SURFACTANT PROTEIN–D AMINO ACID VARIANTS AND THEIR ASSOCIATIONS WITH METABOLIC, OXIDATIVE STRESS, INFECTION AND QUALITY OF LIFE PARAMETERS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS IN PAKISTAN

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LIST OF SYMBOLS

α	Alpha
β	Beta
γ	Gamma
≤	less than or equal to
>	Greater than
µ	Micro

LIST OF ABBREVIATIONS

ADA	American Diabetes Association
AFH	Aziz Fatimah Hospital
AFMDC	Aziz Fatimah Medical and Dental College
AIDS	Acquired Immunodeficiency Syndrome
α2M	Alpha-2 Macroglobulin
APC	Antigen-Presenting Cell
BP	Bodily Pain
CD	cluster of differentiation
CI	Confidence Interval
Cm	Centimeter
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Corona Virus Disease- 2019
CRD	Carbohydrate Recognition Domain
DNA	Deoxyribonucleic Acid
dNTPs	deoxynucleotides triphosphates
ddNTPs	dideoxynucleotides triphosphates
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme Link Immuno Assay
ER Ca2	Endoplasmic Reticulum Calcium
ESE	Exonic Splicing Enhancers
FBS	Fasting Blood Sugar
FFA	Free Fatty Acids
GH	General Health
GOD	Glucose Oxidase
HbA1c	Glycated Hemoglobin
HCoV	human coronavirus
HIV	Human Immunodeficiency Virus
HOCl	Hypochlorous Acid
HWE	Hardy -Weinberg Equilibrium
IDF	International Diabetes Federation
IFN γ	Interferon Gamma
IL	Interleukin
IQR	Intra Quartile Range
JNK	Jun N-Terminal Kinase
LAIR-1	Leukocyte-Associated Immunoglobulin-Like Receptor 1
LPS	Lipopolysaccharide
MAP kinase	Mitogen-Activated Protein Kinase
MCS	Mental Component Summary
MDA	Malondialdehyde
MH	Mental Health
MHC	Major Histocompatibility Complex
MPO	Myeloperoxidase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Center For Biotechnology Information
NFkβ	Nuclear factor kappa B cells
NK cells	Natural Killer Cells

O_2	Molecular Oxygen
O_2^-	Superoxide Anions
OPD	Outpatient Department
OR	Odds Ratios
PCR	Polymerase Chain Reaction
PCS	Physical Component Summary
PF	Physical Functioning
POD	Peroxidase enzyme
PPR	Pattern Recognition Receptor
QOL	Quality of Life
RAND	Research and Development.
RE	Role Limitations Due to Emotional Problems
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RP	Role Limitations due to Physical Health
RSV	Respiratory Syncytial Virus
SARS -CoV	Sever acute respiratory syndrome coronavirus
SD	Standard Deviation
SF	Social Functioning
SF36	Short Form 36
SFTPD	SP-D Gene
SIRPα	signal inhibitory regulatory protein alpha
SNP	Single Nucleotide Polymorphism
SOD	Superoxide Dismutase
SP-D	Surfactant Protein–D
SPSS	Statistical Package for The Social Sciences
T2DM	Type 2 Diabetes Mellitus
TBA	Thiobarbituric Acid
Th1	T-Helper 1 Cells
Th2	T-Helper 2 Cells
TNFα	Tumor Necrosis Factor Alpha
UPR	Unfolded Protein Response
UTI	Urinary Tract Infection
VT	Vitality
WHO	World Health Organization
WST-1	Water Soluble Tetrazolium Salt
XO	Xanthine Oxidase

LIST OF APPENDICES

VARIAN ASID AMINO PROTEIN SURFAKTAN- D DAN KAITANNYA DENGAN PARAMETER METABOLIK, TEKANAN OKSIDATIF, JANGKITAN DAN KUALITI HIDUP DALAM KALANGAN PESAKIT DIABETES MELLITUS JENIS 2 DI PAKISTAN

ABSTRAK

Diabetes mellitus jenis 2 (T2DM) adalah lazim di Pakistan, dan sering dikaitkan dengan jangkitan berulang dan kualiti hidup yang rendah. Tekanan oksidatif, surfaktan protein- D (SP-D), dan rs721917 SNP mungkin menyumbang kepada risiko ini, tetapi tiada kajian telah mengkaji perkaitan ini di Pakistan. Oleh itu, kajian ini mengkaji varian rs721917 SNP dan kaitannya dengan parameter metabolik, tekanan oksidatif, jangkitan dan kualiti hidup dalam pesakit T2DM. Kajian kawalan kes telah dijalankan di Hospital Aziz Fatimah, Faisalabad, Pakistan. Menggunakan teknik persampelan bertujuan bukan kebarangkalian, 170 peserta (86 T2DM, 84 Kawalan; berumur 30-65 tahun) telah diambil. Sampel darah telah diuji untuk surfaktan protein-D (SP-D), gula darah puasa (FBS), hemoglobin glikat (HbA1c), malondialdehid (MDA), dan aktiviti superoksida dismutase (SOD). DNA telah diekstrak dan dikembangkan oleh tindak balas rantai polimerase. Penjujukan Sanger dilakukan untuk mengenal pasti genotip rs721917SNP. Genotip TC adalah lebih lazim dalam pesakit T2DM (p<0.001). Tahap SP-D lebih rendah pada pesakit T2DM (p=0.014), dengan, HbA1c (kedua-dua p<0.001), dan tahap MDA (p=0.012) yang lebih tinggi, dan aktiviti SOD (p=0.022) yang lebih rendah berbanding kawalan. SP-D menunjukkan perkaitan negatif yang signifikan dengan FBS (p=0.001), HbA1c (p=0.032), dan MDA (p=0.014). Genotip TC mempunyai tahap SP-D (p=0.040) dan aktiviti SOD (p=0.044) yang lebih rendah berbanding genotip TT dan CC. Tahap FBS, HbA1c, dan MDA adalah lebih tinggi dalam TC, diikuti

