PHARMACOMETRICS TIME-TO-EVENT AND DISEASE PROGRESSION MODELLING OF DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE II DIABETES MELLITUS IN MALAYSIA

SOHAIL AZIZ

UNIVERSITI SAINS MALAYSIA

2024

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by

SOHAIL AZIZ

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

July 2024

ACKNOWLEDGEMENT

I would like to begin by dedicating this work to my beloved parents, whose unwavering prayers and constant guidance have been a source of inspiration and strength throughout my academic journey. Their love, encouragement, and sacrifices have played a pivotal role in shaping the person I am today.

I dedicate this work to my esteemed supervisor Dr Siti Maisharah Sheikh Ghadzi, whose invaluable guidance and unwavering support have been instrumental in shaping the trajectory of my academic pursuits. Her expertise, patience, and encouragement have been instrumental in shaping the direction of my research and guiding me through the challenges along the way. Your mentorship has illuminated my path and enriched my scholarly journey beyond measure.

I would also like to express my gratitude to my co-supervisors, Dr Sabariah Noor Harun, and Prof Syed Azhar Syed Sulaiman, for their valuable insights, constructive feedback, and contributions to my research. Their expertise and guidance have been instrumental in enriching the depth and quality of my work.

I am indebted to my field supervisor, Dr Shalini C Sree Dharan and Dr. Nor Azizah Binti Aziz, for her invaluable guidance and support throughout my research project. Her expertise and dedication in the field have provided me with valuable insights and enriched my understanding of the subject matter.

I would like to extend my appreciation to Dr Orwa Albitar and Dr Marwa Elsaeed Elhefnawy for their thorough assistance, technical support, and valuable discussions throughout this journey. Their contributions have been invaluable in enhancing the quality and rigor of my research.

Furthermore, I would like to acknowledge the financial support provided by the USM Fellowship, which has enabled me to pursue my research endeavours. Their generosity and support have been crucial in facilitating the successful completion of this work.

I would like to express my sincere gratitude to Jawatankuasa Etika Penyelidikan (Manusia) (JEPeM), USM, and MREC (Medical Research and Ethics Committee) for granting me permission and ethical approval for data collection in my PhD research. Their invaluable support and cooperation have been crucial in ensuring the ethical conduct of my study and upholding the rights and well-being of the participants involved. I am deeply appreciative of their guidance and assistance throughout this important phase of my research.

Finally, I express my gratitude to all my friends, colleagues, and loved ones who have provided support, encouragement, and understanding throughout this challenging yet rewarding journey. Your presence and belief in me have been a constant source of motivation.

Words cannot fully express my appreciation for all those who have contributed to my academic and personal growth. I am forever grateful for their presence in my life and their impact on my journey towards obtaining this PhD degree.

Sohail **A**ziz

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LIST OF ABBREVIATIONS

ARBs	Angiotensin Receptor Blockers
ACEi	Angiotensin Converting Enzyme Inhibitors
BMI	Body Mass Index
BSV	Between Subject Variability
CVD	Cardiovascular Disorder
CCB	Calcium Channel Blockers
DN	Diabetic Nephropathy
DP	Disease Progression
eGFR	Estimated Glomerular Filtration Rate
FSI	Fasting Serum Insulin
FBS	Fasting Blood Sugar
GOF	Goodness of Fit
HBA1c	Glycated Haemoglobin
HR	Hazard Ratio
KM-VPC	Kaplan Meier Visual Predictive Check
OFV	Objective Function Value
RSE	Relative Standard Error
RUV	Residual Unexplained Variability
SBP	Systolic Blood Pressure
T2DM	Type 2 Diabetes Mellitus
TTE	Time-to-Event
UAE	Urinary Albumin Excretion
VPC	Visual Predictive Check

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PEMODELAN FARMAKOMETRIK "TIME-TO-EVENT" DAN PERKEMBANGAN PENYAKIT BAGI NEFROPATI DIABETIK DALAM PESAKIT DIABETES JENIS II DI MALAYSIA

ABSTRAK

Nefropati diabetis (DN) adalah komplikasi yang paling umum untuk penyakit diabetes mellitus jenis 2 (T2DM) serta menimbulkan beban yang besar kepada sistem penjagaan kesihatan di seluruh dunia. Kajian ini bertujuan untuk membangunkan model masa ke kejadian, (TTE) dan model perkembangan penyakit (DP) berasaskan farmakometrik untuk mendapatkan gambaran tentang faktor-faktor yang mempengaruhi perkembangan nefropati diabetis dalam kohort pesakit Malaysia, di samping menganalisis perbezaan dalam ciri klinikal antara pesakit dengan dan tanpa nefropati diabetis. Data dikumpulkan dari dua hospital penjagaan tertiari, dan perkembangan pesakit diikuti selama 7.2 tahun. Analisis parametrik TTE telah dijalankan menggunakan perisian NONMEM. Pemboleh ubah kajian ini ditakrifkan sebagai perkembangan proteinuria berterusan untuk tiga lawatan berturut-turut. Tiga model bahaya garis dasar eksponen, model bahaya Gompertz dan Weibull telah diuji. Untuk model asas HbA1c dan eGFR, kedua-dua model perkembangan penyakit linear dan bukan linear telah dinilai. Model linear mewakili model perkembangan penyakit paling mudah yang digunakan dalam kajian ini, dengan mengandaikan kadar perubahan yang berterusan dalam status penyakit dari semasa ke semasa. Perbezaan dalam kumpulan dalam parameter klinikal di bawah pelbagai rawatan ubat telah dinilai menggunakan ujian peringkat bertanda Wilcoxon, manakala analisis antara kumpulan dijalankan menggunakan ujian Mann-Whitney U. Kohort kajian ini terdiri daripada 251 pesakit T2DM, dan data klinikal serta makmal yang komprehensif dikumpulkan untuk pembangunan model. Analisis TTE mendedahkan perkaitan yang ketara antara

