as "Syndrome X" and described it to be a "combination of risk factors for cardiovascular and diabetes". IR or insulin resistance was the word coined by him. Kaplan (Frazer et al., 2015) called Metabolic Syndrome as the "Deadly Quartlet Syndrome" which was combination of glucose intolerance, visceral adiposity, increased triglycerides and increased blood pressure. In 1992, the name given again to Metabolic Syndrome was "the Insulin Resistance Syndrome". The final definition of Metabolic

Syndrome came from National Adult Treatment Panel Cholesterol Education Program (NCEP/ATP) in 2001.

Similarly, a study by (Belete et al., 2021) sought to examine the cytokine CRP association with metabolic syndrome and type 1 diabetes mellitus patients. In a cross-sectional study of 487 subjects using complex bioinformatics to determine protein and protein interaction network, the connection between the variables was assessed. The researchers found that hsCRP levels were significantly higher in patients with a recent diagnosis of metabolic syndrome (0.39 vs. 1.73mg/L) (Hong et al., 2020). Several prospective studies showed that elevated CRP levels contributes to increased cardiovascular risk namely, the Physicians' Health Study (PHS), Women's Health Study (WHS), Atherosclerosis Risk in Communities (ARIC), Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/Texas CAPS) in the United States and Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) and Reykjavik studies from Europe. (Ridker et al., 1998)Ridker et al., 2003) (Ballantyne et al., 2005)(Downs et al., 1998)(Koenig et al., 2008). It was also found that CRP levels positively correlate with a range of metabolic factors which contribute to metabolic syndrome including fasting blood glucose, waist circumferences, blood pressure, and triglyceride levels (Festa et al., 2000). Therefore, using binary logistic regression analysis, the authors determined that CRP levels are related to metabolic syndrome and insulin resistance at a level where it can be used a biomarker. In middle aged diabetic men, elevated CRP levels were found in the West of Scotland Coronary Prevention Study independent of risk factors such as triglycerides levels, body mass index (BMI) and glucose. (Freeman DJ et al.,2002). Pradhan et al had similar findings of elevated CRP in diabetic middle-aged women when compared to non-diabetic counterparts (Han et al.,2002). It was also found in this study that there are gender differences in CRP when associated with T2DM incidence. CRP was raised in Japanese Americans who had diabetes but not in native Japanese people according to study by Nakanishi et al.,2003. Chronic hyperglycemia induces oxidative stress (Nishikawa T et al.,2000). Oxidative stress impairs insulin endocytosis in endothelial cells . This leads to endothelial dysfunction and insulin resistance (Gopaul NR et al.,2001). In Japanese population, elevated CRP levels were found in both males and females after adjusting for IR risk factors (Doi et al.,2005). Both obesity and Metabolic Syndrome have the same prevalence around the world. One-

fifth of the US population is affected by Metabolic Syndrome and a quarter of European population is similarly affected while lowest of the prevalence lies in South Asia which are rapidly reaching numbers as that of the west. Due to increased prevalence of obesity in the world, the Metabolic Syndrome has become quite relevant in CVD related disorders. The mechanisms of Metabolic Syndrome pathogenesis are complicated and not fully understood (Fig 2.2) The major contributors are lack of physical activity and consumption of excess calories. Both leads to visceral adiposity which is the primary triggering event for pathways leading to pathogenesis of Metabolic Syndrome. Its pathogenesis revolves around both genetic and acquired factors that play a pivotal role in the final conclusive pathway of inflammation. There are three underlying pathophysiological mechanisms for the development of Metabolic Syndrome namely lipid toxicity, low grade chronic inflammation and insulin resistance.(Frazer et al., 2015) Over-eating and decreased physical activity causes visceral adiposity which is accumulation of fat stores at the abdominal area. Adipocytes are the fat cells of adipose tissue which release a series of cytokines namely increased levels of Leptin, Tumor Necrosis Factor alpha, IL-6, CRP and Fibrinogen while decreased levels of adiponectin is released. Increasing factors cause chronic inflammation. Increase free fatty acids released from adipocytes causes decreased uptake of glucose by the liver and muscle, increased gluconeogenesis, increased lipogenesis, increased triglycerides and hypoinsulinemia all leading to insulin resistance (Figure 2.2). The hormonal effect of insulin on carbohydrate and fat metabolism is increased uptake of glucose in liver and muscle, inhibition of lipolysis and decreased gluconeogenesis. But as insulin resistance develops in adipose tissue, the lipolysis is not inhibited and increased FFA are released into circulation. Reduced glucose uptake occurs in the liver as protein kinase is activated by FFA in the liver, activated protein kinase promotes gluconeogenesis and lipogenesis. As a consequence, insulin level increases to compensate for the maintenance of normal blood sugar. But insulin levels finally decrease as the compensatory mechanisms fail. FFA are lipotoxic to pancreatic beta cells and hence reduces insulin secretion. Insulin resistance also contributes to hypertension as vasodilator effects of insulin and vasoconstrictor effects of FFA is lost (Frazer et al., 2015). According to a study by DeFranzo and Abdul Ghani (2011), impaired insulin signaling pathways in target tissue of insulin namely liver, skeletal muscle and adipose tissue constitute the

insulin resistance which leads to defective and decreased uptake of glucose by cells, increased hepatic glucose production which leads to raised blood glucose levels and this persistent hyperglycemia is a pre-requisite for development of diabetes. Kahn et al (2019) found a low-grade chronic inflammation as the underlying pathogenesis of type 2 diabetes mellitus. It contributes to insulin resistance because the cytokines produced in the inflammatory process, impair insulin signaling and promote insulin resistance. Obesity continues to be a major public health concern worldwide. According to a five-year literature review on obesity, numerous factors contribute to the development and progression of the condition. These factors include genetic predisposition, environmental cues for overeating, sedentary lifestyle, and psychological distress. Studies have also identified the role of diet and lifestyle interventions in reducing obesity incidence, although more research is needed to determine their long-term effectiveness. (Doupis et al., 2017) Furthermore, the literature suggests that addressing obesity requires a multifaceted approach that involves not only individual-level interventions but also changes in food environments and policies at the population level. Finally, the literature emphasizes the importance of addressing obesity as a public health issue, given its significant healthcare costs and negative impact on quality of life. Numerous studies have also identified disparities in obesity prevalence based on race, ethnicity, socioeconomic status, and geographic location. (Pietrobelli et al., 2021). Obesity has become a major public health issue worldwide, affecting individuals of all ages and socioeconomic backgrounds. The prevalence of obesity has more than doubled in the last few decades and is associated with a multitude of health problems, including chronic inflammation. Chronic inflammation is a key factor in the development of several metabolic disorders such as type 2 diabetes, cardiovascular disease and certain types of cancers. (Elshamy et al., 2021) Evidence suggests that obesity-induced inflammation is likely caused by adipose tissue dysfunction, which results in the secretion of various cytokines and chemokines. These adipose-derived factors, such as TNF-α, IL-6 and MCP-1, promote the recruitment of immune cells into adipose tissue, leading to a state of chronic low-grade inflammation. One such study found that obese individuals have higher levels of circulating cytokines than non-obese individuals, indicating a pro-inflammatory state. (Ramirez et al., 2014) Many other studies have confirmed this observation, and a significant consensus has been reached in the

literature that obesity is a state of chronic inflammation. Furthermore, it has also been found that individuals with a higher degree of obesity have a greater level of systemic inflammation. In addition to obesity-induced inflammation, the role of dietary habits in promoting chronic systemic inflammation has also been investigated. (Mirzaei et al., 2022) Diets high in saturated and trans fats, refined sugars, and processed foods have been associated with increased levels of inflammation markers. However, a plant-based diet rich in anti-inflammatory nutrients has been shown to have beneficial effects on weight management and chronic inflammation. Overall, the literature clearly demonstrates a strong relationship between obesity and chronic inflammation. It is important to recognize that obesity is not just a cosmetic issue but rather a serious health concern with significant social and economic implications. Therefore, it is crucial to address the underlying causes of obesity and chronic inflammation with measures such as regular physical activity, healthy dietary habits, and various interventions such as bariatric surgery for individuals with severe obesity. In conclusion, obesity is not only a matter of excessive weight gain but also involves an intricate interplay between adipose tissue dysfunction and chronic inflammation. This relationship has been established through numerous studies, indicating that obesity-induced inflammation contributes significantly to the development of metabolic disorders. (Cibella et al., 2015).

OPERATIONAL DEFINITIONS:

1. METABOLIC SYNDROME: Any patient having Waist circumference of ≥80 cm for women and ≥90 cm for men, high blood pressure of ≥130/85 mm of Hg, Low HDL <50 mg/dl for women and <40mg/dl for men, High triglycerides ≥150 mg/dl and high FBG≥100 mg/dl/ 2hour oral glucose >140mg% IGT or HOMAIR
   (Fahed et al., 2022)

2. OBESE: All patients having BMI> 30kg/m^2
   (Lee et al., 2019)

3. NON-OBESE: All patients having BMI <30kg/m^2
   (Lee et al., 2019)

4. TYPE 2 DIABETES MELLITUS: All patients with FBG of ≥126 mg/dl or HbA1C of ≥6.5%.
   (Rajput et al., 2019)

5. HYPERTENSION: All patients with BP≥130/85 mm of Hg.
   (Saeed et al.,2019)

6. VISCERAL ADIPOSITY: Waist circumference ≥80 cm for women and ≥90 cm for men.
   (Belete et al., 2021)
7. DYSLIPEDEMIA: All patients with HDL-C <50g/dl for women and <40mg/dl for men, LDL-C≥ 140 mg/dl, Triglycerides ≥ 150 mg/dl.
   (Fahed et al., 2022)

8. C-REACTIVE PROTEIN (CRP): CRP is a ring-shaped protein found in blood plasma whose circulating concentrations rise during inflammation. (Sproston & Ashworth et al., 2018)

9. FASTING VENOUS SAMPLING: After an overnight fast of ≥10-12 hours, the venous blood sampling will be done.
   (Rajput et al., 2019)

CHAPTER 3

METHODOLOGY

3.1 Study Design: Comparative Cross-sectional study

In the comparative cross-sectional study, the initial hypothesis is "there is a difference between the two population proportions of the study of interest". In this case 120 type 2 diabetes mellitus patients with metabolic syndrome were taken and divided into equal groups of 60 participants in obese (>30kg/m2) and non-obese(<30kg/m2) groups. The 2005 revised NCEP-ATP III criteria were used to determine participants with Metabolic Syndrome. Quantative data was collected by the subject evaluation forms and objectivity was observed as measured variables are analyzed. Data was collected from the two groups and lipid profiles, glycemic parameters, CRP and demographic variables were compared.

3.2 Subjects: A total of 120 Type 2 diabetes mellitus patients (obese and non-obese) with metabolic syndrome were recruited from Medical OPD National Medical Center, Karachi. Both genders were included.

3.3 Setting: The study was conducted in Bahria University Health Sciences Campus, Karachi in collaboration with National Medical Center, Karachi.

3.4 INCLUSION CRITERIA:

1. Age >20 years
2. Both males and females with type 2 diabetes mellitus were included.
3. Fasting blood glucose (FBG) ≥126 mg/dl, or glycated hemoglobin (HbA1c) ≥ 6.5%
4. HTN will be defined as blood pressure (BP) ≥130/85mm Hg.
5. Obesity states divided into obese (>30 kg/m^2) and non-obese (<30kg/m^2).
6. Patients who gave consent

3.5 EXCLUSION CRITERIA:

1. Patients with fever (oral temperature≥38.0°C), abnormal leukocyte (<4.0 or >10.0 × 109/L) or
2. Platelet counts (<150 or >350 × 109/L) on their complete blood counts, or abnormal liver, kidney, or thyroid function.