oleh CC, berbanding TT (p=0.006, 0.001, 0.037 masing-masing). Kedua-dua genotip TC dan CC menunjukkan risiko tekanan oksidatif tiga kali ganda lebih tinggi daripada jenis liar TT (p=0.044, 0.041 masing-masing). Kadar jangkitan adalah lebih tinggi pada pesakit T2DM (p<0.001), terutamanya di kalangan pembawa TC, yang mempunyai risiko jangkitan empat kali ganda lebih tinggi (p=0.013). Pesakit T2DM, terutamanya wanita, menunjukkan kualiti hidup yang rendah dengan Ringkasan Komponen Fizikal dan Ringkasan Komponen Mental<50. Tiada kaitan yang signifikan bagitahap SP-Ddan rs721917 kepada kualiti hidup. Kesimpulannya, genotip TC rs721917 SP-D adalah genotip yang paling lazim dan peramal bebas tekanan oksidatif dan jangkitan dalam pesakit T2DM dalam populasi Pakistan yang dikaitkan dengan tahap surfaktan protein-D dan aktiviti SOD yang rendah serta tahap FBS, HbA1c dan MDA yang tinggi. Pesakit T2DM juga menunjukkan kualiti hidup yang rendah yang tidak dikaitkan dengan tahap SP-D dan rs721917 SNP. Oleh itu, adalah munasabah untuk mencadangkan bahawa melakukan analisis gen SP-D boleh menyediakan pengurusan awal dan perlindungan terhadap jangkitan dalam T2DM dan kajian lanjut diperlukan untuk menilai potensi penggunaan SP-D sebagai terapi berguna untuk pencegahan atau rawatan jangkitan dalam T2DM.

SURFACTANT PROTEIN–D AMINO ACID VARIANTS AND THEIR ASSOCIATIONS WITH METABOLIC, OXIDATIVE STRESS, INFECTION AND QUALITY OF LIFE PARAMETERS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS IN PAKISTAN

ABSTRACT

Type 2 diabetes mellitus (T2DM) is prevalent in Pakistan, and often linked to recurrent infections and poor quality of life. Oxidative stress, surfactant protein-D (SP-D), and its rs721917 SNP may contribute to this risk, but no studies have investigated this association in Pakistan. Hence, this study investigated rs721917 SNP variants and their links with metabolic, oxidative stress, infection, and quality of life parameters in T2DM patients. A case-control study was conducted at Aziz Fatimah Hospital, Faisalabad, Pakistan. Using non-probability purposive sampling technique, 170 participants (86 T2DM, 84 Controls; aged 30–65 years) were recruited. Blood samples were tested for SP-D-, Fasting Blood Sugar (FBS), glycated haemoglobin (HbA1c), malondialdehyde (MDA), and superoxide dismutase (SOD) activity. DNA was extracted and amplified by polymerase chain reaction. Sanger sequencing was done to identify the rs721917SNP genotypes. The TC genotype was significantly more prevalent in T2DM patients (p<0.001). SP-D levels were lower in T2DM patients (p=0.014), with higher FBS, HbA1c (both p<0.001), and MDA levels (p=0.012), and reduced SOD activity (p=0.022) compared to controls. SP-D showed significant negative associations with FBS (p=0.001), HbA1c (p=0.032), and MDA (p=0.014). The TC genotype had significantly lower SP-D levels (p=0.040) and SOD activity (p=0.044) compared to TT and CC genotypes. FBS, HbA1c, and MDA levels were higher in TC, followed by CC, versus TT (p=0.006, 0.001, 0.037

respectively). Both TC and CC genotypes showed a three-fold higher oxidative stress risk than TT wild type (p=0.044, 0.041 respectively). Infection rates were higher in T2DM patients (p<0.001), particularly among TC carriers, who had a four-fold higher infection risk (p=0.013). T2DM patients, especially females, showed poor quality of life with Physical Component Summary and Mental Component Summary < 50. No significant impact of SP-D levels and rs721917 was found on quality of life. In conclusion, TC genotype of rs721917 of SP-D was the most common genotype and an independent predictor of oxidative stress and infections in T2DM patients in Pakistani population which were linked with low SP-D levels and SOD activity as well as with high FBS, HbA1c and MDA levels. In addition, T2DM patients also exhibited poor quality of life which was not associated with SP-D levels and rs721917 SNP. Hence, it is plausible to suggest that doing SP-D gene analysis can provide early management and protection against infections in T2DM and further studies are needed to assess the potential use of SP-D as a useful therapy for prevention or treatment of infections in T2DM.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Type 2 Diabetes mellitus (T2DM) is global health challenge, however the Southeast Asia region bear a substantial proportion of the global diabetes epidemic burden (Ganasegeran *et al.*, 2020; Trikkalinou *et al.*, 2017). T2DM prevalence in the world is 9% and in Pakistan is 13.1 to 26.7% as documented by numerous Pakistani studies (Azeem *et al.*, 2022; Basit *et al.*, 2018). This prevalence is alarmingly high and escalated rapidly with each passing year throughout the world (Azeem *et al.*, 2022; Ganasegeran *et al.*, 2020). In Pakistan, many patients remained undiagnosed due to lack of primary healthcare facilities in remote areas because unfortunately provision of health care facilities are never being an area of priority for the policy makers of primary health care systems in Pakistan (Azeem *et al.*, 2022; Sadat and Lopes, 2021). Ignorance of regular medical checkup among Pakistani population, driven by high inflation and financial instability, is an additional factor contributing to the underestimation of prevalence of non-communicable diseases including T2DM in Pakistan (Fazal *et al.*, 2023). Although the life span of patients with diabetes mellitus increases due to better hyperglycemic control by using oral hypoglycemic drugs and insulin administration, however complete cure is still not possible globally. T2DM is usually associated with chronic complications often leading to disabilities along with significant social, mental, psychological, and physiological stress, which ultimately compromising the quality of life of these patients (Carey *et al.*, 2018; Tariq *et al.*, 2022). Moreover, due to its rising prevalence, debilitating nature and high mortality rates, it imposes an excruciating emotional and economic burden,

not only on the patients and their caregivers, but also imposes huge socioeconomic burden on the country's economy across the globe (Ganasegeran *et al.*, 2020). Low and middle-income countries such as Pakistan are already at a crisis in provision of best health care facilities and demands a novel approach in shaping medical resources but these countries are not able to bear the extra expenses for raising prevalence of chronic diseases (Sadat and Lopes, 2021). Global expenditure for the management of diabetes mellitus for patients aged 18 to 99 was estimated at 850 billion USD in the year 2017, and expected to rise by 7% approaching 958 billion USD by the year 2045 (Ganasegeran *et al.*, 2020).