tekanan darah sistolik (SBP) dan kandungan gula darah semasa puasa (FBS) dengan peningkatan risiko untuk perkembangan nefropati diabetis. Berdasarkan model akhir, garis dasar risiko ialah 1.006/tahun manakala risiko meningkat separuh selepas setiap 0.7 tahun. Setiap satu unit peningkatan eGFR melebihi 85.20 ml/min/1.73m2 mengurangkan risiko sebanyak 5%. Manakala setiap peningkatan unit dalam FBS melebihi 7.4 mmol/L meningkatkan risiko sebanyak 25% dan setiap peningkatan unit dalam SBP melebihi 132 mmHg meningkatkan risiko bahaya sebanyak 7%. Model linear ialah model DP paling mudah yang digunakan dalam kajian ini, di mana kadar perubahan status penyakit dianggap berterusan dari semasa ke semasa. Model perkembangan penyakit mengenal pasti FBS, umur, dan indeks jisim badan (BMI) sebagai faktor risiko yang memburukkan status glisemik dalam pesakit diabetes. Garis asas HbA1c ialah 10.1%, manakala setiap kenaikan unit dalam FBS melebihi 7.4 mmol/L dikaitkan dengan penurunan sebanyak 0.06% dalam HbA1c dalam masa setahun. BMI mempunyai impak tertinggi terhadap perkembangan penyakit T2DM berkaitan HbA1c (0.12%/tahun). Selain itu, dalam DP berdasarkan eGFR, gangguan kardiovaskular (CVD) bersamaan dengan diabetes mengakibatkan penurunan eGFR sebanyak 1.05 ml/min/1.73m^2/tahun. Setiap kenaikan unit FBS melebihi 7.4 mmol/L dikaitkan dengan penurunan eGFR sebanyak 0.043 ml/min/1.73m^2/tahun dan ARB telah menunjukkan peningkatan eGFR sebanyak 0.4 ml/min/1.73m^2/tahun. FBS muncul sebagai alternatif yang berpotensi untuk menilai profil glisemik apabila pemeriksaan HbA1c tidak tersedia. Selain itu, analisis konvensional mendedahkan perbezaan dalam parameter klinikal yang berkaitan dengan penggunaan ubat. Pesakit DN menunjukkan paras HbA1c dan SBP yang tinggi ($p<0.001$) berbanding pesakit bukan DN, seiring dengan peningkatan ketara secara statistik dalam BMI daripada garis dasar dalam pesakit DN ($p=0.034$). Peningkatan yang ketara dalam kedua-dua

HbA1c dan BMI direkodkan pada pesakit bukan DN yang sedang menjalankan terapi metformin. Walau bagaimanapun, pengurangan ketara dalam tahap HbA1c diperhatikan pada pesakit yang menggunakan insulin, dengan peningkatan ketara dalam BMI yang diperhatikan pada pesakit bukan DN. Tiada perbezaan ketara dalam parameter klinikal antara kohort pesakit yang menerima rawatan dengan ARB atau ACEi (perencat enzim angiotensin). Walau bagaimanapun, penurunan ketara dalam eGFR juga diperhatikan pada pesakit yang diberikan CCB (penghalang saluran kalsium). Secara ringkasnya, kajian ini memberikan penemuan yang berharga tentang pemodelan berasaskan farmakometrik TTE dan perkembangan penyakit nefropati diabetis dalam kalangan pesakit T2DM. Penemuan ini menekankan kepentingan pemantauan secara rapi, pengurusan intensif glisemia, dan campur tangan berkala untuk faktor risiko yang boleh diubah suai untuk melambatkan permulaan dan perkembangan nefropati diabetis. Kajian ini juga lebih menyerlahkan potensi terapeutik untuk meningkatkan hasil pesakit dan menekankan kepentingan strategi pengurusan yang lebih peribadi atau individu untuk populasi yang berisiko tinggi ini**.**

PHARMACOMETRICS TIME-TO-EVENT AND DISEASE PROGRESSION MODELLING OF DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE II DIABETES MELLITUS IN MALAYSIA

ABSTRACT

Diabetic nephropathy (DN) is a significant complication of type 2 diabetes mellitus (T2DM) and poses a considerable burden on healthcare systems worldwide. This study aims to develop pharmacometrics-based time-to-event (TTE) and disease progression (DP) models to gain insights into the factors influencing the progression and development of diabetic nephropathy in a cohort of Malaysian patients, while also analysing the differences in clinical characteristics between patients with and without diabetic nephropathy. The data were collected from two tertiary care hospitals, and the patients were followed for 7.2 years. Parametric TTE analysis was conducted using NONMEM software. The event was defined as the development of persistent proteinuria for three consecutive visits. Three baseline hazard models exponential, Gompertz, and Weibull hazard models were tested. For the baseline HbA1c and eGFR model, both linear and non-linear disease progression models were assessed. The linear model represents the simplest disease progression model utilized in this study, assuming a constant rate of change in disease status over time. Within-group differences in clinical parameters under various drug treatments were evaluated using the Wilcoxon signed-rank test, while between-group analyses were conducted using the Mann-Whitney U Test. The study cohort consisted of 251 T2DM patients, and comprehensive clinical and laboratory data were collected to inform the model development. The TTE analysis revealed significant associations between systolic blood pressure (SBP) and fasting blood sugar (FBS) with an increased hazard of developing diabetic nephropathy. Based on the final model the baseline hazard was

1.006/year while the hazard increases by half after every 0.7 year. Every one unit increase in eGFR above 85.20 ml/min/1.73m^2 decrease the hazard by 5%. While Every unit increase in FBS above 7.4 mmol/L increases hazard by 25% and every unit increase in SBP above 132 mmHg increase the hazard by 7%. Linear model is the simplest DP model used in the present study, which assume a constant rate of change of disease status over time. The disease progression models identified FBS, age, and body mass index (BMI) as risk factors for worsening glycaemic status in diabetic patients. Baseline HbA1c was 10.1%, while every unit rise in FBS above 7.4 mmol/L was associated with 0.06% incline in HbA1c per year. BMI has the highest impact on HbA1c associated disease progression of T2DM (0.12%/year). Additionally, in the DP based on eGFR, cardiovascular disorders (CVD) concurrent to diabetes resulted in decrease of eGFR by 1.05 ml/min/1.73m^2/year. Every unit rise in FBS above 7.4 mmol/L was associated with decrease in eGFR by 0.043 ml/min/1.73m^2/year and ARBs have shown to improve the eGFR by 0.4 ml/min/1.73m^2/year. FBS emerged as a potential alternative for evaluating glycaemic profiles when HbA1c examinations are not readily available. Additionally, the conventional analysis revealed differences in clinical parameters associated with the use of drugs. DN patients exhibited elevated levels of HbA1c and SBP ($p<0.001$) compared to non-DN patients, along with statistically significant escalation in BMI from baseline in DN patients ($p=0.034$). Considerable improvement in both HbA1c and BMI was recorded in non-DN patients on metformin therapy. However, significant reduction in HbA1c level was observed in patients on insulin, with a substantial increase in BMI observed in non-DN patients. There was no significant variance in clinical parameters between the patient cohorts receiving treatment with ARBs or ACEi (angiotensin converting enzyme inhibitors). However, a notable decline in eGFR was observed in patients administered with CCBs

(calcium channel blockers). In summary, this study provides valuable insights into the pharmacometrics-based modelling of TTE and disease progression in diabetic nephropathy among T2DM patients. The findings underscore the importance of close monitoring, intensive management of glycaemia, and timely intervention in modifiable risk factors to delay the onset and progression of diabetic nephropathy. The study also highlights potential therapeutic avenues for improving patient outcomes and emphasizes the significance of personalized management strategies for this high-risk population.