3. Patients with chronic inflammatory diseases and autoimmune diseases

3.6 DURATION OF STUDY:

3.6.1 Individual study period:3 months

3.6.2 Total period of study: 6 months

3.7 Sample size calculation:

Sample Size for Frequency in a Population

Population size (for finite population correction factor or fpc) (*N*):	240
Hypothesized % frequency of outcome factor in the population (*p*):	20%+/-5
Confidence limits as % of 100(absolute +/- %) (*d*):	5%
Design effect (for cluster surveys-*DEFF*):	1

Sample Size(*n*) for Various Confidence Levels

Confidence	**Level (%)**	**Sample Size**
95%		122
80%		74
90%		101
97%		134
99%		154
99.9%		179
99.99%		193

Equation

Sample size $\boldsymbol{n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1) +p*(1-p)]}$

Results from Open Epi, Version 3, open-source calculator—SS Propor

3.8 Sampling technique: Non-probability convenience sampling.

3.9 Human subjects and consent: A consent form was designed for this study to educate the patients about the research, the benefits that they would acquire from it. They were informed about their right to participate and withdraw from the study and that they would not be paid to enroll in the study. Patients had to sign the consent form in order to be inducted in the study. The consent form was both available in English and Urdu. It is attached in Annexure C.

3.10 MATERIALS:

3.10.1 Questionnaire / Subject Evaluation Form: Attached as annexure

3.10.2 Culture Media: N/A

3.10.3 Drugs: N/A

3.10.4 Equipment: c501 Roche chemical analyzer.

3.11 PARAMETERS OF THE STUDY:

CLINICAL AND LABORATORY MEASUREMENTS:

- Height
- Weight
- BMI
- Waist circumference
- BP

Fasting lipid profile

- Triglycerides
- Cholesterol
- LDL-C
- HDL-C

Glycemic parameters

- FBG
- HbA1C
- CRP

3.12 PROTOCOL OF STUDY:

After ethical review by the ethical committee the following methodology was used. After their consent, the patients were given the questionnaire to be filled by them.

A total of 120 type 2 diabetes mellitus patients with metabolic syndrome were included, divided into two groups of 60 obese (>30kg/m^2) and 60 non-obese (<30 kg/m^2) groups. The participants were recruited from Medical OPD National Medical Center for a duration of six months period. The 2005 revised NCEP-ATP III criteria were used to determine participants with Metabolic Syndrome. Subjects with three or more of the following conditions were considered to have Metabolic Syndrome.

1. Central adiposity (Waist circumference ≥ 80cm for women and ≥90 cm for men)
2. Triglycerides≥150 mg/dl
3. HDL-cholesterol (<50 mg/dl for women and <40 mg/dl for men)
4. FBG (>100mg/dl) 2-hour oral glucose >140mg% IGT or HOMA IR
5. BP (≥130/85 mm of Hg or history of taking antihypertensive medication.

ANTHROPOMETRIC AND BIOCHEMICAL MEASUREMENTS:

Anthropometric measurements included Height and Weight which was noted while patients were wearing light clothes with no shoes. BMI was calculated by the formula; Weight divided by square of height in meters.

At the end of normal expiration, the waist circumference (WC) in cm was measured midway between the iliac crest and costal margin. After ≥ 5 min, BP was recorded from the right arm, using digital sphygmomanometer with an appropriate cuff size. After an overnight fast, fasting morning venous blood samples were drawn from antecubital vein into vacuum tubes and analyzed by central certified lab at NMC. Fasting glucose, serum TG, low density lipoprotein cholesterol, HDL-C and CRP were measured by c501 Roche analyzer.

For clinical chemistry, c501 module is a mid-volume analyzer comprising of a photometric unit for a broad range of clinical chemistry and an ISE unit for ion-selective electrode

determinations of sodium, potassium and chloride in serum, plasma or urine. The module is also capable of measuring HbA1c levels in whole blood.

The core unit manages the transport of samples to each assigned analytical module. It allows continuous loading and unloading of up to 150 samples and features a dedicated port for STAT samples. The simple to operate unit processed up to 600 samples/hour.

SPECIFICATIONS:

TESTING CAPABILITIES:

Clinical Chemistry

ISE

HbA1c (whole blood measurement).

Throughput:

Up to 1000 tests/hour

(Combined photometric and ISE tests).

Samples:

Serum

Plasma,

Whole blood,

Urine,

Cerebrospinal fluid (CSF)

Supernatant (Hemolysate)

Automated sample rerun and dilution

Test integrity measures:

Contact free ultrasonic mixing

Determination of serum indices (lipemic, hemolytic and icteric)

Clot and liquid level detection

Reagents:

Up to 60 reagents cassette positions

Automated cassette management system (i-e registration, internal transportation, placement and disposal).

Automated reagent cassette loading and unloading.

PHOTOMETRY:

The principle of photometry is to measure the intensity of light by converting light into electricity. This is done by using the photons to dislodge electrons in the photometer, which produces the electric signals.

It refers to study of the phenomenon of light absorption by molecules in solution. It is designed to measure the intensity of a beam of light. Most routine clinical chemistry reactions involve linking a chemical or enzymatic reaction to the development of a colored product that is measured.

When a monochromatic light with an original intensity (I_o) passes through a solution, it may be reflected, absorbed or transmitted (I_s).

The nature of light absorption in a solution is governed by Beer Lambert Law.

BEER'S LAW: The amount of light transmitted through a colored solution decreases exponentially with increase in the concentration of the colored substance.

$A \alpha C$

LAMBERT'S LAW:

The amount of transmitted light decreases exponentially with increased thickness of the layer of solution through which the light passes.

$A \alpha L$

It is convenient to use absorbance (A) or optical density (OD) which is directly proportional to concentration.

OD and transmittance are related reciprocally.

Mathematical Expression;

At a given wavelength, OD =A=kct

Where K=constant

C=concentration of colored substance.

t= thickness of layer.

Thickness is constant in instrument.

OD is proportional to concentration.

TYPES:

Where absorbed or transmitted light is measured, it is called colorimeter and spectrophotometer.

Where emitted light is measured, it is called Flame emission photometer.

COLORIMETER:

It is an instrument used for the measurement of colored substance in solution. It is of the simplest type and measures visible region of spectrum (320-800nm). The specific wavelengths of light are isolated with interchangeable filters.

SPECTROPHOTOMETER:

It is more sophisticated and measures ultraviolet region (180-320nm) as well as the visible range. Glass prism or quartz prism are used instead of filters. Quartz cell in place of glass cuvette. It estimates the substance which are more or less colorless in the visible spectrum.

FLAME PHOTOMETER:

It deals with quantitative measurement of electrolytes such as Na, K etc.

PARTS OF PHOTOMETER:

1. Light source
2. Monochromator
3. Sample cuvette
4. Optical path (Single or double beam).
5. Photosensitive detectors.
6. Read out devices.

Instrumentation

There are 7 essential parts of a spectrophotometer

Light source – In spectrophotometer three different sources of light are commonly used to produce light of different wavelength. The most common source of light used in the spectrophotometer for the visible spectrum is a tungsten iodide lamp. For **Ultraviolet radiation,** commonly used sources of are the hydrogen lamp and the deuterium lamp.

Monochromatic – To select the particular wavelength, prism or diffraction grating is used to split the light from the light source.

Sample holder – Test tube or Cuvettes are used to hold the colored solutions. They are made up of glass at a visible wavelength.

Beam splitter – It is present only in double beam spectrophotometer. It is used to split the single beam of light coming from the light source into two beams.

Mirror – It is also present only and double beam spectrophotometer. It is used to the right direction to the splitted light from the beam splitter.

Photo-detector system – When light falls on the detector system, an electric current is generated that reflects the galvanometer reading.

Measuring device – The current from the detector is fed to the measuring device – the galvanometer. The meter reading is directly proportional to the intensity of light.

Digital Display

The scale reading on the instrument is a measure of (proportional to) optical density of a colored solution, and according to Beer-Lambert's law, to concentration of colored substance, so the scale reading appearing on digital display are proportional to concentration of substance under consideration.

Components of spectrophotometer:

- **Spectrometer:** for producing light of any selected color (wavelength),
- **Cuvette:** to place sample.
- **Photometer:** for measuring the intensity of light.
- **Galvanometer:** convert voltage signals send by photometer into amount into a reading.

HOW TO CALCULATE:

The instrument is set to zero (0) with the blank.

Concentration of the test (T)=OD of test/OD of standard x concentration of standard.

PREPERATION OF SOLUTION FOR INVESTIGATION:

In colorimetric estimation it is necessary to prepare 3 solutions.

a. Blank (B)
b. Standard (S)
c. Test (S)

Blank: to eliminate the effect of light absorption by the reagent used.

a. Water blank
b. Reagent blank

Standard: Solution of known concentration of the substance.

a. Both OD and concentration are known.
b. Therefore, concentration of the unknown can be calculated.

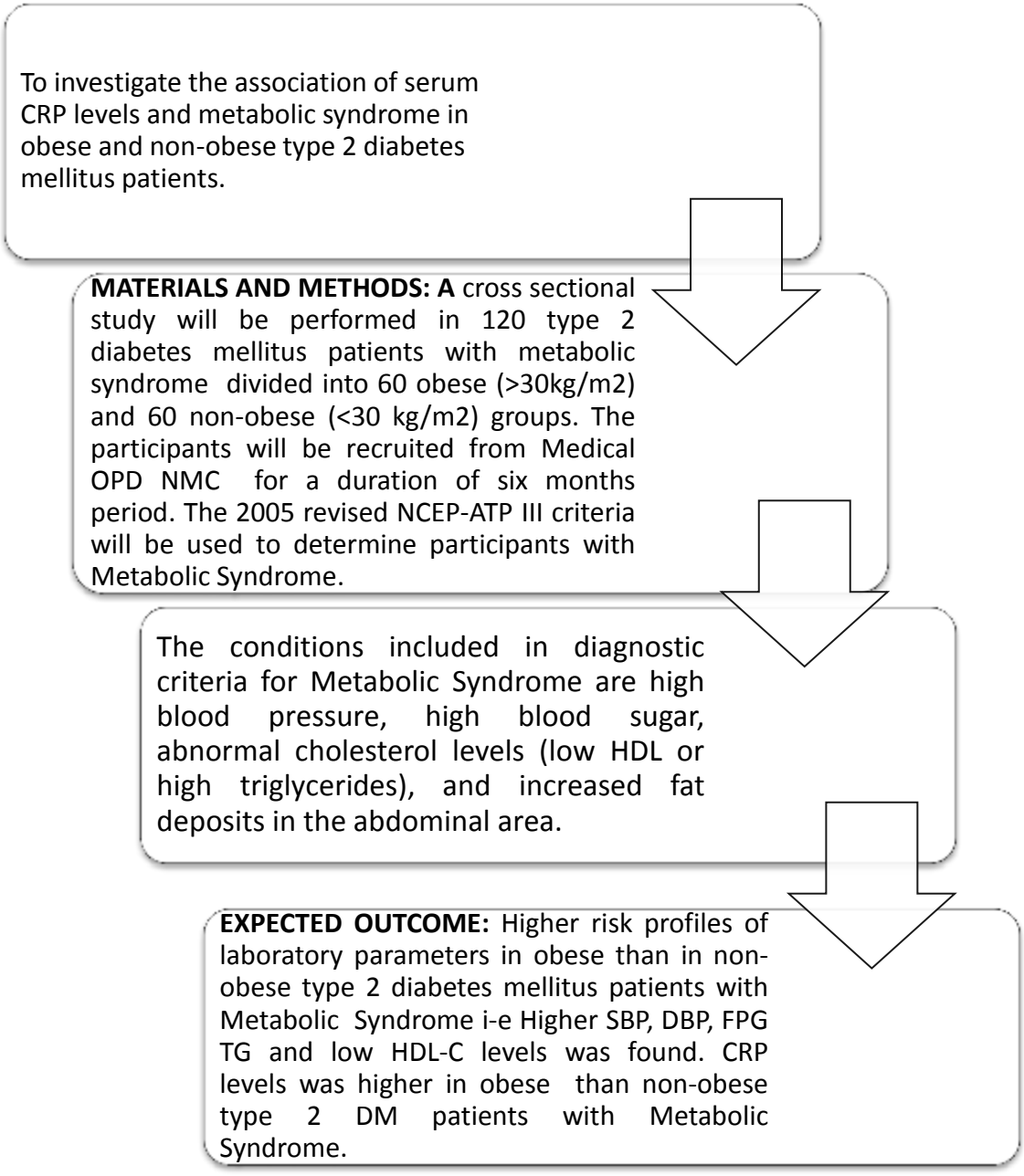
Test:

a. Test solution is made by treating a specific volume of the test sample with reagents (as per procedure).