T2DM is strongly associated with recurrent pulmonary and extrapulmonary infections, leading to poor outcomes and prolonged hospitalization (Jawed *et al.*, 2021). These infections stem from immune dysregulation due to impaired antioxidant defenses and suppressed innate and adaptive immunity (Frydrych *et al.*, 2017; Patchett *et al.*,2021)**.** Insulin resistance and hyperglycemia exacerbate this dysfunction, creating a cycle of oxidative stress, inflammation, and excess reactive oxygen species (ROS) that drive lipid peroxidation, apoptosis, and β-cell loss, thereby worsening T2DM (Oguntibeju, 2019;Nair and Nair, 2017; Shabalala *et al.*, 2022). Surfactant protein-D (SP-D) is an immunoregulator belonging to the collectin family Surfactant protein-D (SP-D), encoded by the SFTPD gene on chromosome 10q22.2–23.1, is a 43 kDa collectin secreted mainly by type II alveolar and Clara cells but also expressed in extrapulmonary tissues including pancreas, gut, adipose tissue, and blood (Liao *et al.*, 2019). SP-D enhances innate immunity by promoting opsonization, phagocytosis, chemotaxis, and microbial aggregation while regulating inflammation through interactions with bacterial lipopolysaccharides, fatty acids, and oligosaccharides (Javed *et al.*, 2024; Ortega *et al.*, 2013;van Moorsel *et al.*, 2021).

Reduced circulating SP-D has been linked to obesity, insulin resistance, T2DM, and dyslipidemia (Javed *et al.*, 2024; van Moorsel *et al.*, 2021; Ortega *et al.*,2013). Genetic variants of SFTPD, particularly rs721917, have been associated with lung disease, kidney disorders, and recurrent aphthous stomatitis in Asian populations (Horimasu *et al.*, 2014; Liu *et al.*, 2020; Rizvi *et al.*, 2023), and with T2DM in a Spanish cohort (Pueyo *et al.*, 2013).). However, in literature no Asian study was found to explore this SNP in T2DM. This missense SNP substitutes methionine with threonine (Met11Thr) in the N-terminal domain, disrupting oligomerization, reducing SP-D levels, and impairing pathogen clearance (Foreman *et al.*, 2011; Liao *et al.*, 2019). A Pakistani study further showed that T2DM patients, particularly those with recurrent infections, exhibit significantly lower SP-D levels compared to healthy controls (Jawed *et al.*, 2021). Thus, exploring rs721917 in the Pakistani T2DM population is warranted.

Moreover, the only one recent human study identified in the literature on the impact of oxidative stress on SP-D, demonstrated that SP-D becomes dysfunctional due to irreversible oxidation in the alveolar spaces. This dysfunction results from carbonylation and nitrosylation caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS) respectively, in an oxidative stress environment in the lungs of patients with Human Immunodeficiency Virus (HIV) infection. However, author of this aforementioned recent study warranted subsequent future studies for further exploration of impact of oxidative stress on SP-D in depth (Akhter *et al.*, 2024). This aforementioned finding highlights a significant gap in the literature, as no studies have yet been explored the association of oxidative stress markers including malondialdehyde (MDA) and superoxide dismutase (SOD)

activity with SP-D. Therefore, it is crucial to explore this specific association to better understand the impact of oxidative stress on SP-D.

A previous animal study by Matalon *et al*., (2009*),* had demonstrated that ROS adversely affects the bacterial aggregating activity of SP-D and host defence by altering its structure and damaging the carbohydrate recognition domain (CRD) (Matalon *et al.,* 2009). Notably, CRD is very important for the full functionality of SP-D, as this is a ligand recognition pattern for recognizing and binding with microbes for clearance. Blood glucose is the preferred ligand for the carbohydrate recognition domain, and its higher levels hampers the CRD to bind with a broad spectrum of microorganisms and thus reduces their clearance from the body leading to recurrent infections in diabetic subjects (Jawed *et al.*, 2021). Hence, the oxidative stress, hyperglycemia, SP-D and related SNPs seems to be linked and merits exploration. rs721917 SNP is previously studied by some researchers for establishing their association with risk for chronic obstructive pulmonary disease in the Asian population and proved to be significant (Liao *et al.*, 2019; Shakoori *et al.*, 2012). However, to date, SNPs in *SFTPD* in diabetic subjects has not been explored in Asia including Pakistan and this provides the rationale for future research. Based on the previous studies describing the significant association between lower SP-D levels with T2DM and associated infections, it can be a candidate gene for studying diabetic subjects with recurrent pulmonary as well as extrapulmonary infections (Jawed *et al.,* 2015). Furthermore, the association between SP-D and oxidative stress is also not well established, so there is a need to elucidate whether the SP-D is on the crossroad of the inflammation, oxidative stress and T2DM.

In a previous study, it was documented that T2DM subjects with pulmonary and extrapulmonary infections have lower SPD levels but the actual

role of SP-D in developing infections is still unclear (Jawed *et al.*, 2021, 2015). Furthermore, clinical management of diabetic patients can help for better hyperglycemic control, however, the ultimate goal of diabetic care is to improve the quality of life of these patients**,** that should be addressed. Hence, the present study was proposed to evaluate the SP-D amino acid variants (rs721917 SNP) and their associations with SP-D levels, metabolic, oxidative stress, infection and quality of life parameters in patients with T2DM in Pakistan.

1.2 Problem Statement

Incidence of T2DM is continuously increasing in Asia including Pakistan and is proved to be a significant risk factor for recurrent infections. Despite the huge number of previous studies, the pathophysiology of recurrent pulmonary as well as extrapulmonary infections in diabetic subjects is still elusive. In a previous study, it was documented that diabetic subjects with recurrent infections have lower SPD levels which might be associated with oxidative stress, however the actual role of SP-D is uncertain. A significant knowledge gap still exists concerning mechanistic link between T2DM, oxidative stress, infection and SP-D at genetic level. Since there is no data for Asian countries particularly Pakistan, hence, this study was planned to determine SP-D amino acid variants (rs721917 SNP), SP-D levels, metabolic and oxidative stress parameters, infection rate and quality of life in T2DM patients and their possible associations. The present study is providing an insight into the role of SP-D in the prevention of serious infections associated with T2DM and will open a new horizon for discovering the therapeutic strategies for the prevention of recurrent infections in immuno-compromised patients with T2DM in order to improve their quality of life. Additionally, the present study also helps in identifying the rs721917 SNP in SP-D gene in Pakistani population that

could be contributed to the increased risk of T2DM and associated infections because still there is no study conducted in Asia including Pakistan concerning this SNP in T2DM patients. This inherited gene of SP-D and its polymorphism can be used for screening and early diagnosis in families with a history of T2DM that could be helpful in management and prevention of progression of disease and recurrent infections.

1.3 Objectives

1.3.1 General Objective

To determine Surfactant Protein-D (SP-D) amino acid variants and their associations with metabolic, oxidative stress, infection and quality of life parameters in patients with T2DM in Pakistan.