CHAPTER 1

INTRODUCTION

1.1 Background

Diabetes is a chronic multi-system disorder characterised by hyperglycaemia condition. Hyperglycaemia is associated with either insulin secretion or insulin action, or both. Persistent high blood glucose level in T2DM leads to multifaceted complications and indelible damages to multiple organs like diabetic retinopathy (eye), diabetic nephropathy (kidney), diabetic neuropathy (nerves), and cardiovascular (blood vessels and heart) (Saraco *et al.*, 2021).

According to the guidelines by the American Diabetes Association (ADA), diabetes is categorized into several types, including type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes, and other specific types of diabetes that are linked to various causes such as monogenic diabetes syndrome, diseases affecting the exocrine pancreas, and diabetes induced by drugs or chemicals. T1DM also referred to as insulin dependent diabetes mellitus exists in 5-10% of diabetic patients and is juvenile onset usually leads to absolute insulin deficiency. Cellular mediated autoimmune degeneration of pancreatic beta-cells leads to this type of diabetes and may sometime be idiopathic in nature causing insulinopenia. Such patients with idiopathic causes are usually prone to ketoacidosis and without proper evidence of autoimmunity (Saraco *et al.*, 2021).

T2DM is complicated in nature and usually leads to gradual decline in production of insulin by beta cell accompanied by insulin resistance. T2DM is also known as non-insulin dependent and accounts for 90 to 95% of overall diabetic population. T2DM usually does not have a specific aetiology but unlike T1DM it is

not caused by autoimmune destruction of beta cells. Overweight or obesity is one of the prime factors of most of the diabetic patients and it has been known as excess weight itself can lead to some degree of insulin resistance. Certain individuals may not exhibit obesity or overweight status according to conventional standards, such as BMI estimates. However, they may possess a higher proportion of body fat, mostly concentrated in the abdomen area (Karpe *et al.*,, 2011). T2DM often remains undetected for extended periods due to the slow progression of hyperglycaemia, which seldom reaches a severity that leads to the manifestation of diabetic symptoms.

Patients with T2DM may appear to have normal or elevated insulin levels, but if their beta cell function were normal, an increase in blood glucose levels would have resulted in even higher insulin levels (Saraco *et al.*, 2021). This finding illustrates that the insulin secretion in these individuals is inadequate in counteracting insulin resistance. Weight reduction and/or the use of anti-hyperglycaemic medications have the potential to ameliorate insulin resistance; nonetheless, it is uncommon for individuals to fully restore normal insulin levels. Obesity, age, and sedentary lifestyle all augment the likelihood of developing T2DM (Saraco *et al.*, 2021).

Based on the findings of the International Diabetes Federation Diabetes Atlas 2019, it has been projected that the worldwide incidence of diabetes in the year 2019 is around 463 million individuals, with a potential escalation to 578 million by the year 2030 (Saeedi *et al*., 2019). Furthermore, it has also been observed that one in two diabetic patients are unaware of their condition (Saeedi *et al*., 2019). According to the National Health and Morbidity Survey (NHMS 2019) one in five adults have diabetes which equal a whooping 3.9 million Malaysians aged 18 and above (Chong *et al.*, 2022).

Similarly, the National Diabetes Registry of Malaysia 2020, which was introduced in 2009, reported in their recent survey that a mere 0.59% of patients were diagnosed with T1DM, 0.06% had other forms of diabetes, and the remaining 99.33% of patients were found to be associated with T2DM (Chandran *et al.,* 2019). The bulk of the patients fell between the age range of 50 to 54 years, constituting 17.16% of the total sample. Additionally, the reported mean age at diagnosis was 53 years. In terms of comorbidities, hypertension was the most prevalent (80.0%) followed by dyslipidaemia (75.72%). In addition, diabetic nephropathy (DN) was the most prevalent complication associated with T2DM in Malaysian population, increasing from 8.8% in 2013 to 14.6% in 2019 while a slight decrease was observed in 2020 (14.38%) (National Diabetes Registry Report, 2020). The escalating surge in the prevalence of DN is closely associated with the concurrent growth in the prevalence of end-stage renal disease (ESRD), necessitating the implementation of appropriate measures to tackle this issue (Chandran *et al.,* 2019).

1.2 T2DM Disease Progression

T2DM is progressive and is associated with complications related to hyperglycaemia, which includes cardiovascular disorders (CVD), microvascular complications, and mortality. The progression of T2DM is characterised by a gradual reduction in beta cell function and an exacerbation of insulin resistance. This progression is accompanied by the deterioration of many indices, including HbA1c levels, FBS levels, and postprandial glucose levels (Nichols *et al*, 2007).

The progression from a state of normal beta cells' function to the onset of diabetes may be elucidated by the five phases of gradual reduction in beta cell functionality. The first phase, sometimes referred to as the compensation phase, is

distinguished by an elevation in the production of insulin by beta cells as a reaction to an escalation in insulin resistance, with the objective of preserving optimal levels of blood glucose. During this stage beta cells mass either remains normal or increased. An illustrative instance of this phase is the presence of insulin resistance linked to obesity, characterised by an elevated rate of insulin secretion and a sudden rise in insulin secretion in response to glucose stimulation after an intravenous glucose infusion (Qu *et al*., 2018). In stage 2, the compensatory mechanism of beta cells cannot maintain the normoglycaemia, hence the glucose level rise and alterations in beta cell functioning manifest as a decrease in acute glucose-stimulated insulin production. Individuals in this stage may not develop diabetes for many years. In the subsequent phase (unstable early decompensation), beta cell mass decreases significantly and insulin resistance increases in the cells and therefore, the glucose level no longer remains in the prediabetic range.

Once an individual reaches the unambiguous diabetes of stage 4, although insulin secretion is sufficient to prevent ketoacidosis, the beta cell mass decreases by nearly 50 %. In most of T2DM cases, this stage remains lifelong while those with rapid autoimmune destruction of beta cells in T1DM may progress rapidly to stage 5. Marked loss of beta cells occur in the 5^{th} stage (severe decompensation) and individuals become ketotic and truly dependent on insulin. Typically, this condition affects patients with T1DM, where beta cells have been destroyed by autoimmune response and very rarely occurs in T2DM patients (Weir & Bonner, 2004).