COLORIMETR USES:

1. Blood glucose
2. BUN
3. Serum creatinine
4. Serum proteins
5. Serum cholesterol
6. Serum bilirubin
7. Urine creatinine

3.13 FLOW-CHART/ ALGORITHM OF STUDY:



3.14 Statistical Analysis:

Data were stored and analyzed using IBM-SPSS version 23.0; counts with percentages were reported on baseline characteristics and co morbidities, mean with standard deviation was given on age, height, weight, BMI, WC, HC, SBP, DBP and lipid profiles. Association of metabolic syndrome was tested using Pearson Chi Square test with baseline characteristics and metabolic syndrome parameters, Independent sample t-test was used to compare the mean differences between two groups, Mann Whitney U test was used to compare median CRP of obese and non-obese diabetic samples with and without metabolic syndrome samples, Pearson Correlation analysis was used to test the correlation of CRP with lipid profile and parameters of Metabolic syndrome, Multivariable regression analysis was used to measure the effect of studied parameters on CRP. P-values less than 0.05 were considered statistically significant. Bar diagrams and pie charts were also used to give graphical presentation of study outcomes.

CHAPTER 4

RESULTS

Table- 4.1 reports the baseline characteristics of studied samples, in the present study there were one hundred and twenty samples, 50.8% were female gender, 96.7% were married, 50.8% up to secondary education, 12.5% were reported for smoking, 30% for exercise, 71.7% with normal sleep, 86.7% had more than two years diabetes duration, 95.6% were on oral hypoglycemic and 52.5% were on antihypertensive treatment.

Table-4.2 reports the descriptive on quantitative baseline characteristics and lipid profile the mean age was 51.6 (SD=±12.4) years, mean height was 162.3(SD=±9.8) cm, mean weight was 79.0 (SD=±16.2) kg, mean BMI was 29.9(SD=±5.6) kg/m2, mean waist circumference was 100.7(SD=±12.1) cm, mean hip circumference was 104.6(SD=±14.8) cm, mean systolic blood pressure was 139.3(SD=±15.4) mm hg, mean diastolic blood pressure was 89.1(SD=±7.7) mm Hg, mean fasting blood sugar was 146.7(SD=±55.3) mg/dl, mean HbA1c was 8.9(SD=±9.5) percent, mean Triglycerides was 147.4(SD=±56.1) mg/dl , mean LDL was 99.2(SD=±34.6) mg/dl, mean HDL was 44.7(SD=±8.6) mg/dl and mean CRP was 3.9 (SD=±5.6) mg/I.

Table-4.3 reports the association of Metabolic syndrome with baseline characteristics, among samples with no metabolic syndrome 43.4% were male gender, 93.3% were married, 30% up to secondary education, 3.3% were smoker, 26.7% do exercise, 66.7% reported normal sleep, for co morbidities 83.3% were reported for diabetes, 3.3% for MI, 10% for stroke and 43.3% for hypertension. There were 86.7% samples had duration of diabetes more than 2-years, 93.3% were on oral hypoglycemic and 56.7% were on antihypertensive treatment, whereas among samples with metabolic syndrome 51.1% were male gender, 97.8% were married, 57.8% up to secondary education, 15.6% were smoker, 31.1% do exercise, 73.3% had normal sleep, for co morbidities 72.2% were reported for diabetes, 11.1% for MI, 4.4% for stroke and 55.6% for hypertension. There were 86.7% samples had duration of diabetes more than 2-years, 96.7% were on oral hypoglycemic and 51.1% were on antihypertensive treatment. The association of Metabolic Syndrome with Martial status and Education was found statistically significant with $p<0.05$.

Table 4.1: Baseline Characteristics of Studied Samples (n=120)

Characteristics		***n***	***%***
Gender	Male	59	49.2
	Female	61	50.8
Marital status	Single	2	1.7
	Married	116	96.7
	Widowed	2	1.7
Education	Primary	26	21.7
	Secondary	61	50.8
	Graduate	28	23.3
	Masters	5	4.2
Smoking	Smoker	15	12.5
	Non-Smoker	105	87.5
Exercise	Exercise	36	30.0
	Don't Exercise	84	70.0
Sleep	Normal	86	71.7
	Disturbed	34	28.3
Duration of Diabetes>2 Years	Yes	104	86.7
	No	16	13.3
Oral Hypoglycemic	Yes	115	95.8
	No	5	4.2
Antihypertensive	Yes	63	52.5
	No	57	47.5

Table 4.2: Descriptive on Quantitative baseline Characteristics and Lipid Profile

Parameters	***Mean***	***SD***
Age (years)	51.6	12.4
Height (cm)	162.3	9.8
Weight (kg)	79.0	16.2
BMI Kg/m^2	29.9	5.6
Waist circumference (cm)	100.7	12.1
Hip circumference (cm)	104.6	14.8
Systolic blood pressure (mm hg)	139.3	15.4
Diastolic blood pressure (mm hg)	89.1	7.7
Fasting Blood Sugar (Mg/Dl)	146.7	55.3
HbA1c (%)	8.9	9.5
Triglycerides (Mg/Dl)	147.4	56.1
LDL (mg/dl)	99.2	34.6
HDL (mg/dl)	44.7	8.6
CRP (mg/l)	3.9	5.6

Table 4.3: Association of Metabolic Syndrome with Baseline Characteristics

Characteristics		***Metabolic Syndrome***				***p-value***
		No		***Yes***		
		n	***%***	***n***	***%***	
Gender	Male	13	43.3	46	51.1	0.46
	Female	17	56.7	44	48.9	
Marital status	Single	2	6.7	0	0.0	0.03*
	Married	28	93.3	88	97.8	
	Widowed	0	0.0	2	2.2	
Education	Primary	11	36.7	15	16.7	0.03*
	Secondary	9	30.0	52	57.8	
	Graduate	8	26.7	20	22.2	
	Masters	2	6.7	3	3.3	
Smoking	Smoker	1	3.3	14	15.6	0.08
	Non-Smoker	29	96.7	76	84.4	
Exercise	Exercise	8	26.7	28	31.1	0.64
	Don't Exercise	22	73.3	62	68.9	
Sleep	Normal	20	66.7	66	73.3	0.48
	Disturbed	10	33.3	24	26.7	
Diabetes	Yes	25	83.3	65	72.2	0.22
	No	5	16.7	25	27.8	
MI	Yes	1	3.3	10	11.1	0.20
	No	29	96.7	80	88.9	
Stroke	Yes	3	10.0	4	4.4	0.26
	No	27	90.0	86	95.6	
Hypertension	Yes	13	43.3	50	55.6	0.24
	No	17	56.7	40	44.4	
Duration of Diabetes>2 Years	Yes	26	86.7	78	86.7	0.99
	No	4	13.3	12	13.3	
Oral Hypoglycemic	Yes	28	93.3	87	96.7	0.42
	No	2	6.7	3	3.3	
Antihypertensive	Yes	17	56.7	46	51.1	0.59
	No	13	43.3	44	48.9	
*p<0.05 was considered statistically significant using Pearson Chi Square test						

Table-4.4 reports the comparison of baseline characteristics and lipid profile, results showed samples with no metabolic syndrome having mean age 49.7 (SD=±13.8) years, mean height 160.2 (SD=±7.8) cm, mean weight 75.6(SD=±13.4) kg, mean BMI was 29.6(SD=±5.7) kg/m2, mean waist circumference was 93.9(SD=±8.8) cm, mean hip circumference was 104.3(SD=±20.7) cm, mean systolic blood pressure was 127.8(SD=±6.4) mm hg, mean diastolic blood pressure was 87.3(SD=±8.1) mm hg, mean fasting blood sugar was 124.0(SD=±35.9) Mg/Dl, mean HbA1c was 7.7(SD=±1.7) percent, mean Triglycerides was 112.2(SD=±23.2) Mg/Dl , mean LDL was 93.0(SD=±37.9) mg/dl, mean HDL was 47.2(SD=±7.3) mg/dl and mean CRP was 4.2 (SD=±3.0) mg/I, whereas among samples with metabolic syndrome mean age was 52.2 (SD=±12.0) years, mean height 163.0 (SD=±10.3) cm, mean weight 80.2(SD=±17.0) kg, mean BMI was 30.1(SD=±5.6) kg/m2, mean waist circumference was 103.0(SD=±12.3) cm, mean hip circumference was 104.8(SD=±12.5) cm, mean systolic blood pressure was 143.2(SD=±15.6) mm hg, mean diastolic blood pressure was 89.7(SD=±7.5) mm hg, mean fasting blood sugar was 154.3(SD=±58.7) Mg/Dl, mean HbA1c was 9.3(SD=±11.0) percent, mean Triglycerides was 159.1(SD=±59) Mg/Dl , mean LDL was 101.4(SD=±33.5) mg/dl, mean HDL was 43.9(SD=±9.0) mg/dl and mean CRP was 3.9 (SD=±6.3) mg/I. Independent sample t-test did give a significant mean differences for waist circumference, systolic blood pressure, fasting blood sugar, and triglycerides between metabolic syndrome and non-metabolic syndrome samples with $p<0.05$.

Table-4.5 reports the association of serum CRP levels and metabolic syndrome in obese and non-obese type –2 diabetes mellitus patients, among non-metabolic syndrome samples the median CRP of non-obese samples was 1.8 with IQR (1.0 – 2.9), and of obese samples median CRP was 3.86 with IQR (3.6 – 6.5), the difference in median CRP of obese and non-obese samples of non-metabolic samples was statistically significant with $p=0.01$ using Mann Whitney U test. Similarly, among metabolic syndrome samples the median CRP of non-obese samples was 1.0 with IQR (0.5 – 3.5), and of obese samples median CRP was 3.99 with IQR (3.0 – 5.8), the difference in median CRP of obese and non-obese samples of metabolic samples was also statistically significant with $p=0.01$ using Mann Whitney U test.

Table 4.4: Comparison of baseline Characteristics and lipid profile with Metabolic Syndrome

Parameters	***Metabolic Syndrome***				***P-value***
	No		***Yes***		
	Mean	***SD***	***Mean***	***SD***	
Age (years)	49.7	13.8	52.2	12.0	0.34
Height (cm)	160.2	7.8	163.0	10.3	0.12
Weight (kg)	75.6	13.4	80.2	17.0	0.18
BMI Kg/m^2	29.6	5.7	30.1	5.6	0.67
Waist circumference (cm)	93.9	8.8	103.0	12.3	<0.01*
Hip circumference (cm)	104.3	20.7	104.8	12.5	0.90
Systolic blood pressure (mm Hg)	127.8	6.4	143.2	15.6	<0.01*
Diastolic blood pressure (mm Hg)	87.3	8.1	89.7	7.5	0.41
Fasting Blood Sugar (FBS mg/dl)	124.0	35.9	154.3	58.7	<0.01*
HbA1c (%)	7.7	1.7	9.3	11.0	0.41
Triglycerides (TG mg/dl)	112.2	23.2	159.1	59.0	<0.01*
LDL (mg/dl)	93.0	37.9	101.4	33.5	0.25
HDL (mg/dl)	47.2	7.3	43.9	9.0	0.07
CRP (mg/l)	4.2	3.0	3.9	6.3	0.74
*p<0.05 was considered statistically significant using independent sample t-test					

Table 4.5: Association of serum CRP levels and metabolic syndrome in obese and non-obese type 2 diabetes mellitus patients

Metabolic Syndrome	**BMI levels**				**p-value**
	Non - obese		**Obese**		
	Median	**IQR**	**Median**	**IQR**	
No	1.80	1.0 – 2.9	3.86	3.6 – 6.5	0.01*
Yes	1.00	0.5 0 3.5	3.99	3.0 – 5.8	<0.01*
*p<0.05 was considered statistically significant using Mann Whitney U test					

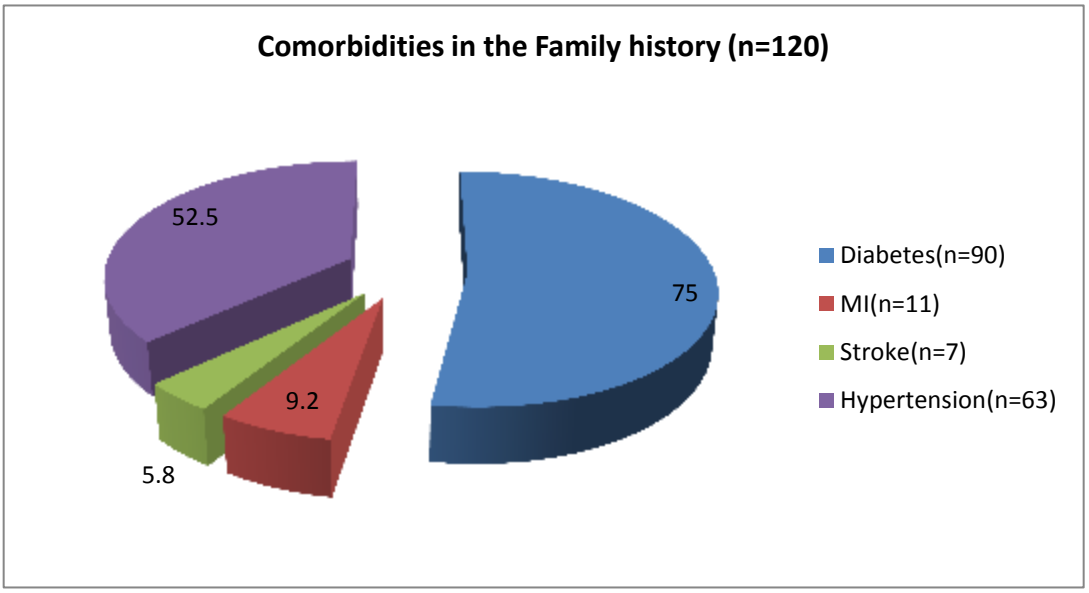
Pie diagram in Figure 4.1, is showing the co morbidities in the family history of the patients, 75% were reported for diabetes, 52.5% for hypertension, 9.2% reported for MI and 5.8% were reported for stroke.