1.3.2 Specific Objectives

1. To determine frequency of SP-D amino acid variants (rs721917 SNP) in patients with T2DM in Pakistan.
2. To determine serum levels of SP-D, metabolic (fasting blood sugar [FBS] and glycated hemoglobin [HbA1c]), and oxidative stress (MDA and SOD) parameters in patients with T2DM in Pakistan.
3. To assess associations of SP-D levels with metabolic and oxidative stress parameters in patients with T2DM in Pakistan.
4. To evaluate association of rs721917 SNP with serum SP-D levels, metabolic and oxidative stress parameters in patients with T2DM in Pakistan.
5. To assess infection rates and their associations with rs721917 SNP, SP-D levels and oxidative stress in patients with T2DM in Pakistan.
6. To evaluate quality of life (QOL) and its association with SP-D levels and genotypes of rs721917 SNP in patients with T2DM in Pakistan.

1.4 Hypothesis

1. Patients with T2DM have significantly higher percentages of of SPD amino acid variants (rs721917 SNP) compared to healthy control participants.
2. Patients with Type 2 Diabetes Mellitus (T2DM) have significantly lower SP-D level and SOD activity, and higher MDA level than healthy control participants.
3. SP-D has significant negative associations with FBS, HbA1c, and MDA, and a significant positive association with SOD activity.
4. rs721917 SNP is significantly associated with serum SP-D levels, as well as with metabolic and oxidative stress parameters in patients with T2DM.
5. Patients with T2DM in Pakistan exhibit higher infection rates, which are significantly associated with specific genotypes of rs721917 SNP, lower SP-D levels and increased oxidative stress**.**
6. Patients with T2DM in Pakistan have poorer QOL and is significantly associated with lower serum SP-D levels and genotypes of rs721917 SNP.

CHAPTER 2

LITERATURE REVIEW

2.1 Type 2 Diabetes Mellitus

2.1.1 Overview of Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is the fastest growing health challenge across the world (Hasan and Siddiqui, 2024). The prevalence of T2DM in the world is 9% and in Pakistan is 26.1% which is continuously rising (Azeem *et al.*, 2022; "International Diabetes Federation," 2021). Hyperglycemia with impaired carbohydrate, protein and fat metabolism caused by lack of insulin secretion or decreased sensitivity of the tissues to insulin or both are the hallmark of this chronic condition**.** Evidences from previous epidemiological studies indicated that numerous cases of T2DM have a strong genetic predisposition and attributed to non-modifiable risk factors including ethnicity and family history. However, obesity, physical inactivity, unhealthy diet, carbohydrate-rich diet and modernized life style are modifiable risk factors for its pathogenesis (Galicia-Garcia *et al.*, 2020; Tariq *et al.*, 2022). These factors are the common causes of T2DM in Pakistani population (Tariq *et al.*, 2022).

2.1.2 Pathophysiology

Complex network of interactions between genetic, epigenetic, metabolic and environmental factors are implicated in T2DM pathophysiology. Disturbance in feedback loops between insulin secretion and action results in hyperglycemia. Insulin resistance is a hallmark of T2DM, but the disease only develops when the pancreatic beta cells can no longer compensate for the insulin resistance by producing enough insulin (Galicia-Garcia *et al.*, 2020). On the other hand, decreased physical activity and obesity lead to insulin resistance in which receptors

are not responding to insulin due to release of pro-inflammatory mediators from adipose tissues and reduced leptin levels (Himanshu *et al.*, 2020). Insulin resistance contributes to increased hepatic glucose production, and decreased glucose uptake by the muscle and adipose tissues. It also enhances lipolysis in adipose tissue and hence increases free fatty acid levels (Galicia-Garcia *et al.*, 2020; Himanshu *et al.*, 2020). Surplus free fatty acids and hyperglycemia lead to lipotoxicity and glucotoxicity, respectively, both activating apoptotic unfolded protein response pathways that disrupt endoplasmic reticulum-calcium homeostasis, which is essential for insulin production and processing. This impairment in β-cell dynamics reduces insulin secretion, leading to β-cell dysfunction (Galicia-Garcia *et al.*, 2020; Yamamoto *et al.*, 2019). Insufficient insulin secretion limits the body's ability to regulate blood glucose, resulting in severe glucotoxicity leading to severe complications. The combination of insulin resistance and β-cell dysfunction exacerbates hyperglycemia, accelerating the progression of T2DM. (Galicia-Garcia *et al.*, 2020). Genetic predisposition has a key role in its pathogenesis. Previous T2DM genome-wide association studies have documented complex polygenic nature of diabetes mellitus, and many genetic loci in association with diabetes have been identified by previous researchers (Halban *et al.*, 2014; Himanshu *et al.*, 2020). Most of genetic variants primarily effect insulin secretion, and some act through reducing insulin action and causing insulin resistance (Galicia-Garcia *et al.*, 2020). Advanced studies based on whole-genome sequencing and exome sequencing technology on different ethnicities are still under way and researchers are doing extensive efforts to identify loci and genetic variants accounting for risk and pathological basis of T2DM (Himanshu *et al.*, 2020). In future, these studies will prove to be advantageous in early diagnosis and management of this serious

public health challenge. The diverse interaction between genetic and epigenetics factors causing insulin resistance and T2DM are summarized in Figure 2.1.