1.3 Microvascular and Macrovascular Complications

The pathophysiology of vascular complications in diabetes patients is primarily influenced by hyperglycaemia, dyslipidaemia, epigenetic regulation, and genetics.

Vascular complications may be categorized into two main types: macrovascular complications, such as coronary artery disease and CVD, and microvascular, including neuropathy, nephropathy, and retinopathy. Elevated level of blood sugar leads to the aforementioned complications and intense management of blood sugar with anti-diabetic agents decrease the onset as well as the progression of diabetic vascular complications (Barrett *et al*., 2017; Paneni *et al*., 2013). Barret *et al*. (2017) have underscored the notion that hyperglycaemia may not be the only instigator of diabetic vascular problems, since lesser-known variables such as hereditary and endogenous protective factors also play a significant role. Furthermore, it has been observed that multiple contributing factors like cellular signalling, environmental phenotypes and epigenetic regulations may play their role in diabetes associated vascular complications (Burg *et al.*, 2015). As a matter of fact, endothelial, mesangial and retinal cells are better adapted than other cells to handle hyperglycaemia. Even with glucose level below the diagnostic threshold of diabetes, the detrimental effects exist which can be explained by the concept of glycaemic continuum during prediabetes and then diabetes. The occurrence of elevated blood glucose levels in the early stages, caused by insulin resistance linked to obesity or impaired insulin production, is primarily correlated with changes in the structure and function of the blood vessel wall. These abnormalities contribute to the development of vascular problems in individuals with diabetes (Paneni *et al*., 2013).

1.4 Diabetic Nephropathy

Diabetic nephropathy (DN), also known as diabetic kidney disease (DKD), is a very prevalent and commonly observed complication of diabetes mellitus. Diabetes mellitus is known to cause DN, which is characterised by the occurrence of

albuminuria and a progressive deterioration of renal function. DN is a highly prevalent complication associated with diabetes, with an incidence ranging from 30% to 40% among individuals. The prevalence of DN may vary across various populations (Sagoo & Gnudi, 2020). Not all individuals diagnosed with T2DM necessarily develop DN; nonetheless, among those who do develop DN, the progression exhibits variability. The onset of end-stage renal disease (ESRD) and the progression of DN are initiated by the synthesis and dissemination of advanced glycation end products (AGEs), as well as glomerular haemodynamic and hormonal changes linked to increased growth factor levels resulting from diabetes mellitus. The aforementioned alterations result in the production of reactive oxygen species (ROS) and inflammatory mediators, ultimately leading to glomerular hyperfiltration, renal hypertrophy, a modified glomerular filtration rate (GFR), and clinically evident albuminuria and hypertension. Furthermore, it is important to note that significant vascular consequences, including cardiac disease and mortality resulting from cardiac arrest, may manifest at any stage of the evolution of T2DM, ranging from diabetes mellitus to early DN (Umanath & Lewis, 2018). DN is often diagnosed by clinical assessment of many factors, including the estimated glomerular filtration rate (eGFR) level, albuminuria measures, and other clinical criteria such as the duration of diabetes and the existence of diabetic retinopathy (Ahmad, 2015 ; Levin *et al*., 2013). Based on KDIGO guidelines Clinically DN is characterised by the presence of continuously increased levels of albumin in the urine and/or a prolonged reduction in eGFR below60 ml/min/1.73m^2. Microalbuminuria is operationally defined as the quantitative measurement of urine albumin excretion (UAE) falling within the range of 30–300 mg/day, 20–200 μg/min in timed urinary collection, or 30–300 mg albumin/g creatinine in a spot specimen. The urine albumin-to-creatinine ratio (ACR) is considered the optimal diagnostic test

for albuminuria and is recommended to be performed on a spot urine sample, ideally collected in the morning (Chugh & Bakris, 2007;Levin *et al.*, 2013). The eGFR is calculated by evaluating the concentration of serum creatinine. Patients with T2DM who display the presence of microalbuminuria are acknowledged as having incipient nephropathy. However, the development of macroalbuminuria in these patients is indicative of the presence of clinical or overt nephropathy (Tuttle *et al.*, 2014). The natural progression of DN in T2DM patients is intricate due to challenges in accurately determining the precise onset of T2DM. Consequently, patients may exhibit proteinuria and diabetic nephropathy upon kidney biopsy prior to diagnosis of T2DM (Samsu, 2021 ; Umanath & Lewis, 2018).

Diabetes has reached an epidemic state and the resulted DN has become the most frequent cause of ESRD in most part of the world. From 2009 till 2011, it has been estimated that 60% of patients in Singapore, Malaysia, and Mexico had diabetes associated ESRD. Similarly, the incidence of ESRD was 40-50% in countries like Korea, Hong Kong, Philippines, Japan, the US, Israel, and New Zealand (Narres *et al.*, 2016). Furthermore, older age diabetic patients are at a higher risk of developing ESRD as observed in the US, where the patients in age group 65-74 years had the highest incidence of 584 per million, which was in accordance with the Australian Diabetes, Obesity and Lifestyle Study (AusDiab), comprising of 11,247 diabetic patients (Lim, 2014).

Accordingly, the prevalence of DN in Chinese and German population was found to be 20-30%, while the study using National Diabetes Registry of Saudi Arabia reported the prevalence of DN was10.8%. The difference in the prevalence can be associated with the racial and ethnical differences which may contribute to the

development of DN (Spanakis & Golden, 2013). Similarly, in Malaysian population, there was an observed rise in prevalence of CKD to 15.48% and diabetic nephropathy accounts for 58% of the new dialysis patients. Furthermore, the prevalence of microalbuminuria reported in Malaysia in a study was 25.4% among T2DM patients (Shahrir *et al.*, 2022). The associated factors contributing to the differences in prevalence may involve the standards of living, BMI and prevalence of hypertension which can be associated to increased salt sensitivity in Blacks and Asians, low responsiveness to renin-angiotensin in blacks, decreased potassium excretion, and variations in renal vasculature and nephron number, although some postulated mechanisms remain controversial (Zhang *et al.*, 2020;Young *et al*., 2005).

1.4.1 Risk Factors Contributing to Development of DN

The development of DN in individuals with T2DM exhibits considerable variability and complexity. In the context of DN, the development may be attributed to two main categories of risk factors: modifiable and non-modifiable. The modifiable risk factors consist of hyperglycaemia, hypertension, and dyslipidaemia, while the non-modifiable risk factors include age, race, and genetic profile (Lim, 2014).