Pie diagram 2 in Figure 4.2, is showing in the present study there were 75% samples were found with metabolic syndrome.

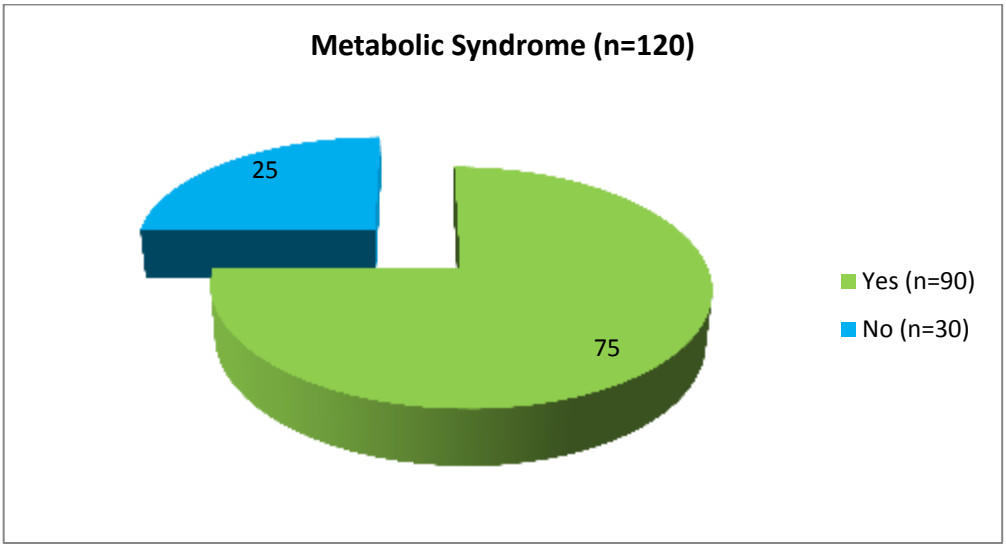
Table-4.6 reports the association of metabolic syndrome with CRP and other studied parameters, in non-metabolic syndrome samples 56.7% were obese, 16.7% were abnormal SBP, 73.3% were abnormal DBP, 43.3% were pre diabetic from FBS, 63.3% were pre diabetic from HbA1C, 3.3% were borderline high TG, 6.7% were abnormal LDL, 3.3% were abnormal HDL and 63.3% were found with high risk CRP, whereas among samples with metabolic syndrome 47.8% were obese, 77.8% were abnormal SBP, 76.7% were abnormal DBP, 30% were pre diabetic from FBS, 13.3% were pre diabetic from HbA1C, 28.9% were borderline high TG, 3.3% were abnormal LDL,10% were abnormal HDL and 48.9% were found with high risk CRP. Pearson Chi Square test did give a significant association of metabolic syndrome with SBP, FBS, HbA1C, and TG with $p<0.05$.

Table-4.7 reports the association of CRP and individual components of metabolic syndrome and lipid profile using multivariate linear regression analysis, results showed increase in WC gives 0.13-time positive impact on CRP ($p<0.01$), R-square showed 4.9% variation in CRP was explained by these parameters.

Table-4.8 reports the correlation analysis of CRP and parameters of metabolic syndrome, results showed CRP gives 26% positive correlation with BMI, 22% positive correlation with WC, 23% positive correlation with HC, considered statistically significant with $p<0.05$, whereas correlation of BMI with WC, HC, and DBP, correlation of WC with HC, SBP, and DBP, correlation of HC with DBP, correlation of SBP with DBP, correlation of FBS with HbA1c, TG, and correlation of TG with LDL, HDL, were also found statistically significant with $p<0.05$.



(Figure 4.1): Pie Diagram 1



(Figure 4.2): Pie Diagram 2

Table 4.6: Association of Metabolic Syndrome with CRP and Other Studied parameters

Parameters		Metabolic Syndrome				p-value
		No		Yes		
		n	%	n	%	
BMI	Non-obese	13	43.3	47	52.2	0.39
	Obese	17	56.7	43	47.8	
SBP	Normal <130	25	83.3	20	22.2	<0.01*
	Abnormal >130	5	16.7	70	77.8	
DBP	Normal <80	8	26.7	21	23.3	0.71
	Abnormal >80	22	73.3	69	76.7	
FBS	normal	7	23.3	4	4.4	<0.01*
	Pre-diabetic 100-125	13	43.3	27	30.0	
	Diabetic >126	10	33.3	59	65.6	
HbA1C	Normal <5.7	1	3.3	2	2.2	0.04*
	Pre-diabetic 5.7-6.45	10	33.3	12	13.3	
	Diabetic>6.5	19	63.3	76	84.4	
TG	Normal <150	29	96.7	42	46.7	<0.01*
	Borderline High 150-199	1	3.3	26	28.9	
	High 200-499	0	0.0	22	24.4	
LDL	Normal <100	18	60.0	45	50.0	0.22
	Near Optimal 100-129	8	26.7	21	23.3	
	Borderline High 130-159	2	6.7	21	23.3	
	High >160	2	6.7	3	3.3	
HDL	Normal	29	96.7	81	90.0	0.25
	Abnormal	1	3.3	9	10.0	
CRP	Low risk<1	3	10.0	24	26.7	0.15
	Moderate risk 1-3	8	26.7	22	24.4	
	High risk >3	19	63.3	44	48.9	
*p<0.05 was considered statistically significant using Pearson Chi Square test						

Table 4.7: Association between CRP and individual components of metabolic syndrome and Lipid profiles using multivariable linear regression analysis

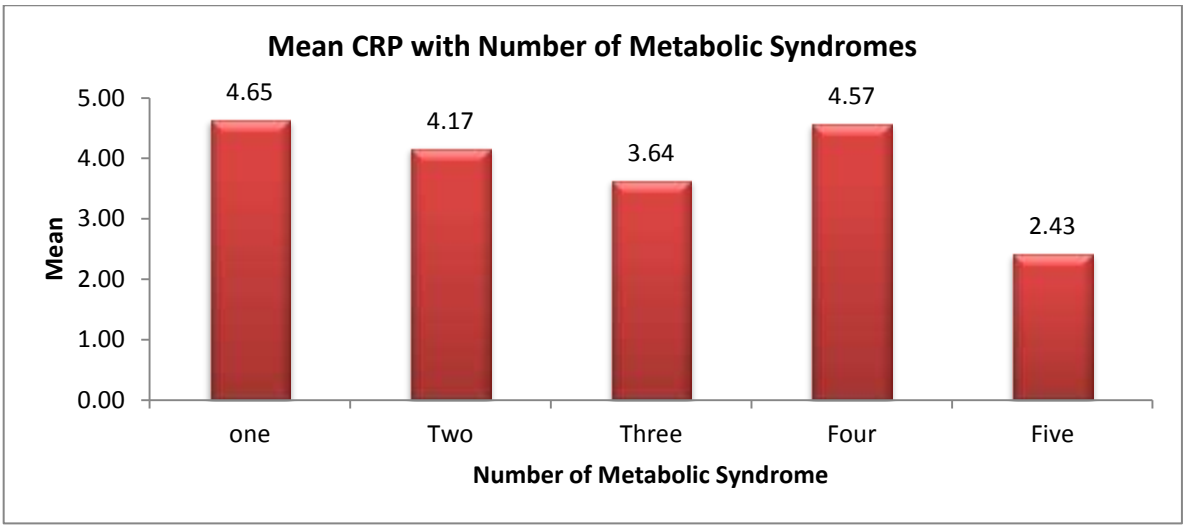
Parameters	Beta Coefficient	Standard Error	p-value
Number of Metabolic Syndrome	0.21	086	0.80
FBS	0.001	0.01	0.92
HDL	0.02	0.07	0.71
TG	-0.009	0.01	0.48
WC	0.13	0.04	<0.01*
SBP	-0.09	0.05	0.056
DBP	-0.006	0.08	0.94
LDL (mg/dl)	0.008	0.01	0.63
Dependent Variable: CRP Adjusted R-Square 4.9% *P<0.05 was considered Statistically significant*			

Bar Chart 4 in Figure 4.3 is showing the mean CRP with number of Metabolic Syndrome, samples with one metabolic syndrome have mean CRP 4.65 units and samples with five metabolic syndromes have 2.43 units mean CRP, the difference in CRP with number of metabolic syndromes was statistically insignificant with $p>0.05$ using one way ANOVA.

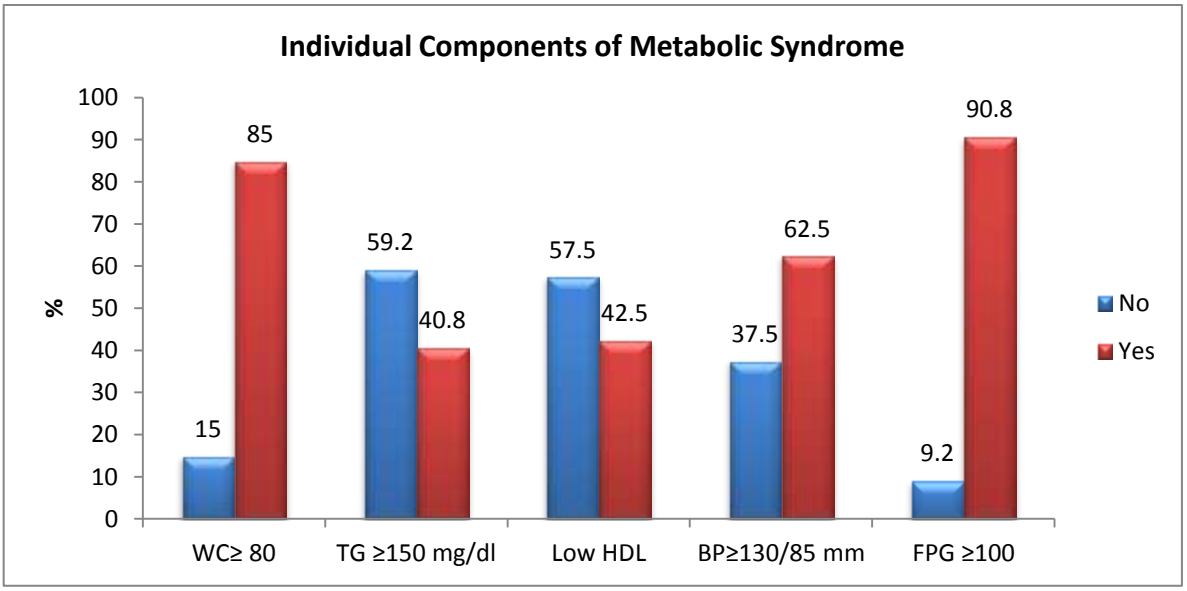
Bar Chart 5 in Figure 4.4 is showing the descriptive on individual components of metabolic syndrome, there were 85% samples having WC≥80 cm, 40.8% samples having TG≥150 mg/dl, 42.5% samples with low HDL, 62.5% samples with BP>130 / 85 mm, and 90.8% samples with FPG≥100 units.