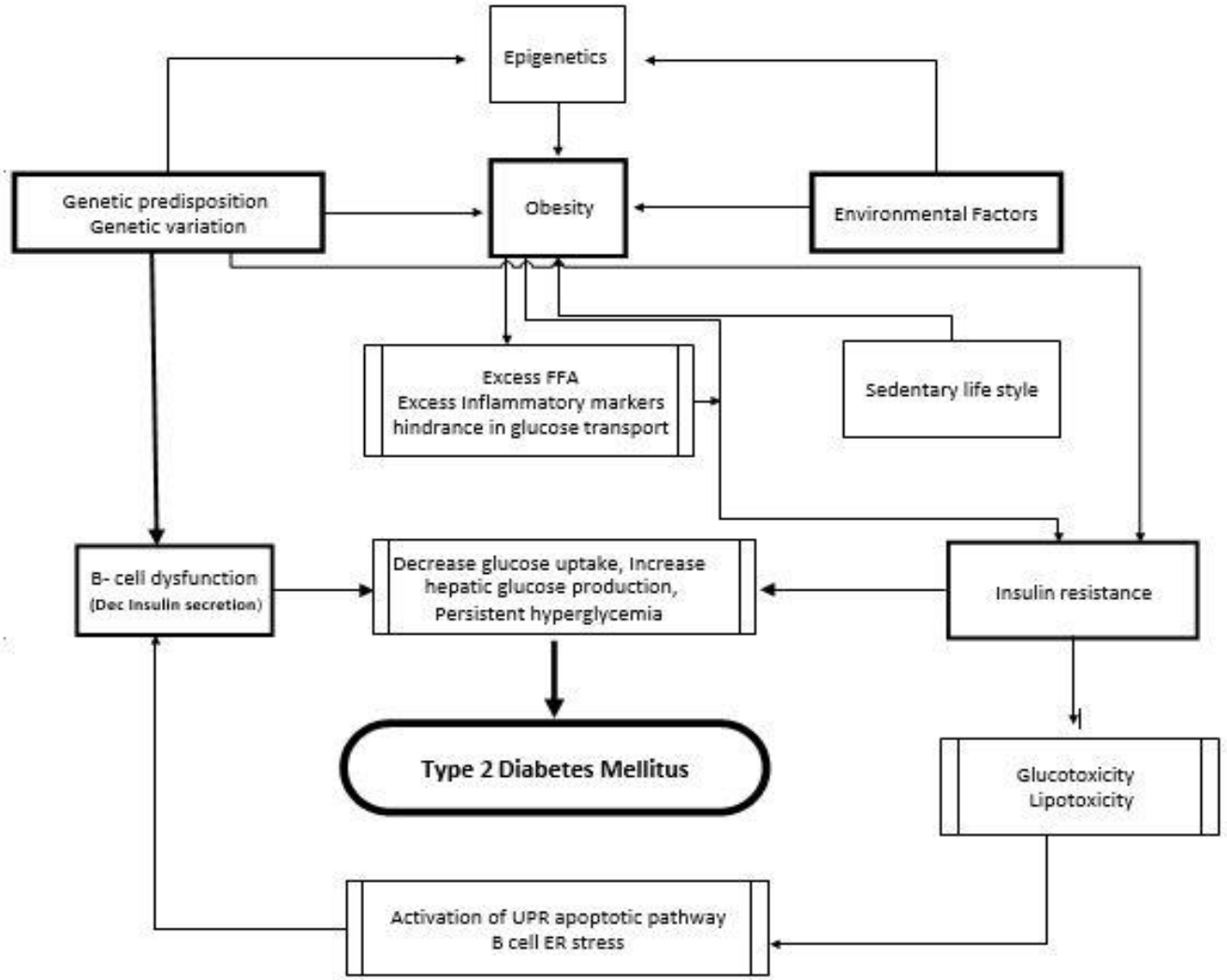

Figure 2.1 Diverse Interaction between Genetic and Epigenetics Factors Causing Insulin Resistance and Type 2 Diabetes Mellitus.

UPR, Unfolded response protein; ER, Endoplasmic reticulum; FFA, free fatty acid.

2.1.3 Diagnosis and Clinical Evaluation

Diagnosis of T2DM is based on classical symptoms of hyperglycemia such as polyphagia, polyuria, polydipsia, unexplained weight loss plus blood glucose and glycated hemoglobin levels as per American Diabetic Association. Cut-off point of fasting blood sugar and HbA1c levels for diagnosing diabetes are described below in Table 2.1 (American Diabetes Association, 2022).

Table 2.1 American Diabetic Association Diagnostic Criteria for Type 2 Diabetes Mellitus

Glucose Indices	**Blood glucose levels**
Random plasma sugar	⩾200 mg/dl on 2 more occasions
Fasting plasma sugar	⩾ 126 mg/dl on 2 more occasions
Two hours post glucose load (75 g) plasma glucose	Plasma glucose ⩾200 mg/dl
Glycated haemoglobin	> 6.5%

Glycated hemoglobin (HbA1c) is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. HbA1c estimates average plasma glucose of 2 to 3 months, is more reliable index and is considered to be a superior index for chronic hyperglycemia than plasma glucose levels. HbA1c > 6.5% can be used as a diagnostic test for diabetes mellitus according to latest recommendation of WHO (World Health Organization, 2020).

2.1.4 Prevalence of Type 2 Diabetes Mellitus

Prevalence of T2DM is varies widely depending on the ethnicity and geographical regions globally and is steadily rising at alarming rate leading to increased overall healthcare burden and the health expenditure for countries globally (Vicks *et al.*, 2022). Therefore, it is great socioeconomic burden

particularly for low to middle income countries with limited resources like Pakistan.

International Diabetes Federation (IDF) has reported 9.8% global prevalence of diabetes mellitus among adults with age ranged 20–79 years, affecting 536,600 million adults in 2021. IDF has estimated that by the year 2030, global prevalence is projected to rise up to 10.8%, affecting 642,800 million adults and it is expected to increase to 11.2% affecting 783,700 million adults all around the world by the year 2045 (Bhatti *et al.*, 2022; International Diabetes Federation, 2021).

Asian countries such as Pakistan, India, China, Sri Lanka and Bangladesh accounts for 60% of the global population with diabetes mellitus (Misra *et al.*, 2023). IDF is expecting 68% increase in numbers of people with T2DM in South-East Asia Region reaching 152 million population by 2045 and the prevalence of diabetes will increase 30% reaching 11.3% in year 2045 (International Diabetes Federation, 2021). According to the latest report of IDF in 2021, prevalence of diabetes in Pakistan was 26.7% affecting approximately 32,964,500 of adults' population (International Diabetes Federation, 2021). Both incidence and prevalence have been gradually rising over the past few decades at alarming rates. Pakistan is one of the more susceptible Asian countries to diabetes-related morbidities and death (Ahmed *et al.*, 2024; Azeem *et al.*, 2022).