The presence of HbA1c in blood serves as an indicator of an individual's mean blood glucose concentrations over the preceding two to three months, a period approximating the half-life of erythrocytes. Presently, the HbA1c test is widely acknowledged as the preferred method for diagnosing and monitoring diabetes, particularly T2DM. HbA1c serves as a reliable indicator of chronic hyperglycaemia and exhibits a strong association with the likelihood of enduring diabetic complications, establishing it as the preferred diagnostic tool for ongoing monitoring and chronic management of diabetes. However, from a diagnostic standpoint, the

HbA1c cut-off value remains disputed (Sherwani *et al.*, 2016). The bonding of glucose to the haemoglobin molecule has a concentration-dependent relationship, wherein the extent of bonding increases with elevated blood glucose levels in patients with diabetes. HbA1c is indicative of a person's average blood glucose level and are directly associated, as glucose binds to haemoglobin and becomes glycated (Herman & Fajans, 2010).

HbA1c not only serves as a significant biomarker for assessing long-term glycaemic control, but also exhibits predictive capabilities for evaluating lipid profile (Kidwai *et al.*, 2020); hence, using HbA1c for monitoring glycaemic control can have an added advantage of identifying the patients at risk of developing cardiovascular disorders. Thus, a single HbA1c test provides valuable insight for effective management of chronic conditions (Khan *et al.*, 2007).

In addition to aforementioned risk factors, susceptibility factors including age, gender, race, and family history, as well as initiation factors such as hyperglycaemia and acute kidney injury, and progression factors such as hypertension, dietary factors, and obesity, collectively contribute to the pathogenesis of DN. The most prevalent and well-established risk factors for DN are hypertension and hyperglycaemia. (Alicic *et al.*, 2017).

1.4.1(a) **Hyperglycaemia**

Hyperglycaemia is a key factor in the development of DN due to its effects on glomerular and mesangial cells; however, it is not the sole contributory factor. Mesangial cells are essential for the maintenance of glomerular capillary structure and smooth-muscle activity-mediated regulation of glomerular filtration. Hyperglycaemia is associated with increased mesangial cell proliferation and hypertrophy, as well as

increased matrix synthesis and thickness of basement membrane (Dronavalli *et al.*, 2008). Increased mesangial cell matrix production and mesangial cell apoptosis have been associated with hyperglycaemia. The proliferation of mesangial cells appears to be partially mediated by an increase in the glucose concentration of mesangial cells. Overexpression of glucose transporters such as glucose transporter 1 (GLUT1) and GLUT4, which increases glucose entrance into the cells, can induce comparable alterations in mesangial function under normal glucose conditions (Lin *et al*., 2006; Mishra *et al*, 2005). Hyperglycaemia may also boost vascular endothelial growth factor (VEGF) expression in podocytes, resulting in a significant increase in vascular permeability (Chen *et al.*, 2007). However, hyperglycaemia is not solely responsible for the development of diabetic nephropathy. Experiments in which non-diabetic kidneys were transplanted into diabetic patients revealed that nephropathy developed irrespective of glucose control. Thus, hyperglycaemia alone may be responsible but insufficient to produce renal injury (Thomas *et al.*, 2015). To elucidate the pathogenic effects of hyperglycaemia on tissue, three distinct processes have been postulated: non-enzymatic glycosylation leading to the formation of advanced glycosylation end products, activation of PKC, and augmentation of the aldose reductase pathway. Oxidative stress is shown as a common factor throughout all three pathways (Dronavalli *et al.*, 2008).

The glycosylation of tissue proteins has a role in the progression of diabetic nephropathy and associated microvascular complications. Chronic hyperglycaemia induces some of the excess glucose to bind to free amino acids on circulating or tissue proteins. Binding of glucose to amino acids leads to the development of advanced glycation end products (AGEs). AGEs can accumulate in the glomerular membrane and surrounding tissues, promoting inflammation, oxidative stress, and fibrosis, all of

which contribute to the progression of DN (Woodhams *et al.*, 2021). These sophisticated products have the potential to impact the pathogenesis of diabetic nephropathy by altering signal transduction pathways via the modulation of soluble signals, including cytokines, hormones, and free radicals (Singh *et al.*, 2014). As AGEs are normally eliminated in the urine, their levels in the blood are increased in patients with diabetes, particularly those with renal failure. The overall outcome is tissue accumulation of AGEs (partially via collagen cross-linking), which contributes to the associated renal and microvascular complications. Furthermore, AGEs products interact with the AGE receptor, resulting in a dose-dependent decrease in nitric oxide concentrations (Dronavalli *et al.*, 2008; Yamagishi & Matsui, 2010).

Polyols are thought to be involved in the pathogenesis of diabetic nephropathy. Multiple studies have demonstrated a reduction in urine albumin excretion in animals given aldose reductase inhibitors, however these agents have not been extensively examined in humans, and the results are ambiguous (Dronavalli *et al.*, 2008).

1.4.1(b) **Hypertension**

Patients with diabetes are almost twice as likely to have hypertension as the general population (Petrie *et al.*, 2018). According to International society of Hypertension (ISH) in the presence of diabetes, hypertension is defined as systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥80 mmHg (Unger *et al.*, 2020). In T2DM, hypertension frequently precedes renal dysfunction. The observed association between glucose intolerance and hypertension might potentially be elucidated by the presence of common risk factors, such as obesity. In a study, 58% of newly diagnosed T2DM patients (without proteinuria) were already hypertensive, while in other studies the proportion was as high as 70%. Hypertension accelerates the

progression of renal disease and contributes to the elevated prevalence of cardiovascular disease in diabetic population (Van Buren & Toto, 2011). The elevation of blood pressure and the onset of hypertension in individuals with diabetes and nephropathy may be attributed to a multitude of factors. The primary mechanisms contributing to hypertension in individuals with T2DM are the expansion of volume resulting from enhanced renal sodium reabsorption and the constriction of peripheral blood vessels owing to dysregulation of factors that regulate peripheral vascular resistance. In this context, hypertension is caused by the activation of the RAS, the overexpression of endothelin-1 (ET-1), the upregulation of reactive oxygen species, and the downregulation of nitric oxide (NO). Significantly, a number of these pathogenic variables have local non-haemodynamic effects that can exacerbate kidney disease and cardiovascular disease in diabetic and kidney disease patients (Sugahara *et al.*, 2021).