Bar Chart 3 in Figure 4.5 is showing the outcomes on BMI, SBP, DBP and lipid profile, there were 50% obese samples, 62.5% were abnormal SBP, 75.8% were abnormal DBP, 57.5% were diabetic from FBS, 79.2% were diabetic from HbA1C, 22.5% were borderline high TG, 24.2% near optimal LDL, 8.3% were abnormal HDL and 52.5% were found with high-risk CRP.

Bar diagram 6 in Figure 4.6 is showing the mean CRP with individual metabolic syndrome components, these means were compared using independent sample t-test and were found statistically insignificant with $p>0.05$.



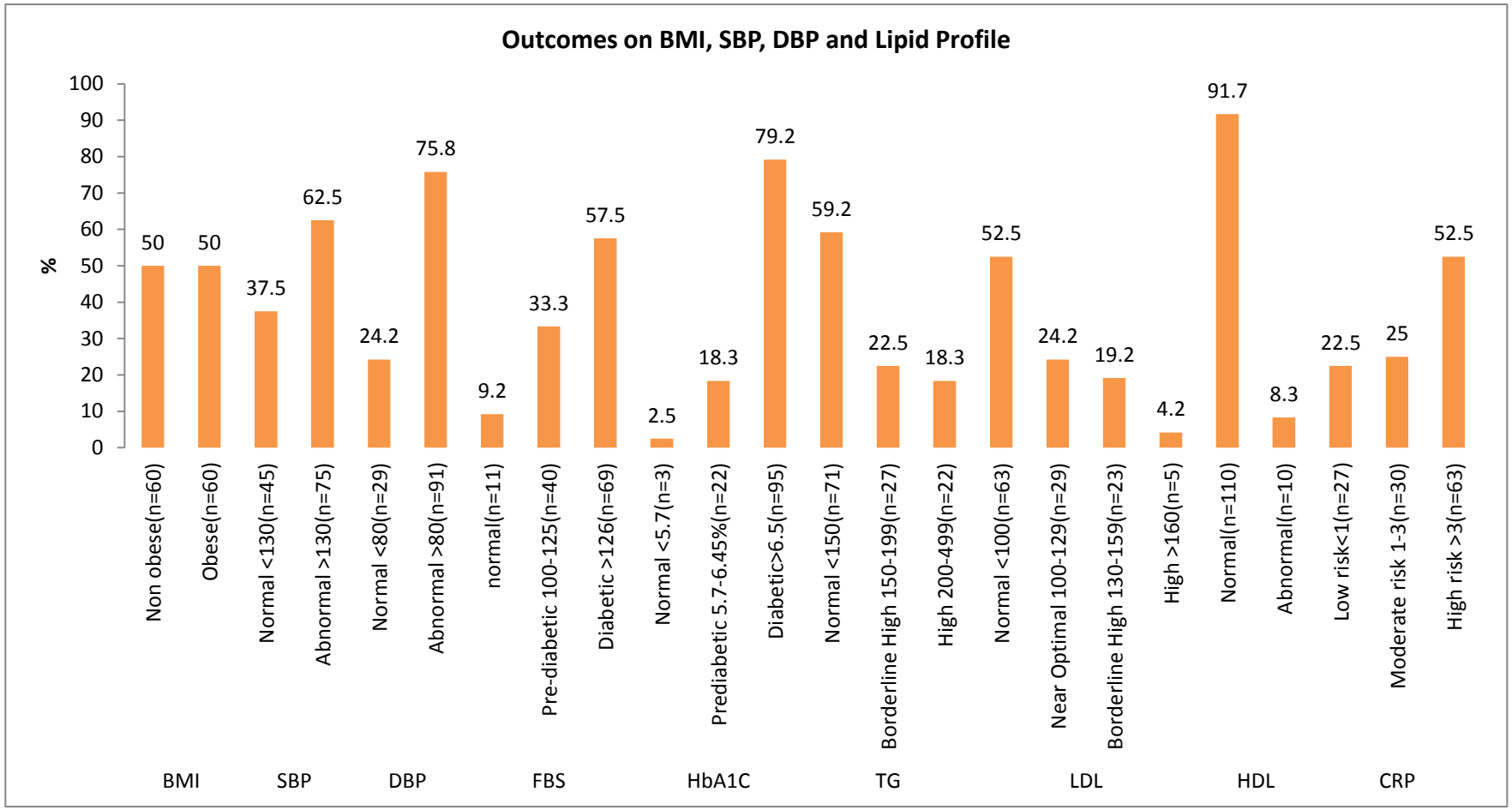
(Figure4.3): Bar Diagram 4



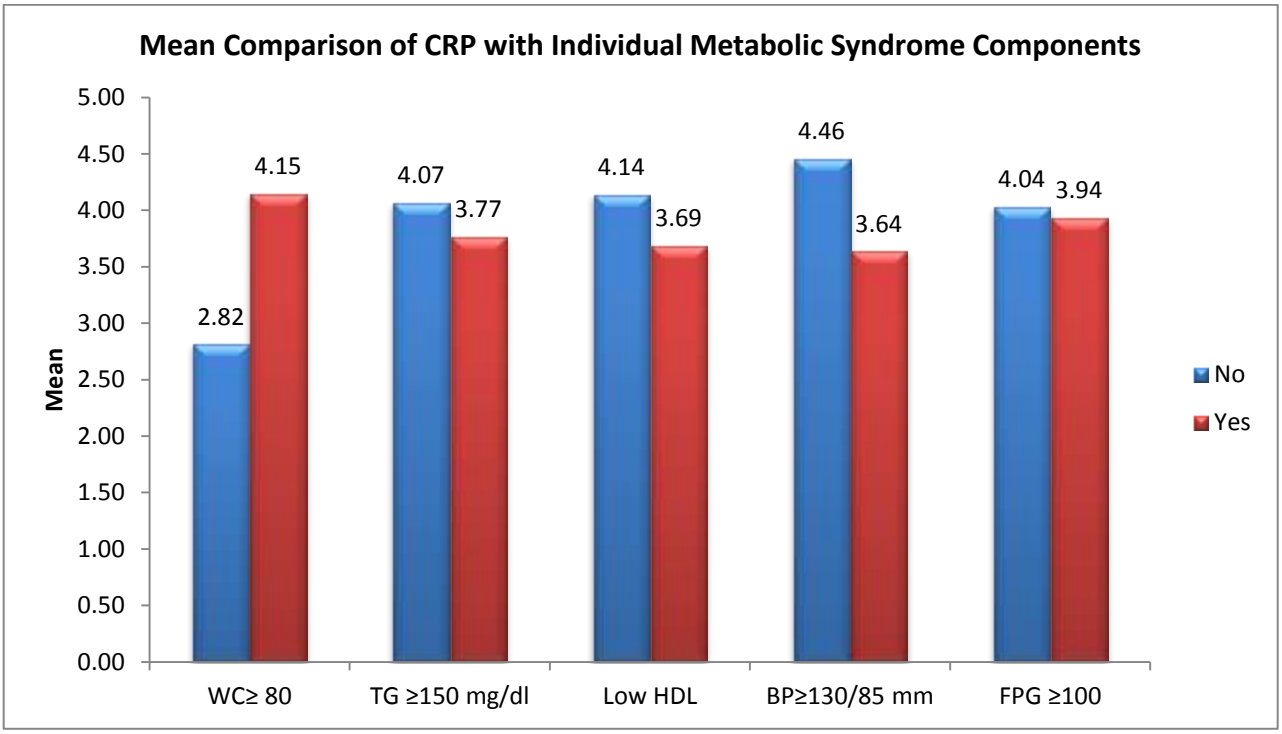
(Figure 4.4): Bar Chart 5

Table 4.8: Correlation Analysis of CRP and Parameters of Metabolic Syndrome

Parameters	BMI	WC	HC	SBP	DBP	FBS	HbA1c (%)	TG	LDL	HDL	CRP
BMI	1										
WC	0.66*	1									
HC	0.61*	0.62*	1								
SBP	0.12	0.29*	0.17	1							
DBP	0.19*	0.18*	0.31*	0.56*	1						
FBS	-0.17	-0.05	-0.16	0.08	0.03	1					
HbA1c (%)	-0.07	-0.14	-0.10	-0.04	-0.08	0.36*	1				
TG	-0.09	0.10	-0.06	0.07	0.02	0.19*	0.04	1			
LDL	-0.02	-0.04	-0.17	0.01	-0.21*	0.04	0.15	0.24*	1		
HDL	0.16	0.05	0.12	0.24*	0.17	0.03	-0.10	-0.23*	0.14	1	
CRP	0.26*	0.22*	0.23*	-0.15	-0.10	-0.03	-0.03	-0.04	0.02	0.006	1
*Correlation with $p<0.05$ was considered statistically significant											



(Figure 4.5): Bar Chart 3



(Figure 4.6): Bar Diagram 6

CHAPTER 5

DISCUSSION

5.1 SEQUENCE OF DISCUSSION EXPERIMENT/HYPOTHESIS WISE:

Diabetes has persistently elevated chronic hyperglycemia which is due to disturbances in carbohydrate, protein and fat metabolism. Several mechanisms are studied to be the underlying pathophysiology of diabetes and its complications namely deranged lipid profile, platelet activation and altered endothelial metabolism (Taskinen SR et al., 2003). It is a metabolic disorder with a pro-inflammatory pathogenesis in its development with increased levels of circulatory cytokines (Wang X et al., 2013).

The mean age in the present study was 51.6(SD=±12.4) years, which is in accordance with a study (Bellfki et al., 2011), where patients with T2DM were older (57±9.0) years than those without T2DM. Recent research articles have provided conflicting evidence regarding the mean age of individuals diagnosed with type 2 diabetes mellitus. Some studies suggest that the mean age is above fifty years, while others contradict this finding. Another study agrees with our study where their patient's mean age was (54.89±12.11) years. (Ali Maleki et al., 2014). A study found that the mean age was > 50 years for T2DM with high risk of raised CRP in both genders (Suganya Kanmani et al., 2019). Type 2 diabetes mellitus is higher in the older age group due to a combination of factors. Firstly, as individuals age, their body's ability to produce and effectively use insulin, a hormone that regulates blood sugar levels, decreases. This age-related decline in insulin sensitivity can lead to the development of insulin resistance, a key characteristic of type 2 diabetes. Additionally, older adults are more likely to have other health conditions such as obesity, high blood pressure, and high cholesterol, which further increase their risk of developing type 2 diabetes. Furthermore, lifestyle factors such as sedentary behavior, poor diet, and lack of physical activity, which are more prevalent in older age groups, can contribute to the development of type 2 diabetes. Lastly, genetic factors also play a role, as there is evidence to suggest that certain genetic variations are more common in older individuals and may predispose them to developing type 2 diabetes. Overall, the higher prevalence of type 2

diabetes in older age groups can be attributed to a combination of age-related physiological changes, coexisting health conditions, lifestyle factors, and genetic predisposition. The possible reasoning of occurrence of type 2 diabetes mellitus in the older age group is in accordance with the scientific proof of this disease prevalence in the group >50 years of age. A European study found that T2DM patients had a risk of having a raised CRP in women aged 55-74 years old. (Kim D et al., 2011). Ahmed et al reported 56% of the patients with obesity in type 2 diabetes mellitus patients were aged above 40 years. (Ahmed et al.,2010).