2.1.5 Pathways for Oxidative Stress in Type 2 Diabetes Mellitus

Oxidative stress in T2DM is owing to excess production of oxygen and nitrogen free radicals, such as reactive oxygen species (ROS) which includes hydrogen peroxide, superoxide, hypochlorous acid and hydroxide, whereas nitric oxides and peroxynitrite are important reactive nitrogen species (RNS) (Oguntibeju, 2019). ROS or RNS are produced by mitochondria during electron transfer reactions

by losing or accepting electrons normally within the physiological limits (Jomova *et al.,* 2024). Hyperglycemia in T2DM can trigger the production of ROS by hyper-activation of numerous biochemical pathways such as glucose auto-oxidation, non-enzyme glycation of protein, advanced glycation end product, polyol pathway flux, hexosamine pathway and activation of protein kinase C isoforms. Excess generation of ROS accompanied by the amplified expression of nicotinamide phosphate adenine dinucleotide oxidase causes high lipid peroxidation of polyunsaturated fatty acid and subsequent release of excess oxidant end product malondialdehyde (MDA) reflecting high oxidative tissue damage (Al-Sayyar *et al.*, 2022; Nair and Nair, 2017; Nita and Grzybowski, 2016)

Superoxide dismutase (SOD) is the crucial component of antioxidant system and is proven first line of defence against oxidative tissue damage (Tavares *et al.*, 2019). It helps in elimination of hydrogen peroxide from superoxide that can be later on decomposed into the water and oxygen by the catalase enzyme, hence protects tissues from oxidative damage (Nair and Nair, 2017; Nna *et al.,* 2019). Increases glycosylation of SOD due to hyperglycemia reduces its activity and limits its capacity to detoxify oxygen radicals causing oxidative stress and activation of apoptotic cascade leading to deoxyribonucleic acid (DNA) damage and cell death (Briggs *et al.*, 2016; Madi *et al.*, 2016; Oguntibeju, 2019; Sharma *et al.*, 2019).

In summary, hyperglycemia impairs antioxidant defence system which could result in inactivation of antioxidant enzymes (Oguntibeju, 2019), on the other hand its results in abnormally high lipid peroxidation and production of oxidants as reported in diabetic conditions. Imbalance between oxidant and antioxidant systems in T2DM triggers the production of ROS promoting oxidative stress (Nna *et al.,* 2019; Oguntibeju., 2019).

Augmented non-enzymatic and auto-oxidative glycosylation is important principal mechanism that contribute to the generation of disproportionate free radicals and subsequent lipid peroxidation in hyperglycemic-induced oxidative stress in T2DM. Oxidative stress play an important role in the progression and development of diabetic complications. Free radicals are unstable molecules having free unpaired electrons which can react rapidly with the cell proteins, lipids and DNA causing injurious effect on cells in high concentration. However, free radicals in low concentration have beneficial effects on physiological functions and helps in maintenance of redox homeostasis, regulation of important transcription factors and defence against infectious agents (Jomova *et al.*, 2024; Oguntibeju, 2019; Soliman, 2008).

Higher circulating levels of low-density lipoprotein-cholesterol and triglycerides are the hallmark of T2DM, which are subjected to be attacked by free radicals including ROS and RNS molecules giving rise to lipid peroxidation (Shabalala *et al.*, 2022). Malondialdehyde (MDA) is highly toxic end product of lipid peroxidation, its toxicity attributed to high reactivity of free radicals towards proteins and DNA (Shabalala *et al.*, 2022). MDA is produced due to the degradation of cell membrane phospholipids by ROS during oxidative stress. The process begins with the release of arachidonic acid due to break down of cell membrane phospholipids by the action of phospholipase A2. This released arachidonic acid is subsequently attacked by ROS, primarily the hydroxyl radical, produced in the mitochondria. This reaction forms lipid endoperoxide, which then spontaneously ruptures, resulting in the formation of MDA in the intracellular spaces and eventually releases into extracellular spaces (Lorente *et al.*, 2013). Numerous studies reported higher MDA levels in T2DM, interacting both reversibly and irreversibly with phospholipids,

proteins and DNA leading to oxidative damage to these cellular components and detrimental effects in these patients (Shalash *et al.*, 2020; Soliman, 2008). Lipid peroxidation plays a key role in the development of lethal complications leading premature death in T2DM patients. This whole process depletes intracellular antioxidants such as SOD activity leading to failure of neutralization or elimination of excess ROS causing severe oxidative stress in T2DM (Shabalala *et al.*, 2022). Exposure of pancreatic beta cells to oxidative stress have been proven to cause beta cell dysfunction and inhibit promotor activity of mRNA expression of insulin gene, leading to insulin resistance and further progression of T2DM. Chronic sustained hyperglycemia is the main key factor of oxidative stress in beta cells (Oguntibeju, 2019). Mitochondria are inevitable source of ROS in the cells and generate ROS through electron transport chain within physiological limits which can be neutralized and eliminated by endogenous antioxidant systems (Bhatti *et al.*, 2022). It has been evident that the alterations in mitochondrial membrane could lead to activation of complexes in electron transport, hence contributing to exacerbated oxygen radical production. Additionally, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is believed to be the principal source for production of glucose-induced ROS in cells and tissues of diabetic patients (Oguntibeju, 2019). Glucose and its metabolites have been documented to react with hydrogen peroxide in the presence of iron and copper to produce hydroxyl radical during auto-oxidation in diabetes mellitus, thereby, promoting the production of ROS and development of severe diabetic complications (Oguntibeju, 2019).

2.1.6 Link between Inflammation and Oxidative Stress in Type 2 Diabetes Mellitus

Recurrent infections in T2DM are attributed to immune dysregulation due to depression of the antioxidant system, a decline in cytokine production, and suppression of T cell and humoral immunity. Moreover, neutrophil dysfunction affecting innate immunity of the body is also a contributing factor (Shalash *et al.*, 2020). Oxidative stress and inflammation are believed to be a major pathway for alteration in immune systems and subsequent recurrent infection and other diabetic complications in T2DM patients (Carey *et al.*, 2018). These alterations in the immune system are provoked by insulin resistance and hyperglycemic environment (Pueyo *et al.*, 2013). Hypersecretion of proinflammatory mediators such as tumor necrosis factor-alpha (TNF α) and gut-derived lipopolysaccharide due to ongoing systemic inflammation in indivduals with T2DM leads to an increase progression of insulin resistance. This results in poorer glycemic control in T2DM patients, ultimately worsening their condition (Andreasen *et al.*, 2011). Binding of proinflammatory mediators to their receptors activates nuclear factor kappa B cell (NF-κB) and Jun N -terminal kinase (JNK) signaling pathways, which upregulate infilamatory markers like interleukin -6 (IL-6) and interleukin -β (ILβ). This initiates a positive feedback loop that amplifies inflammation and exacerbates insulin resistance (Andreasen *et al.*, 2011). In T2DM, elevated ROS levels from oxidative stress activate signaling pathways that trigger a vicious cycle of inflammation and ROS production. This promotes hypersecretion of pro-inflammatory cytokines and sustains pro-inflammatory cells like T lymphocytes and NK cells in the bone marrow (Andreasen *et al.*, 2011). The resulting inflammation-ROS loop impairs T and microglial cell function, increases cytokine release, enhances lipid peroxidation, and activates apoptotic pathways, leading to cell death (Pangrazzi *et al*., 2020; Patergnani

et al., 2021). Additionally, worsening glycemic control and dyslipidemia with a long duration of disease causes excess ROS production and reduction in superoxide dismutase (SOD) activity. This is caused by rising glucokinase, reduced exocytosis of insulin granules and reduced glucose transporter membrane levels. Ultimately, this leads to mitochondrial stress and beta cell dysfunction and further progression of T2DM leading to severe consequences (Promyos *et al.*, 2023).