The RAS is vital for blood pressure and fluid homeostasis. Additionally, disruption of the system can increase tissue injury in chronic disorders such as hypertension, heart failure, and kidney disease. These mechanisms through which the RAS promotes disease development are most evident in diabetic nephropathy. The role of RAS is implicated in diabetic nephropathy based on animal model studies and randomised clinical trials demonstrating the effectiveness of angiotensin-converting enzyme inhibitors (ACEis) and angiotensin-receptor blockers (ARBs) in slowing the progression of renal disease (Gurley & Coffman, 2007). Angiotensin II (ANG II) produced locally exerts a variety of significant haemodynamic effects. Sodium reabsorption is enhanced in the proximal tubule, and predominantly causes efferent arteriolar vasoconstriction and raises the pressure and permeability of glomerular capillaries. Local, non-haemodynamic outcomes include increased cytokine

production; proliferation of glomerular and tubular cells; accumulation of extracellular matrix; and the generation of reactive oxygen species (ROS). When combined with the effects of hyperglycaemia, which itself promotes ANG II release, this provides a potent mix for the development and progression of diabetic kidney disease (Satirapoj & Adler, 2014).

1.4.1(c) **Dyslipidaemia**

Additionally, there is substantial evidence that the atherogenic mixed dyslipidaemia profile associated with increased triglycerides and low high-density lipoprotein (HDL) plays a crucial role in the development and progression of diabetes-related microvascular problems. Raised triglyceride levels and triglyceride-rich very low density lipoprotein (VLDL) appear to play a significant role in the progression of retinopathy and albuminuria (Kaysen & Eiserich, 2004). Additionally, elevated levels of apolipoprotein C-III, a non-competitive inhibitor of lipoprotein lipase activity, and increase in VLDL and impair arterial relaxation are predictive of renal disease and CVD development. Notably, it has been demonstrated that elevated plasma levels of apolipoprotein C-III are highly associated with proteinuria in individuals with T1DM (Klein *et al.*, 2004). Microalbuminuria and decreased GFR associated with decreased renal function contribute to endothelial dysfunction as well. As the renal function declines, hepatic synthesis of apolipoprotein A-I that is the primary apo-lipoprotein in HDL, diminishes, leading to a decline in plasma HDL cholesterol level. Apolipoprotein A-I is also a significant activator of lecithin–cholesterol acyltransferase, which is required for HDL maturation, and hence substantially impairs the quality of HDL. Additionally, inflammation results in further structural and functional abnormalities in HDL. Thus, key atheroprotective properties of HDL are

compromised, increasing the risk of oxidative damage to the vasculature. Oxidative stress plays a significant role in the pathogenesis DN by promoting inflammation, endothelial dysfunction, and fibrosis in the kidneys. (Brown, 2008; Misra *et al.*, 2003).

Furthermore, obesity has been linked to an increased incidence of diabetic nephropathy. Abdominal obesity, as measured by waist circumference, was linked with a greater prevalence of albuminuria but did not predict a reduction in GFR in the Diabetes Control and Complications Trial (DCCT). Weight loss, on the other hand, decreases urine albumin excretion and avoids GFR reduction (Saiki *et al*., 2005). In both T1DM and T2DM patients, smoking is related to an increase in albuminuria and a decrease in GFR (Chuahirun *et al*., 2004). Advanced age patients with T1DM and T2DM have an increased risk of nephropathy. This relationship appears to be unrelated to the duration of diabetes. Female sex was related to a lower risk of progression from mild to severe albuminuria or end-stage renal disease (ESRD) in the DCCT/EDIC (Epidemiology of Diabetes Interventions and Complications) trial. (De Boer *et al*., 2011; Nathan, 2014).

1.4.2 Diagnosis of Diabetic Nephropathy

DN is clinically diagnosed using eGFR and albuminuria measures, in combination with clinical criteria including the duration of diabetes and the existence of diabetic retinopathy (Alicic *et al.*, 2017). DN is defined clinically as the presence of a persistent albumin-to-creatinine ratio of ≥30 mg/g in the urine and/or a prolonged reduction in eGFR below 60 ml/min/1.73 m^2 (Selby *et al.*, 2020). DN screening should begin five years following diagnosis for individuals with T1DM and at the time of diagnosis for all individuals with T2DM. The prevalence of diabetic retinopathy in individuals with albuminuria is highly indicative of DN. The urine albumin-to-

creatinine ratio is considered the optimal diagnostic test for detecting albuminuria, and it is recommended to be performed using a spot urine sample, ideally collected in the morning. The estimated glomerular filtration rate (eGFR) is determined using the serum creatinine concentration (Tuttle *et al*., 2014). Although the chronic kidney disease-Epidemiologic Prognosis Initiative (CKD-EPI) equation is more accurate, particularly for eGFR values in the normal or near-normal range, clinical laboratories commonly report the Modification of Diet in Renal Disease (MDRD) equation. (Johnson *et al*., 2012).

The initial MDRD Study equation was created using 1628 participants with non-diabetic kidney disease predominately. It was based on six variables: age, gender, ethnicity, serum levels of creatinine, urea, and albumin. To ease clinical application, a 4-variable equation comprised of age, sex, ethnicity, and serum creatinine levels was presented. This equation is now widely accepted, and numerous clinical laboratories publish GFR estimates using it (Levey *et al.*, 2006). Extensive study of the MDRD Study equation reveals that it performs well in people with lower GFR levels but performs inconsistently in populations with higher GFR levels. Variability between clinical laboratories in the calibration of blood creatinine assays contributes error in GFR estimates, particularly at high levels of GFR, and may partially account for the inferior performance in this range (Michels *et al.*, 2010).

The accuracy of the MDRD equation in predicting GFR is most reliable for individuals with modest renal impairment, considering that the equation was developed using a population characterised by suboptimal kidney function. In individuals with a normal eGFR >90 mL/min/1.73m^2, MDRD tends to underestimate renal function. In 2009, the CKD-EPI developed and verified a novel equation with

the aim of achieving comparable precision to the MDRD equation for GFR values below 60 mL/min/1.73m^2, while also providing enhanced accuracy for higher GFR values. This was done in order to mitigate the issue of over-diagnosing CKD by employing the MDRD equation (Florkowski & Harris, 2011). The revised CKD-EPI equation was derived from 8254 data points collected from six trials and four clinical populations, with original serum creatinine values recalibrated using the Roche enzymatic approach. The CKD-EPI equation consists of log serum creatinine (modelled as a 2-slope linear spline with sex-specific knots at 62 mol/L in women and 80 mol/L in men), with gender, race, and age on the natural scale. Therefore, there are effectively four equations for whites (men, women, above the knot value, below the knot value) and four equations for African-Americans, where a separate component is applied. In the subgroup with eGFR <60 ml/min/1.73m^2, the CKD-EPI equation was found to be as accurate as MDRD, and much more accurate in the subgroup with eGFR >60 ml/min/1.73m^2 (Levey *et al.*, 2009; Florkowski & Harris, 2011). In certain scenario CKD-EPI may be superior over MDRD especially when eGFR is above 60 mL/min/1.73 m² or in populations without significant kidney impairment, but still literature is suggestive of the equality in overall performance of MDRD when compared to CKD-EPI. CKD-EPI has demonstrated superior accuracy in estimating eGFR in individuals with normal to near-normal kidney function, reducing the risk of overestimating kidney function compared to MDRD. CKD-EPI has been shown to perform better in estimating eGFR in older adults compared to MDRD (Matsushita *et al*., 2012; Matsushita *et al.,* 2010; Michels *et al*., 2010; Stevens *et al*., 2010). Present study incorporated MDRD for the measurement of eGFR. The reason behind using MDRD was that the equation has been in use for a longer time and has been extensively validated in various populations. Furthermore, the study centres mostly used MDRD

for calculating eGFR. Lastly, in stable eGFR MDRD and CKD-EPI offer almost the same accuracy.