Recent research articles have provided both favoring and contradicting remarks on why CRP levels are high in women. Higher levels of CRP were found in women than men, even after controlling for factors such as age and body mass index (BMI) (Albert et al., 2004). Another study found that this gender difference in CRP levels may be due to differences in sex hormones, as women with higher levels of estrogen tend to have higher CRP levels (Ridker et al., 2000). Estrogen may increase inflammation. Studies on postmenopausal women who are taking hormone replacement therapy have shown to have higher CRP levels than those who do not take HRT. There are possible hormonal changes during pregnancy and in menstruation contributing to higher CRP levels in women. Secondly, women tend to be obese as they have higher levels of body fat than men in their body. Adipose tissue then has a tendency to undergo inflammation and produce cytokines that can trigger inflammation throughout the body. The relationship between CRP and gender may vary depending on the population being studied. For example, one study found that while women had higher CRP levels than men in a general population sample, this gender difference was not observed in a sample of individuals with type 2 DM (Ford et al., 2003).

In our study, the co-morbidities in the family history of study participants were diabetes (75%) and hypertension (52%).The results of a study show that a positive family history is a sensitive indicator of the presence of diabetes with much high sensitivity than obesity, a well -established risk factor: use of family history in combination with obesity almost doubles the positive predictive value of each risk factor alone, representing a substantial reduction in false positive rates for screening. The consequences of undiagnosed diabetes cases are known to affect the society and individuals and absence of accurate screening

tools, family history in addition to obesity, gives an inexpensive screening tool to identify at risk groups, also persons with undiagnosed diabetes, for evaluation, detection and risk reducing interventions (Hariri S et al.,2006).

The mean BMI found in present study was 29.9 (SD±5.6) which falls in the category of overweight according to the classification of BMI. (Ali Maleki et al., 2014) also found BMI in the range of overweight (26.75±4.92) kg/m2. Similarly, high BMI (31.7±7.33) was found in another study (Ellulu et al., 2022). A study found that with every unit increase in BMI, CRP increased by 37% amongst Indian children (Dayal et al.,2014) as BMI directly correlates with CRP, hence obese children have higher CRP levels. While considering BMI, when postmenopausal obese women were compared with postmenopausal normal weight women, CRP was higher in the formal category (Klisic A.N. et al., 2014). A similar study done amongst Spanish obese adults found higher CRP levels compared to non-obese counterparts as measured by the category of BMI (Warnberg J et al., 2004). In a prospective case control study done between diabetic US women and non-diabetic US women, BMI was >30kg/m2 in diabetics than non-diabetic (25kg/m2). Zhang C et al.,2020 contradicts. Thus, High BMI was associated with T2DM disease. This suggests that with an increase in the BMI, the level of inflammation also increases as proven by increase in the adipose tissue inflammation. Meshram et al found that diabetic individuals had higher BMI($p<0.006$) (Meshram et al.,2013). About 75% of the subjects who participated in the study were overweight/obese (BMI $\geq$ 23kg/m2) which is a prime determinant of Metabolic Syndrome occurrence. Studies have identified that Asian Indians tend to develop central obesity rather than generalized obesity. This could be the reason for the development of Metabolic Syndrome among type 2 diabetes mellitus patients according to IDF criteria. (Merlit James et al.,2020).

The mean waist circumference was higher (>100 cm) for both genders in our study in patients with type 2 diabetes mellitus. In a cross-sectional study done amongst patients having type 2 diabetes mellitus in an outpatient clinic in RDF India, there abdominal obesity prevalence percentage was 76.47% which was significantly higher in women compared to men (Singh P et al.,2022). Similar study showed that in an urban slum in a community in Nairobi, Kenya; patients with diabetes have 2 times more chance of having

elevated waist circumference. (Ayah R et al.,2013). The most relevant type of body fat is intraabdominal adipose tissue and its increase shows strong association with metabolic complications such as T2DM and hyperinsulinemia (Mehdad S et al.,2012). This is evident by the fact that waist circumference has a negative influence on blood glucose levels. Changes in living style due to urbanization have arisen a major global concern called obesity that leads to several metabolic complications. Obesity measured chiefly by BMI is a well characterized risk factor of diabetes (Guh DP et al.,2009). Increased BMI is suggestive of increased WHR and WC. It is widely accepted that visceral obesity plays a pivotal role in mediating insulin resistance that finally culminates in diabetes. Obesity is associated with low-grade inflammation due to expansion of adipose tissue chiefly of visceral origin. The presence of abnormal adipocytes at ectopic sites affects overall body metabolism. Further, the infiltration of macrophages in adipose tissue stimulates hepatic insulin resistance and chronic inflammation. (Shanmugam N et al.,2003). Several studies have confirmed that low-grade inflammation for a prolonged duration with an increase in circulating inflammatory markers will facilitate the development of pre-diabetes and diabetes. It is also a well-known fact that most of the pre-diabetic individuals finally progress to diabetes; however, the individuals who are overweight or obese with sedentary lifestyle are more prone to progress.

The fasting blood glucose was in the diabetic range (146mg/dl) in the classification of normal, prediabetic and diabetic. Although majority (93%) patients were on oral hypoglycemic agents, yet their FBS was uncontrolled, which makes us look for factors contributing to its deranged values. Yordanos et al (2021) found in their study that among the associated factors of raised fasting blood glucose level, age, body mass index (BMI), marital status, education level, systolic and diastolic blood pressure, and regime of medications had significant effects on progression of fasting blood glucose level ($p < 0.05$). Hence, patients who were not educated, had higher BMI levels, were married, had high SBP and DBP and used oral treatment were better not suited to control and reduce their fasting blood glucose level in their body overtime. Laura et al 2021, had low FBS in their study.

Recent research articles have shown a significant association between marital status and education in patients with type 2 diabetes mellitus. This finding is supported by several studies, including a study by (Li et al., 2019) that found a higher prevalence of diabetes among unmarried individuals with low education levels. Another study by (Wang et al.,2018) also found a significant association between marital status and education in diabetes patients. The study showed that patients who were married and had higher education levels had better glycemic control and lower rates of complications compared to those who were unmarried and had lower education levels.

In our study, the CRP level was found to be 3.9mg/L. In the Copenhagen study, the Mean CRP was 1.53mg/L (Allin KH et al., 2011) as compared to US study, the CRP levels were 4.3mg/L (Woloshin S et al.,2005). But in a Korean Study, the mean CRP levels were lower than the western counterparts (Timpson MJ et al., 2011). BMI is strongly correlated to CRP; therefore, low CRP in Korean population could be due to Low BMI in Asian population than western people. In a large population based Korean cohort study, CRP level was associated with increased risk of developing T2DM and association was apparent among the older age group (>50 years). A study done on Chinese population reported that CRP level was higher in T2DM patients than normal subjects (Wen J et al.,2010). This suggested that CRP is an independent predictor of incident T2DM. However, in a Singapore base Chinese population study found that in 571 T2DM cases and 571 matched controls, CRP was not positively associated with higher risk of incident diabetes (Pan A et al.,2017). The mechanism of association between CRP and T2DM is still not known in detail. There are explanatory factors including oxidative stress (which is believed to implicate low grade inflammation (Nishikawa T et al.,2000) and genetic factors such as family history of T2DM. (Panncciulli N et al.,2002).

In our study, FBS was 146.7mg/dl which is diabetic in classification of normal, prediabetes and diabetes. Fasting blood glucose levels are positively correlated with CRP which is in accordance with NHANES study where CRP was more in diabetic patients than with non-diabetics (Ford ES et al., 1999, Lee S H et al.,2019). Chronic hyperglycemia stimulates pancreatic β cells to secrete insulin to the point of exhaustion of these cells which along with insulin resistance leads to development of T2DM. Obesity mediates the relationship

between inflammation and insulin resistance (Shoelson S.E et al., 2006). As inflammatory markers were found to be increased in type 2 diabetes mellitus with high FBG and increased BMI (Kawamoto et al., 2011). (Lee et al., 2011) also showed that as FBG levels also increased. In another study amongst Japanese persons with FBG levels were >100mg/dl had high levels of CRP than those persons with FBG in the range of <100mg/dl) (Kawamoto at al., 2011). This is also true for Portuguese adults who had higher CRP when FBG levels were elevated along with occurrence of Metabolic Syndrome (Santos AC et al., 2005.Frohlich M et al.,2000).

In our study, the mean glycated Hb was found to be raised (8.9%) among majority of type 2 diabetes mellitus patients. Our study participants were on oral hypoglycemic agents, but despite that majority were found to have poor glycemic control. This is in favor of an Ethiopian study, where mean HbA1c and RBS level of the participants were 7.6% and 280mg/dl respectively. Having high HbA1c makes these patients at high risk of developing complications (Cheneke W et al.,2016). There was another study with a high HbA1c in the range of 11.3% (Gill G et al.,2008). It is already established fact that glycated hemoglobin level greater than 7%, fasting blood sugar level greater than 126mg/dl and random blood sugar greater than 200 mg/dl are considered as poor glycemic control of the study subjects. Poor glycemic control can arise from a number of factors such as dietary non-compliance, lack of physical exercise, poor storage and use of drugs, poor quality of drugs and poor prescription of drugs and any other. These factors are categorized as patient factors, medication factors and health care provider (professional) factors. Different research (Maysaaa K et al., 2010, Gill G et al., 2008) around the world indicated that older age, female sex, ethnic variation, higher BMI, smoking, longer duration of diabetes, lower physical activity, shortage of drugs and insulin, lack of diabetic care team and lack of adherence to diabetes management are contributing factors to poor glycemic control.

In our study, majority of the type 2 diabetic patients had metabolic syndrome (75%). Some studies suggest that a majority of type 2 diabetic patients also have metabolic syndrome while others present conflicting findings. The results of one study showed that MS is a common occurrence (68.6%) among people with T2DM who attended routine clinics in suburban hospitals in the middle-belt region of Ghana. (Abagre et al.,2022). In contrast,

(Moegre et al.,2014) recruited 200 participants with T2DM for a cross-sectional study at the Tamale Teaching Hospital and found that the prevalence of Metabolic Syndrome was 24.0% among participants using IDF definition of Metabolic Syndrome. A similar study conducted among participants with T2DM in India reported a MS prevalence of (71.9%). (Bhatti GK et al.,2016). Another study reported a prevalence of 42.8% according to IDF criteria (Jones M et al.,2020). A study found out that the prevalence of Metabolic Syndrome (according to IDF criteria) to be 66.5%, which was higher in women (84.7%) then men (44.3%). The reason being females tends to be more obese than men, average WC being 97.3 cm and BMI 29.79 in women having metabolic syndrome (Ahmed N et al.,2010). The prevalence of Metabolic syndrome was higher in women than that in men which could be due to higher rate of abdominal obesity in women. (Shehu A et al.,2015). According to another study done by Sharmila et al, found the overall prevalence of Metabolic Syndrome to be above 77% of the people suffering from diabetes coming from urban southern district of India (Sharmila JB et al.,2018). Again, the prevalence was higher among women (84.6%) as compared to men (72.4%). The prevalence of Metabolic Syndrome in females was found to be twice as common as males. It might be related to their higher rates of obesity and body mass index. It was found that upper body adiposity and abdominal obesity especially deposition of fats in the buttock, hip and limb areas were more common in females than males. The women were also in their post-menopausal stage in this study. (Merlit James et al.,2020). Moreover, the lifestyle factors might be the reason for occurrence of metabolic syndrome in female patients. (White UA et al.,2014). Zafar et al found in their study that the prevalence of Metabolic Syndrome was around 77.3% of the diabetic patients and it was higher in obese and overweight individuals than those with normal BMI. (Zafar KS et al.,2017). The variations in prevalence rates are related to differences in the criteria used in defining the metabolic syndrome, ethnic differences or may be due to combination of genetics and environmental factors such as obesity and decreased physical activity.

There was a statistically significant difference in waist circumference when comparison was done between metabolic syndrome and non-metabolic syndrome patients i-e larger waist circumference was found in type 2 diabetic patients with metabolic syndrome. A study reported that abdominal obesity (increased waist circumference-68.8%), elevated

blood pressure (66.4%) and decreased HDL-c (64.1%) were the most prevalent Metabolic Syndrome risk factors (Mentoor et al.,2018).Studies have further proposed that abdominal obesity might be responsible either independently or through the induction of insulin resistance (via inflammatory mediators released from adipose tissue), to contribute to both the development of systemic hypertension and dyslipidemia (Motalla AA et al.,2011).