2.1.7 Type 2 Diabetes Mellitus and Infections

T2DM can predispose to all kinds of infections which increase morbidity and mortality (Al-Sayyar *et al.*, 2022; Carey *et al.*, 2018). These infections have poorer treatment outcomes with rapid progression to severe forms requiring prolonged hospitalization. Increased susceptibility to infections is being attributed to micro environmental dysmetabolism impairing the immune responses (Holt *et al.*, 2024). Recurrent infections are leading cause of morbidity and mortality in diabetic patients specially in low- and middle-income countries where these patients presented to hospital late when the infection already get worsen and complicated. Notably most of these patients with severe infections are presented to hospitals with previously undiagnosed diabetes (Holt *et al.*, 2024).

It was proposed that hyperglycemia causes immune dysfunction particularly alteration in innate immunity which results in impairment in leukocyte adherence, antigen presentation, and depression of polymorph nuclear leukocyte functions such as chemotaxis, phagocytosis, production of specific antibodies, and efficiency of intracellular killing of microorganisms (Al-Sayyar *et al.*,2022; Joshi *et al.*, 1999). Suppression of cell-mediated immunity is also reported by previous researchers which make the T2DM patients more vulnerable to infections (Eliashiv *et al.*, 1978). Additionally, antioxidant system involved in bactericidal activity is also impaired

due to hyperglycemia and has contribution in causing infections among diabetic subjects (Joshi *et al.*, 1999). Furthermore, in individuals with T2DM, dysregulation of inflammatory cytokines significantly rises the vulnerability to infections. Notably, mononuclear cells and monocytes exhibit a diminished interleukin-1 (IL-1) and IL-6 production in response to lipopolysaccharides, which is attributed to an intrinsic cellular defect. Additionally, the process of glycation inhibits the production of interleukin-10 (IL-10), interferon gamma (IFN-γ), and TNFα, thereby reducing the expression of major histocompatibility complex and ultimately impairing cell-mediated immunity (Casqueiro *et al.*, 2012).

Respiratory tract infections are a significant concern for T2DM patients, who face a higher risk of upper and lower respiratory infections compared to healthy individuals. Severe pneumonia caused by pathogens like *Streptococcus pneumoniae*, influenza, leads to prolonged hospitalization and worse outcomes in diabetics (Al-Sayyar *et al.*, 2022; Casqueiro *et al.*, 2012; Holt *et al.*, 2024). Previous research indicates that T2DM patients are more likely to experience recurrent lower respiratory infections (Al-Sayyar *et al.*, 2022). Respiratory system is consistently exposed to inhaled pathogens, but pulmonary host defence mechanisms can defend the lungs effectively. Innate immunity through its efficient components such as dendritic cells, lymphoid tissue, IgA and IgG, chemotactic factors, macrophages, innate immune proteins including defensins, mucins, surfactant proteins along with adaptive immune responses involving T and B lymphocytes provide protection to respiratory tract from infections. Filtration through nasal cavity, cough and neurological tuberculosis reflexes are additional mechanism that clear the huge foreign particles and protect the respiratory tract from damaging effects of these foreign antigens (Al-Sayyar *et al.*, 2022; Hartl *et al.*, 2018). In T2DM, the increased

predisposition to infection with severe consequences are due to the defects in above mentioned functions at multiple levels (Al-Sayyar *et al.*, 2022).

Oxidative stress from chronic hyperglycemia contributes to this vulnerability by forming advanced glycation end-products, which impair pulmonary defenses and worsen infection prognosis (Al-Sayyar *et al.*, 2022). Additionally, elevated glucose levels activate glycolysis, promoting pro-inflammatory immune responses. Viruses enhance glucose uptake and glycolysis in infected cells, creating an environment conducive to viral replication while suppressing T-cell function. Hyperglycemia-driven ROS production further exacerbates inflammation by releasing pro-inflammatory cytokines, fostering recurrent viral infections (Damen *et al.*, 2022). A similar spectrum of infection outcomes was observed for bacterial infection, specifically tuberculosis, caused by intracellular bacteria *Mycobacterium tuberculi*. T2DM significantly increases the risk of active tuberculosis, especially in South Asian countries, where it contributes to 14-15% of cases. Defective innate and adaptive immune responses in diabetics, along with malnutrition, heighten susceptibility to tuberculosis (Kubiak *et al.*, 2019; Tahir *et al.*, 2016). Major extra pulmonary infections in T2DM have been reported in previous studies. T2DM patients are particularly prone to severe bacterial and fungal urinary tract infections, with common pathogens including *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus species, Group B Streptococcus,* and *Candida albicans* (Casqueiro *et al.*, 2012).

Gastrointestinal motility and sensitivity help defend against infections, but chronic hyperglycemia elevates the risk of gastrointestinal infections by inducing gastric dysmotility, compromising the body's natural defenses. Common gastrointestinal infections among diabetic patients include oral and esophageal

candidiasis, gastritis, and hepatitis B and C (Casqueiro *et al.*, 2012). Additionally, T2DM patients are susceptible to skin and soft tissue infections such as furunculosis, folliculitis, and subcutaneous abscesses. Diabetic foot infections are a leading cause of hospitalization, often resulting in amputations, osteomyelitis, and even death. Diabetic angiopathies and neuropathies are classical risk factors for diabetic foot ulcers (Holt *et al.*, 2024; Tomita *et al.*, 2016). These ulcers can be monomicrobial or polymicrobial, with S*taphylococcus aureus* and *Staphylococcus epidermidis* being the most frequent pathogens, while enterococci, streptococci, and enterobacteria contribute less frequently. Diabetics are also prone to invasive external otitis caused by *Pseudomonas aeruginosa* and *Rhinocerebral mucormycosis*, affecting the nose, sinuses, and brain, particularly in individuals with weakened immune systems (Casqueiro *et al.*, 2012). The summary on the mechanisms associated with the interface T2DM and infections are presented in Figure 2.2.