To confirm albuminuria or a low eGFR, two abnormal readings must be taken at least three months apart. If characteristics not associated with DN are present, alternative causes of renal disease must be investigated. Atypical characteristics include an instant onset of low eGFR or a rapid decline in eGFR, an abrupt rise in albuminuria or the development of nephrotic or nephritic syndrome, refractory hypertension, and signs or symptoms of another systemic disorder, as well as a decrease in eGFR of up to 30% within two to three months following the initiation of RAS inhibitors. (Alicic *et al.*, 2017; Rocco & Berns, 2012).

1.4.3 Biomarkers for Detecting DN

Albuminuria as a measure of DN has limitations since many individuals exhibit GFR decline without change in albuminuria or even normo-albuminuria (Perkins *et al.*, 2010). Moreover, despite normo-albuminuria, histologically established advanced diabetic glomerular lesions can form. Likewise, macroalbuminuria is a better predictor of disease progression than low-grade albuminuria (Perkins *et al*., 2007). As a result, there is interest in developing biomarkers that might be used to detect DN sooner and to assess progression risk. Urine micro ribonucleic acid (RNA) profiling is also of relevance, however studies in that field is still very preliminary (Distefano *et al.*, 2013). Currently, the most promising biomarker is serum Tumour Necrosis Factor Alpha (TNF-α) receptor levels, which may be used to predict the development of CKD and ESRD in type 1 and type 2 diabetic patients. Along with albuminuria, the TNF-α receptor level was of particular importance in patients with T2DM (Niewczas *et al.*, 2012). Serum uric acid is another indicator that may be pathogenic. Conflicting results

have been obtained from studies using tubular biomarkers. These biomarkers have not been shown to contribute value to traditional prediction models in the larger studies. Additional research is required to elucidate the clinical relevance of biomarkers (Lim, 2014).

1.4.4 Management of Diabetic Nephropathy

The development of DN is influenced by multiple risk factors, necessitating the use of multifactorial interventional techniques for the management of patients with DN. The multifactorial strategy includes aggressive approaches in the reduction of blood glucose and blood pressure level, reduction of level of cholesterol along with dietary approaches and smoking cessation (Fineberg *et al.,* 2013).

The major emphasis in mitigating the development of DN has always been on intense treatment of hyperglycaemia. Certain drugs, such as PPAR- γ inhibitors (pioglitazone, rosiglitazone), have shown antifibrotic and anti-inflammatory properties in the renal tissue of diabetic rats, with their ability to reduce glucose levels. In addition, the incorporation of rosiglitazone into metformin treatment for T2DM has shown improvements in albuminuria and blood pressure, regardless of glycaemic control. (Ko *et al.*, 2008; Zhang *et al.*, 2008). In certain DN model, it has been shown that DPP-4 inhibitors have properties that might potentially mitigate inflammation and apoptosis. Similarly, it has been shown that sitagliptin has efficacy in decreasing albuminuria in individuals with T2DM, regardless of HbA1c levels. Conversely, algoliptin has been linked to a decrease in oxidative stress but does not exhibit any beneficial effects on renal function. (Kodera *et al.*, 2014; Mori *etal.,* 2014). Lastly, empagliflozin, sodium glucose cotransporter-2 (SGLT-2) inhibitor) has effects on

tubuloglomerular feedback and may be efficient in reducing hyperfiltration (Cherney *et al.*, 2014).

Additional comprehensive studies are required to determine the comparative efficacy of atypical therapy alternatives which are not implied in clinical settings on conventional basis such as vitamin D in managing hyperglycaemia, in order to ascertain their potential superiority or inferiority to conventional treatment choices. The use of different vitamin D derivatives in the management of renal diseases has been extensively documented throughout history. Several studies have shown that vitamin D compounds may potentially reduce overall death rates in individuals with chronic kidney disease (Yeung *et al*., 2023). There are many potential mechanisms that might explain the ability of vitamin D to reverse the progression of DN. These mechanisms include enhanced glucose metabolism, diminished stimulation of the RAS, and reduced fibrosis. Moreover, the underlying processes that explain the cross-sectional correlation between vitamin D and DN are yet to be elucidated. Animal studies have shown a correlation between the knockout of the vitamin D receptor in diabetic mice and the development of severe albuminuria and glomerulosclerosis. On the other hand, it is possible that vitamin D has the potential to mitigate the advancement of DN via its ability to augment insulin production, impede the deterioration of beta-islet cells, modulate osteocalcin, and therefore promote glucose metabolism (Derakhshanian *et al.*, 2015).

The various effects of the RAS on the development of DN necessitate the early administration of RAS inhibitors as a crucial step in avoiding the onset and progression of nephropathy in individuals with diabetes mellitus. This therapy is already used in clinical practise and should be a part of every diabetes patient's optimal management

(Wolf, 2004). Table 1.1 provides a comprehensive summary of the various pharmacological alternatives to antidiabetic medications, along with their effects on proteinuria and renal function.

Table 1.1 Pharmacological treatment of DN

Drug (s)	**Antiproteinuric**	**Preserve GFR**	**Diabetes type**
ACEi	++	++	T1/T2DM
ARB	++	++	T2DM
ACEi + ARB	+++	-	T1/T2DM
Aldosterone Antagonist	+	?	T2DM
Aldosterone Antagonist + ARB/ACEi	+++	?	T1/T2DM
Renin Inhibitor	++	?	T2DM
Renin Inhibitor + ACEi/ARB	+++	-	T2DM
Non-dihydropyridine CCB	+	?	T2DM
Non-Dihydropyridine CCB +ACEi/ARB	++	?	T2DM
Dihydropyridine CCB	-	-	T2DM
Allopurinol	?	?	T2DM
Statin	+	?	T2DM
Vitamin D	+	?	T2DM

+ data exist to indicate benefit; − data exist to indicate lack of benefit or harm;? insufficient data for conclusion, possible benefit. ACEi (angiotensin converting enzyme inhibitors), ARBs (angiotensin receptor blockers), CCB (calcium channel blocker)

Adapted from Lim, A. (2014). Diabetic nephropathy – Complications and treatment. International Journal of Nephrology and Renovascular Disease, 7, 361–381.