There was statistically significant difference in systolic blood pressure between metabolic syndrome patients and non-metabolic syndrome patients with type 2 diabetes mellitus. Recent research articles have provided evidence of a statistically significant mean difference in systolic blood pressure between metabolic and non-metabolic syndrome patients. Systolic blood pressure was highest in patients with metabolic syndrome with or without diabetes mellitus (Zidek et al.,2009). Their findings also show that blood pressure is influenced by metabolic syndrome, irrespective of presence or absence of diabetes mellitus. There are multiple mechanisms that link hypertension and Metabolic Syndrome including insulin resistance, central /visceral obesity, sympathetic overactivity, activated renin-angiotensin system, oxidative stress, increased inflammatory mediators and obstructive sleep apnea.

In one study, there was a positive correlation between CRP concentration and BMI in obese subjects with T2DM (p=0.001). (Fronczyk et al.,2014). A cross -sectional study done by Cruz et al found that higher CRP levels in overweight and obese adolescents and in those adolescents whose % body fat was higher. (Cruz et al.,2013). Chronic inflammation plays a specific role in people with T2DM and co-existent obesity. The sources of inflammatory cytokines that modulate inflammatory reactions in these patients are both immune cells, activated by hyperglycemia and associated metabolic disorders and adipocytes. In their study they found, non-significant rise in CRP levels in normal weight diabetic individuals suggesting that obesity is a state corresponding to subclinical inflammation. CRP levels were significantly lower in obese non-diabetic individuals in the same study suggesting the presence of diabetes itself; is an independent risk factor increasing the value of CRP. It seems that increased level of CRP in subjects with T2DM and obesity are the result of simultaneous impact of obesity and diabetes. The mechanism of effect of diabetes on the CRP concentration remains unclear, ever more so since there is no correlation between

CRP and glycemic control measured by HbA1c and glucose levels. CRP values also showed no relation to lipid disorders and hypertension which accompany diabetes. The observed results indicate that most important factor determining an increase in the concentration of CRP in obese T2DM patients is excess body fat and presence of diabetes itself.

In the present study, there was a positive correlation of waist circumference with CRP. In the study done by (Pradeep Kumar et al., 2019), it was evaluated, the levels of inflammatory mediators and their association with obesity indices in patient groups (pre-diabetes and diabetes). Adiponectin which is an anti-inflammatory agent was decreased and pro-inflammatory markers such as CRP, IL-6, uric acid and fibrinogen increased significantly compared to control groups. In their case, of diabetic patients, inflammatory mediators correlated significantly with BMI and WC but not with WHR. Similarly, (Sachan et al.,2017) and (Shantaram and Nayak et al.,2014) suggested a positive association of CRP with obesity and diabetes. This is because increased adiposity mediates up-regulation of pro-inflammatory genes through activation of C- Jun-N- terminal kinase and nuclear factor kappa beta, a central regulator of inflammation. These activated genes cause the release of excess number of cytokines, thereby exacerbating inflammatory responses and leading to insulin resistance which is major contributor to pre-diabetes and diabetes (Rajala MW et al.,2003). There were 20 biomarkers studied taken from a pool of cross -sectional studies, related to metabolic, inflammatory and coagulation disorders that have activated low grade inflammation. (Daniel Fernandez Berges et al., 2014). Overeating results in adipose tissue remodeling (Suganami T et al.,2010) which is characterized by increased in size of fat cells, infiltration of macrophages and process of new vessel formation (Nomiyana T et al., 2007, Hosogai N et al.,2007). Remodeling induces chronic inflammation (Schenk S. et al.,2008, Berg AH et al.,2005) which can contribute to development of arteriosclerosis and insulin resistance. During the development of obesity, occurs an “adipose tissue remodeling”, i-e there is an increase in white adipose tissue, caused by hyperplasia and hypertrophy of adipocyte, an increased infiltration of immune cells such as lymphocytes and macrophages and an imbalance between production of pro-inflammatory and anti-inflammatory adipokines. The presence of macrophages infiltration in adipose tissue makes it a source of

inflammatory signals release that act in adipose tissue metabolism and control metabolic changes associated with obesity. (Ogawa Y et al.,2010).

In present study CRP was not positively associated with HbA1C but in another study it was positively associated with it (King DE et al.,2003). In another study, there was no relationship between CRP and degree of glycemic control as assessed by level of HbA1c. Similar results were observed by authors of other studies. (Kahn S. E et al.,2006, Gustavsson C. G et al.,2004). Raised blood sugar levels contribute to inflammation by causing endothelial dysfunction through the tyrosine kinase release mechanism from the spleen (Galeya B et al.,2010). However, there are counter reports indicating a correlation between increased levels of CRP and worse glycemic control. (Streja D.P et al., 2003). Pradhan et al.,2009 also showed that there was a lack of relationship between CRP and glycemic control who after having introduced hypoglycemic therapy that resulted in an improvement of glycemic control, did not observe a decrease in CRP, IL-6 or TNF alpha levels.

In present study, CRP levels were raised in obese diabetic patients than their non-obese counterparts. In a case control study done in Pima Indian population, it was found that there was no relation between CRP and T2DM (Krakoff JT et al., 2003). In another study done in Japanese population, CRP was elevated as a single predictor of diabetes, independently of raised body weight and end-organ resistance to diabetes (Doi YY et al., 2005).

CRP when compared with individual components of metabolic syndrome, it was found that CRP was positively correlated with increase in the individual components of metabolic syndrome. It was positively correlated for abdominal obesity in the form of raised waist circumference in present study.

According to another study raised CRP was associated with high blood pressure (Sesso HD et al., 2003). In present study, all components of metabolic syndrome namely waist circumference, fasting blood sugar, triglycerides levels and blood pressure were raised. All these findings indicate that the components of metabolic syndrome are related to a low-grade inflammation. (James et al.,2020) found out in their study that increased levels of triglycerides, raised BP, increased waist circumference and fasting blood glucose levels were found to be strongly associated with Metabolic Syndrome in diabetic patients of both

genders. In their study, higher BP and waist circumference were the strongest metabolic risk factors of Metabolic Syndrome.

Recent research articles have explored the association between C-reactive protein (CRP) and waist circumference. In present study, there was a significant mean difference for waist circumference between metabolic syndrome and non-metabolic syndrome samples with p value <0.05. A study published in East African Medical Journal (Joshi MD et al.,2008) 66% of recruited type 2 diabetes mellitus patients, had central obesity as defined by waist circumference above NCEP ATP III cut off values. One study found that higher levels of CRP were associated with larger waist circumferences in both men and women (Smith et al., 2018). Another study found that waist circumference was a stronger predictor of CRP levels than body mass index (BMI) (Jones et al., 2019). In a study conducted by (Zhang et al., 2019), it was found that CRP levels were significantly higher in obese individuals with type 2 diabetes compared to non-obese individuals with type 2 diabetes. This suggests that obesity may contribute to the development of chronic inflammation in diabetic individuals. Another study by (Lee et al., 2020) found that CRP levels were also elevated in non-obese diabetic individuals compared to non-diabetic individuals. This indicates that diabetes itself may also contribute to chronic inflammation. The study also found that CRP levels were positively correlated with HbA1c levels, indicating that poor glycemic control may exacerbate inflammation in diabetic individuals. Metabolic syndrome is a cluster of conditions that increase the risk of heart disease, stroke, and diabetes. Recent research articles have shown that C-reactive protein (CRP) levels are significantly higher in patients with metabolic syndrome than those without it. According to a study by Ford et al. (2008), CRP levels were 1.5 times higher in patients with metabolic syndrome compared to those without it. Another study by Kim et al. (2013) found that CRP levels were positively correlated with the severity of metabolic syndrome. Furthermore, high CRP levels have been associated with an increased risk of cardiovascular disease in patients with metabolic syndrome. A study by (Ridker et al., 2008) found that patients with metabolic syndrome and high CRP levels had a 1.7-fold increased risk of cardiovascular disease compared to those with low CRP levels. This highlights the importance of monitoring CRP levels in patients with metabolic syndrome as a potential marker for cardiovascular disease risk. Recent research articles have compared patients with metabolic syndrome to those without

it in terms of waist circumference, systolic blood pressure, fasting blood sugar, and triglycerides. According to these studies, patients with metabolic syndrome have higher levels of all four parameters compared to those without it.

One study found that patients with metabolic syndrome had a significantly larger waist circumference than those without it (Alberti KG et al.,2009) Another study showed that patients with metabolic syndrome had higher systolic blood pressure levels (Ford ES et al.,2002). Additionally, patients with metabolic syndrome had higher fasting blood sugar and triglyceride levels compared to those without it (Grundy SM et al.,2005). Arat et al found that among patients with Metabolic Syndrome had hypertension (44%), raised blood glucose (37%), had hypertriglyceridemia (31%) and Low HDL cholesterol (30%). (Arat et al.,2008). These figures were low in patients without Metabolic Syndrome. Many studies have confirmed the existence of correlation of central obesity with insulin resistance, deranged lipid profile, raised blood pressure and cardiovascular disorders. (Brown LK et al.,2002). Cruz et al observed a correlation between CRP level and Metabolic Syndrome components: BMI, WC, blood glucose, Total cholesterol, HDL-C, LDL-C and SBP, which is in accordance to our study. (Cruz et al.,2013). Increased levels of triglycerides, raised BP, increased BP, increased waist circumference and fasting blood glucose levels and reduced HDL cholesterol were strongly associated with Metabolic Syndrome in diabetic patients in both genders. Higher BP and waist circumference were the strongest risk factors of Metabolic syndrome in this study. (Merlit James et al.,2020). A study done by Zafar et al reported the adverse outcomes which were more common in diabetic women as compared to diabetic men. (Zafar U et al.,2018). Another study showed that type 2 diabetes mellitus patients with Metabolic Syndrome have been associated with higher rates of cardiometabolic risk factors especially abdominal obesity, high BP and Hypertension. (Merlit James et al.,2020). This result is consistent with a published report on Indian population by Sharma et al. (Sharma KK et al.,2018).

(C) IMPLICATIONS OF THE STUDY:

5.2.1 THEORETICAL IMPLICATIONS:

In present study, CRP levels were higher in obese persons with type 2 diabetes mellitus and Metabolic Syndrome. This suggests that obesity has a

role to play in the development of both Type 2 diabetes mellitus and Metabolic Syndrome through the process of increased inflammation. Additionally, CRP levels have been linked with an increased risk of cardiovascular disease in both obese and non-obese patients with type 2 diabetes mellitus. Overall, the theoretical implication in this context is the need to understand the underlying mechanisms of these conditions. Future research is required to understand the relationship between CRP, obesity, metabolic Syndrome and diabetes.

5.2.2 PRACTICAL IMPLICATIONS:

Obese and overweight individuals have the risk of developing Metabolic Syndrome and Type 2 diabetes mellitus. CRP levels are found to be higher in these individuals suggesting an underlying presence of inflammation. This suggests the need for future investigation and potential options to address the cause of inflammation. Therefore, regular monitoring of CRP in these high-risk individuals can also predict their overall cardiovascular health.

5.2.3 POLICY IMPLICATIONS:

The association of CRP in obese and non-obese type 2 diabetes mellitus patients with Metabolic Syndrome has significant policy implications. Higher CRP levels in obese patients with type 2 diabetes mellitus and Metabolic Syndrome, indicate higher risk of cardiovascular disease so policies are needed to focus on preventing and managing obesity, type 2 diabetes mellitus and metabolic Syndrome to reduce the risk of cardiovascular disease. Policies should also be made to investigate the early detection and management of CRP levels in patients with type 2 diabetes mellitus and Metabolic Syndrome especially in obese individuals. This can be achieved through monitoring of CRP and implementing interventions such as lifestyle modifications, medications and weight loss programs.

5.3 LIMITATIONS AND STRENGTHS OF STUDY:

(A) LIMITATIONS:

1. Small sample size therefore results cannot be applied to general population
2. Cross sectional design of study therefore no causal relationship can be formed
3. CRP levels will be measured once and some measurement error can occur simultaneously.
4. Many cross-sectional studies have already been performed on the topic so results might not be new.
5. This biomarker is not a specific marker for chronic low-grade inflammation of adipose tissue metabolism.

(D) STRENGTHS:

1. A sixth indicator in type to diabetes mellitus patients with metabolic syndrome as a predictor or risk factor for CHD and stroke in these patients.
2. Despite the small sample size, WC may be recommended as the single most convenient, feasible measure that could be used across communities for its significant association with T2DM patients with metabolic syndrome.
3. This is the first study done to understand the complex relationship between CRP levels, obesity, type II diabetes mellitus and Metabolic Syndrome.