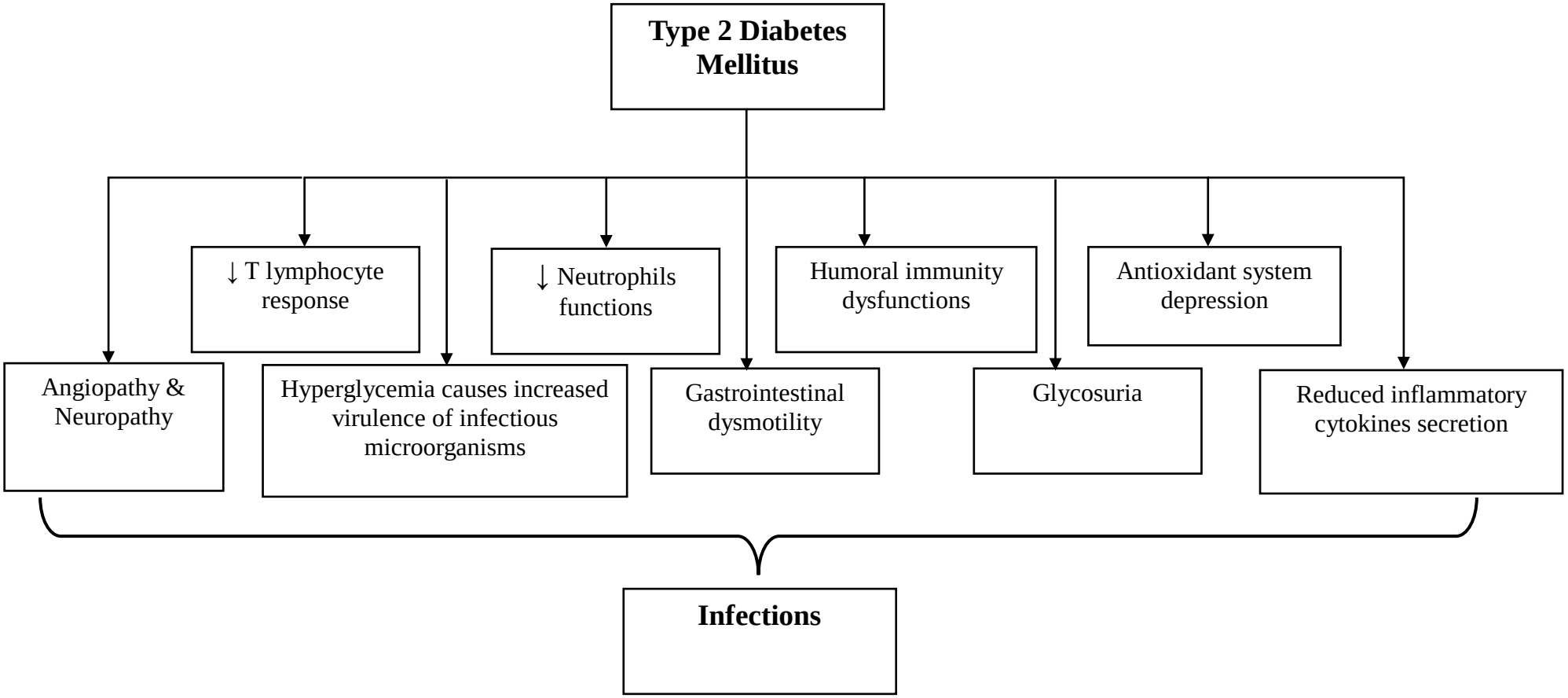

Figure 2.2 Mechanisms of Infections associated with Type 2 Diabetes Mellitus

2.1.8 Type 2 Diabetes Mellitus and Quality of life

Diabetes is a chronic disease recognized globally as an epidemic with significant social and economic consequences (Kehailou *et al.*, 2020). According to the WHO, quality of life (QOL) is defined as "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns (World Health Organization, 2012)." This multidimensional concept encompasses general domains such as physical health, psychological well-being, level of independence, social relationships, environmental factors, and personal beliefs (Karki *et al.*, 2025; Kehailou *et al.*, 2020). T2DM, due to its lifelong management demands and associated complications, can adversely affect many of these general domains physical functioning, mental and cognitive health, emotional stability, and social interaction, leading to a substantial decline in overall QOL (Karki *et al.*, 2025; Takahashi *et al.*, 2006).

Despite advancements in insulin therapy and modern oral hypoglycemic agents, a complete cure for T2DM remains elusive. Patients often experience significant anxiety and stress related to disease management, further intensified by concerns over chronic complications that may result in disability. The need for insulin administration can add to psychological and emotional distress, contributing to a lower QOL compared to healthy individuals. Moreover, recurrent infections are common in individuals with T2DM and can result in serious complications, disability, and physical decline, significantly impairing QOL (Al-Nimer *et al.*, 2019; Jing *et al.*, 2018). Notably, studies have associated surfactant protein-D (SP-D) levels with susceptibility to infections, suggesting that altered SP-D concentrations may increase the risk of severe infections (Jawed *et al.*, 2021). Despite this, no research

has yet explored the direct impact of SP-D on quality of life in T2DM. Understanding the role of SP-D in immune defense could therefore offer important insights into the prevention of infection related disabilities and the improvement of QOL among diabetic patients.

2.2 Surfactant Protein -D

2.2.1 Overview of Surfactant Protein –D

Surfactant Protein-D (SP-D) is crucial constituents of innate immune system and belongs to collagenous subfamily calcium dependent lectins called “collectins” (Crouch, 2000; Watson *et al.*, 2021). SP-D is a large, soluble, hydrophilic protein found on most mucosal surfaces, playing diverse roles in lung homeostasis and innate immunity (Watson *et al.*, 2021). It is primarily synthesized by alveolar type II cells, Clara cells, and bronchiolar epithelial cell, but is also present in extrapulmonary sites such as serum, brain, pancreas, gut, urogenital tract, heart, and amniotic fluid (Sorensen, 2018). Its presence in these sites may result from leakage through alveolar capillaries into systemic circulation. Serum SP-D levels may reflect bronchoalveolar SP-D levels and could serve as a promising biomarker for lung damage in pulmonary diseases (Sorensen, 2018; Leth-Larsen *et al.*, 2005; Sorensen *et al.*, 2006).

Pulmonary collectins like SP-D and mannose-binding lectin act as pattern recognition receptors, binding to viral, bacterial, and fungal surfaces, thereby enhancing phagocytosis by neutrophils and macrophages as a first line of defense (Leth-Larsen *et al.*, 2005; Sorensen, 2018). Additionally, it diminishes inflammation through interacting directly with pathogens and modulating host cell responses through a series of cellular receptors (Sorensen, 2018).