Samsu, N. (2021). Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. BioMed Research International, 2021.

1.4.5 Regression of Proteinuria

As a matter of fact, when morphological changes associated with diabetic nephropathy develop i.e., glomerulosclerosis or interstitial fibrosis, the chances of regression seem very theoretical and the progression to ESRD seems obvious. However, there are a few evidences from certain clinical studies, which indicates that

aggressive control of blood pressure with RAS inhibitors may lead to remission of overt proteinuria. Furthermore, aggressive approaches by incorporating ACE have been found to induce regression in experimental nondiabetic models of glomerulosclerosis (Adamczak *et al.*, 2003; Hovind *et al.*, 2004). Nonetheless, proper understanding of the complex pathophysiology of DN and instigating multifactorial management strategies may effectively help in minimizing the development of DN or inducing regression.

1.5 Pharmacometrics

Pharmacometrics refers to the scientific discipline that involves the development and utilization of mathematical and statistical techniques. Its primary objectives include: (a) the characterization, comprehension, and prediction of the pharmacokinetics and pharmacodynamics behaviour of drugs, (b) the quantification of uncertainty associated with information pertaining to that behaviour, and (c) the rationalization of data-driven decision-making within the contexts of drug development and pharmacotherapy (Williams & Ette, 2006). In a subsequent study conducted in 2008, Barret *et al*. proposed an expanded conceptualization of pharmacometrics as a scientific discipline that encompasses the development of mathematical models to characterise and quantify the interactions between xenobiotics and patients. These models encompass various aspects such as biology, pharmacology, disease, and physiology, and aim to elucidate both the positive therapeutic effects and potential adverse reactions that arise from these interactions (Barrett *et al.*, 2008). Furthermore, the Unites States (US) Food and Drug Administration (FDA) has concisely described pharmacometrics as an emerging science that quantify drug, disease, and trail information to aid efficient drug development or regulatory decision.

Drug models is a description of relationship between exposure (pharmacokinetics (PK)), response (pharmacodynamics (PD)), for both desired and undesired effects, as well as characteristics of the patients. On the other hand, disease models describe the association between biomarkers (covariates) and clinical outcome, disease progression, and placebo effects. In addition, trial models define inclusion and exclusion criteria, patient dropout and adherence. These description shows that pharmacometrics is a broad term, and not necessarily related only on the typical focus of pharmacokinetics/pharmacodynamic and clinical pharmacology (Bhavatharini *et al.*, 2022).

Pharmacometrics analysis often integrates non-linear mixed effect models (NLME), which enable simultaneous estimate of mean and variance of parameters derived from individuals within the research population describing a biological process. (Sheiner & Ludden, 1992). These models provide a means to account for inter-individual variabilities and estimate the mean values of parameters within the populations under investigation. However, it is important to note that these models may also be incorporated into the analysis of joint modelling, particularly in the context of PK and PD data, which pertain to biological responses. All these analyses using NLME models provide significant contributions to the population approach. Maximum likelihood estimation is widely recognized as the primary approach used to get parameter estimates. (Davidian & Giltinan, 2003; Karlsson *et al.*, 1995).

The population approach in pharmacometrics encompasses the use of multilevel modelling or mixed effects modelling techniques. The objective of these models is to estimate the parameter values by leveraging observed data and known covariates (Standing, 2017). Variabilities are inevitable due to the fact that PKPD data

frequently consists of multiple data points from numerous individuals. To explain all of these variations and reduce bias in parameter estimates, mixed effect modelling must be incorporated. In mixed effects models, parameter level variability exists, permitting parameters to vary between individuals. (Upton & Mould, 2014). Random effects consist of three levels of variability, namely interindividual variability (IIV), residual variability, and inter-occasion variability (IOV). IIV refers to the variation in parameter values observed among different individuals. Residual variability represents the differences between individual predictions of parameter values and actual observations. IOV describes the variation in parameter values observed between different occasions within the same individual (Mats O Karlsson *et al*., 1995)**.**

1.5.1 Population Modelling

As explained above, utilizing data obtained from clinical trials, pharmacometrics modelling aims to develop mathematical models that characterise and quantify drug behaviour and action, as well as disease progression. As humans (patients and healthy volunteers) vary, it is necessary to account for these differences in pharmacometrics models. The integration of population modelling into the discipline of PK-PD analysis necessitated the adoption of a two-stage approach for defining PK-PD interactions. The estimation of model parameters is conducted separately for each individual, after which summary statistics are computed on these individual parameters to evaluate population parameters, namely the mean, as well as the variability of the data (Karlsson *et al*., 2010).

Mixed effects modelling is an alternative to the conventional two-stage method, in which data from all individuals are used simultaneously to calculate population parameters (means and variances) (Dingemanse & Dochtermann, 2013).

The term "mixed" pertains to the use of both fixed effects, which characterise average individual (i.e., the mean), and random effects, which define the components of variability in the data. In theoretical terms, random effects include two distinct levels: the residual error, which represents the disparity between individual predictions and observations, and the inter-individual variability (IIV), which denotes the variance seen among individuals. Inter-occasion variability (IOV) is a third degree of variability that can be introduced if individual parameters vary between occasions arbitrarily or as a result of an unknown physiological process. Additional causes of variability, such as inter-study variability, may be accounted for in the same manner as IOV (Zuur *et al.*, 2009).

Nonlinear mixed effects modelling is a sort of population-based analysis used to analyse biologically derived data with nonlinear patterns between dependent and independent variables, example as the nonlinear relationship between hyperglycaemia and eGFR in a disease progression model. NLME models typically have three model components: the structural model, the stochastic model, and the covariate model. Model development often adheres to a fundamental methodology in which the structural and stochastic model is determined first, followed by the construction of covariate models that link relevant elements to model parameters (Owen & Fiedler, 2014).

There are several software packages available for NLME modelling. The use of different estimation methods is the primary difference between the packages. NONMEM (Nonlinear Mixed Effects Modelling) is the most frequently applied software package for population PK/PD/DP modelling (Smith, 2003). It permits model construction and parameter estimation using a population-based method. NONMEM