5.4: FUTURE RESEARCH DIRECTIONS/RECOMMENDATIONS:

The association between CRP levels and Metabolic Syndrome in both obese and non-obese groups with type II diabetes mellitus was studied in the present study. Future research should focus on finding factors contributing to Metabolic Syndrome in these individuals and finding the benefits of reducing CRP levels in these persons by including lifestyle modifications such as healthy diet and exercise as well as drug therapy. Effective interventions will improve health in these individuals and reduce their risk of developing complications associated in type II diabetes mellitus with Metabolic Syndrome.

5.5: CONCLUSION:

Diabetes mellitus is an endocrine disorder with its implications on one's health because of its role in causing complications when faced with poor glycemic control. It is known that majority of type 2 diabetes mellitus patients have Metabolic syndrome. The presence of Metabolic Syndrome in these patients puts them at risk for developing future cardiovascular events. Among the various anthropometric parameters, WC had the best discriminatory power. Hence, WC can be used as a single measure because of its simplicity of measurement and usage both in community and hospital settings. CRP levels were found to be higher in obese group, when comparing obese with non-obese group in type II diabetes mellitus patients with Metabolic Syndrome, so there is a stronger association between CRP and Metabolic Syndrome in obese individuals which suggests that Metabolic Syndrome develops in obese persons through activation of inflammatory pathways. Monitoring CRP levels in these patients may be an investigative tool for early detection and management of Metabolic Syndrome in Type II diabetes mellitus patients. The diagnosis of metabolic syndrome and its association with cardiovascular risk factors may point towards effective prevention of cardiovascular complications in these patients.

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ANNEXURE A

Bahria University
Discovering Knowledge
Health Sciences Campus, Karachi

FACULTY RESEARCH COMMITTEE
BAHRIA UNIVERSITY HEALTH SCIENCES CAMPUS

LETTER OF APPROVAL

Date: 15-09- 2022

To,
Dr. Rabia Siddiqui
M.Phil - Student
Department of Physiology
BUHS-Karachi

Subject: **Faculty Research Committee**
FRC-BUHS Approval of Research Study

Title of Study: C. Reactive Protein (CPR) as a Biomarker of Inflammation in Obese and Non Obese type 2 Diabetes Mellitus Patients with Metabolic Syndrome.

Name of Student: **Dr. Rabia Siddiqui**

Reference No: **FRC-BUHS 50/2022-503**

Dear Dr. Rabia Siddiqui

Thank you for submitting research proposal to FRC-BUHS. The committee has approved your project.

This letter is referred to ERC for approval.
Regards

Dr. Mehreen Lateef,
Associate Professor,
CO- CHAIRPERSON FRC-BUHS

Cc:
DG-BUHS
Principal Medical
Principal Dental
Vice Principal BUHS
Co-chairperson FRC
Secretary

ANNEXURE B

Bahria University
Discovering Knowledge
Health Sciences Campus Karachi

ETHICAL REVIEW COMMITTEE
LETTER OF APPROVAL

Date: 28-Nov-22

Reference:
FRC-BUHS-50/2022-504

PATRON
Prof. Ambreen Usmani
Principal & Dean
Health Sciences(BU)

CHAIRPERSON
Dr. Quratulain Javaid

SECTRETARY
Dr. Ambreen Surti

MEMBERS
Prof M Alamgir
Prof Anis Jafarey
Prof Aisha Qamar
Ms Nighat Huda
Surg Cdre Amir Ejaz
Prof Reza H Syed
Ms Shabina Arif
Mr M Amir Sultan
Prof Dr Rafat Murad
Ms NajmusSahar Ilyas

Dr. Rabia Siddiqui
MPhil Candidate
Department of Physiology
BUHS-Karachi

Subject: Institutional approval of research study

Title of Study: C-reactive protein as biomarker of inflammation in obese & non-obese type 2 diabetes mellitus proteins with Metabolic Syndrome

Principal Investigator: Dr. Rabia Siddiqui

Reference No: ERC 107/2022

Dear Dr. Rabia Siddiqui,

Thank you for submitting the above mentioned study proposal. ERC Bahria University Health Sciences Campus has reviewed this project in the meeting held on 25-Nov-2022 and gives approval. Kindly notify us when the research is complete.

Regards,

28/11/2022
DR. AMBREEN SURTI
Secretary, ERC
BUHS

28/11/2022
DR. QURATULAIN JAVAID
Chairperson, ERC
BUHS

Cc:
- Principal BUHS

ANNEXURE C

WRITTEN INFORMED CONSENT FORM OF PATIENT

You are giving your consent to participate voluntarily and at your own will in the research project that aims for prevention and early diagnosis of Metabolic Syndrome in type 2 diabetes mellitus patients. The project will evaluate markers for early diagnosis of Metabolic Syndrome in obese and non-obese type 2 diabetes mellitus patients and will provide advice of symptomatic treatment to the patients.

You have been explained in detail the nature and significance of participating in the project and you understand the provided explanation.

You have been told that findings of your disease and your data will be kept strictly confidential and will be used only for the benefit of community, publications and paper presentations.

You have been explained that laboratory investigations will be conducted to evaluate your health status and to diagnose and monitor your disease process. For this purpose, you fully agree to give 5cc of your blood sample, to the researcher, drawn from the antecubital vein in the cubital fossa.

RISKS:
You have been informed that there will be no risk to you while drawing blood sample from your antecubital vein in cubital fossa. And if any hematoma forms during the procedure, it will be properly taken care of by a professional health worker.

You also agree to give all relevant information needed, in full and to the best of your knowledge to the researcher. It is clarified to you that no incentive, financial assistance or reimbursement will be provided to you for participating in the study whereas you do have the right to withdraw from the study at any time.

You are advised to contact Dr. _____ on mobile number _____ or visit _____ hospital in case of query/ emergency related to my disease.

I hereby confirm that I have read and understood whatever has been stated and based on the same I voluntarily consent to participate in the study.

Name of Patient: _____ S/o, D/o, W/ o _____

Signature of Patient: _____

Name of Researcher: _____

Signature of Researcher: _____

Date: _____

تحقیق کے لیے رضامندی فارم

آپ رضاکارانہ طور پر اور اپنی مرضی سے تحقیقی منصوبے میں حصہ لینے کی رضامندی دے رہے ہیں جس کا مقصد ٹائپ 2 ذیابیطس میلیٹس مریضوں میں میٹابولک سنڈروم کی روک تھام اور جلد تشخیص کرنا ہے۔ یہ پروجیکٹ موٹے اور غیر موٹے ٹائپ 2 ذیابیطس میلیٹس مریضوں میں میٹابولک سنڈروم کی جلد تشخیص کے لئے مارکرز کا جائزہ لے گا اور مریضوں کو علامتی علاج کا مشورہ فراہم کرے گا۔

آپ کو اس منصوبے میں حصہ لینے کی نوعیت اور اہمیت کی تفصیل سے وضاحت کی گئی ہے اور آپ فراہم کردہ وضاحت کو سمجھتے ہیں آپ کو بتایا گیا ہے کہ آپ کی بیماری اور آپ کے ڈیٹا کے نتائج کو سختی سے خفیہ رکھا جائے گا اور اسے صرف کمیونٹی، اشاعتوں اور کاغذی پیشکشوں کے فائدے کے لئے استعمال کیا جائے گا

آپ کو وضاحت کی گئی ہے کہ آپ کی صحت کی حیثیت کا جائزہ لینے اور آپ کی بیماری کے عمل کی تشخیص اور نگرانی کے لئے لیبارٹری کی تحقیقات کی جائیں گی۔ آپ اپنے خون کے نمونے کا 5 سی سی محقق کو دینے کے لئے مکمل طور پر اتفاق کرتے ہیں، جو کیوبٹل فوسا میں اینٹیکیوبٹل رگ سے تیار کیا گیا ہے

خطرات

آپ کو مطلع کیا گیا ہے کہ کیوبٹل فوسا میں آپ کے اینٹیکیوبٹل رگ سے خون کے نمونے کھینچتے وقت آپ کو کوئی خطرہ نہیں ہوگا۔ اور اگر طریقہ کار کے دوران کوئی ہیماٹوما تشکیل دیتا ہے تو ، اس کی مناسب دیکھ بھال ایک پیشہ ور صحت کارکن کے ذریعہ کی جائے گی۔

آپ تمام ضروری معلومات، مکمل طور پر اور اپنے علم کے مطابق محقق کو دینے پر بھی متفق ہیں۔ آپ کو واضح کیا گیا ہے کہ مطالعے میں حصہ لینے کے لئے آپ کو کوئی ترغیب، مالی امداد یا معاوضہ فراہم نہیں کیا جائے گا جبکہ آپ کو کسی بھی وقت مطالعہ سے دستبردار ہونے کا حق حاصل ہے۔

میں اس بات کی تصدیق کرتا ہوں کہ میں نے جو کچھ بھی بیان کیا گیا ہے اسے پڑھا اور سمجھا ہے اور اسی کی بنیاد پر میں رضاکارانہ طور پر مطالعہ میں حصہ لینے کے لئے رضامند ہوں۔

مریض کا نام..... ولد.....

مریض کے دستخط.....

محقق کا نام.....

محقق کے دستخط.....

تاریخ.....

ANNEXURE D

RESEARCH PROFORMA

SERIAL NO:_____ **DATE:_____**

Name/Address/Contact Number	
Age	
Gender	□Male □ Female
Marital status	□Single □Divorced □ Widowed □Married
Education	□Primary □Secondary □Graduate □Masters
Smoking	□Smoker □Non-smoker
Exercise	□Exercise □Do not exercise
Sleep	□Normal □Disturbed
Family History	□Diabetes □MI □HTN □Stroke
Personal History	□Hypertension □Duration of Diabetes >2 Years
Medications	□Oral Hypoglycemics □Antihypertensives

1. HEIGHT (cm)_____ WEIGHT (Kg)_____BMI Kg/m2_____
2. WAIST CIRCUMFERENCE (cm)_____
3. BLOOD PRESSURE (mm Hg) _____
4. FASTING BLOOD SUGAR (FBS mg/dl) _____
5. HbA1c _____
6. TRIGLYCERIDES (TG mg/dl) _____LDL (mg/dl) _____HDL (mg/dl) _____
7. CRP (mg/l) _____

ANNEXURE E


National Medical Centre
FACILITIES AVAILABLE 24 HOURS
• 64 Slice C.T. Scan
• MRI (Open)
• Laboratory
• Blood Bank
• X-Ray
• Sleep Disorders Laboratory *
• Fluoroscopy *
• Exercise Tolerance Test (ETT)
(11 a.m. to 9 p.m.)
• DEXA Scan
(10 a.m. to 6 p.m.)
• Physiotherapy
(9 a.m. to 9:30 p.m.)
• Thallium Scan *
• Nuclear Medicine *
→ Sunday Open
• BAER / BERA, VEP *
• Ultra Sound 4D & Color Doppler
(9:30 a.m. to 10 p.m.) (Sunday 2 - 5 p.m.)
• Angiography & Angioplasty
• Transesophageal Echocardiography (TEE) *
• Stress Echocardiography *
• Echocardiography (Sunday 2 - 5 p.m.)
• EEG, EMG & NCS *
• Mammography
(9:30 a.m. to 5:30 p.m.)
• Endoscopy, Colonoscopy & Bronchoscopy *
• OPG Dental X-Ray
• Bone Scan *
• ERCP *
• Halter Monitoring *
* Call for Appointment 021-111-222-662 (NMC)
OUT PATIENT DEPARTMENT

ANNEXURE F

ORIGINALITY REPORT

15%	12%	12%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Infectious Diseases Institute of Makerere Student Paper	1%
2	www.ncbi.nlm.nih.gov Internet Source	1%
3	orbi.ulg.ac.be Internet Source	1%
4	link.springer.com Internet Source	1%
5	www.science.gov Internet Source	1%
6	"The Metabolic Syndrome", Wiley, 2011 Publication	1%
7	"Islets of Langerhans", Springer Nature, 2015 Publication	1%
8	www.hindawi.com Internet Source	1%
9	worldwidescience.org Internet Source	1%